

The Importance of Selecting Permanent Pacemaker, Evaluating the Heart and Assessing Quality of Life in Cardiac Patient

Ika Prasetya Wijaya

Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

Corresponding Author:

Ika Prasetya Wijaya, MD., PhD. Division of Cardiology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia-Cipto Mangunkusumo Hospital. Jl. Diponegoro no. 71, Jakarta 10430, Indonesia. Email: ipwijaya@hotmail.com

The use of pacemakers is necessary for patients with symptomatic bradycardia. Pacemaker implantation also acts as a life-saving procedure. However, there are several reports that patients with a pacemaker (the most widely used pacemaker is on the right ventricle, known as single chamber pacemaker) had disturbances in left ventricle contraction lead to left ventricular systolic dysfunction. Global Longitudinal Strain (GLS) Echocardiography can confirm these left ventricular disturbances. Echocardiography examination is best carried out before and after single-chamber permanent pacemaker (PPM) implantation. This study compares PPM placement in apical Right Ventricle (RV) and Right Ventricular Outflow Tract (RVOT) and compares paced QRS duration 150 ms and > 150 ms. Pacing burden >40% causes subclinical left ventricular systolic dysfunction after a month of PPM implantation with decreased GLS in apical RV pacing.¹

This study gives additional information that PPM placement is preferably in RVOT with some specific settings. However, there are many ways to reduce impaired left ventricular function due to lead installation on the PPM single-chamber device. Using the PPM device with a dual chamber, the leads will be placed in RVOT and also in the right atrium with atrial-ventricle synchronization. The aim is to avoid impaired left ventricular pump function² as supported by a systematic review which states the superiority of dual-chamber PPM over single-chamber PPM

in reducing atrial fibrillation and pacemaker syndrome.³

A retrospective study found that regardless of PPM type in subjects with an EF >40%, there was no significant difference in long-term outcome between those with AV block or sinus node disease as etiologies of bradycardia. Otherwise, this study did not report the PPM lead position.⁴

In the importance of quality of life related to heart disease, the Indonesian version of the MacNew questionnaire can be used. This questionnaire assesses the quality of life of patients with coronary heart disease after revascularization surgery and who has undergone rehabilitation. The results were compared with the Indonesian version of the SF36 questionnaire, using Pearson's correlation, with $r = 0.47 - 0.71$. The researchers considered the Indonesian version of the MacNew questionnaire to have good validity and reliability.⁵ One of the challenges researchers face is the subject's understanding of the questionnaire, as there must be a common understanding of the questions. Another important thing is the suitability of the adaptation to the original English version.

REFERENCES

1. Iqbal M, Endamatriza GR, Lampita I, et al. The Influence of right ventricular pacing location, pacing burden and paced QRS duration to subclinical left ventricular systolic dysfunction as shown by Global Longitudinal Strain echocardiography. *Acta Med Indones.* 2021;53(3):245-53.
2. Dawood M, Elsharkawy E, Abdel-Hay MA, Nawar

- M, Alexandria EPS Unit. Comparative study between effect of single and dual chamber pacemaker on stroke volume, cardiac output and strain using 3D echocardiography, pulsed doppler methode and global longitudinal strain (GLS). *Eur Heart J*. 2020; 41(Suppl 2). doi: 10.1093/ehjci/ehaa946.0716.
3. Dretzke J, Toff WD, Lip GYH, et al. Dual chamber versus single cahmber ventricular pacemakers for sick sinus syndrome and atrioventricular block (Review). *Cochrane Database of Systematic Reviews*. 2004; 2. Art.No.:CD003710. Doi: 10.1002/14651858.CD003710.pub2.
 4. Ebert M, Jander N, Minners J, et al. Long term impact of the right ventricular pacing on left ventricular systolic function in pacemaker recipients with preserved ejection fraction: Result from a large single-center registry. *J Am Heart Assoc*. 2016;5:e003485 doi: 10.1161/JAHA.116003485.
 5. Basuki N, El-Ansary D, Hofer S, Dwiputra B, Nualnim N. The validity and reliability of the MacNew heart disease health related quality of life questionnaire: The Indonesian version. *Acta Med Indones*. 2021;53(3):276-81.

The Influence of Right Ventricular Pacing Location, Pacing Burden and Paced QRS Duration to Subclinical Left Ventricular Systolic Dysfunction as Shown by Global Longitudinal Strain Echocardiography

Mohammad Iqbal¹, Gadih R. Endamatriza¹, Irene Lampita¹, Sindy Hendrawansyah¹, Sidhi Laksono², Melawati Hasan¹, Chaerul Achmad¹, Giky Karwiky¹, Hanna Goenawan³, Mohammad R. Akbar¹, Arief S. Kartasasmita⁴

¹ Department of Cardiology and Vascular Medicine, Faculty of Medicine University of Padjadjaran, Bandung, Indonesia.

² Faculty of Medicine, University of Muhammadiyah Prof Dr Hamka, South Jakarta, Indonesia.

³ Departement of Biomedical Sciences, Division of Physiology, Faculty of Medicine University of Padjadjaran, Bandung, Indonesia.

⁴ Faculty of Medicine University of Padjadjaran, Bandung, Indonesia.

Corresponding Author:

Mohammad Iqbal, MD. Department of Cardiology and Vascular Medicine, Universitas Padjadjaran. Jalan Eyckman 38, Bandung 40161, Indonesia. E-mail: mohammadiqbal178@gmail.com; dr_mohammadiqbal@yahoo.com.

ABSTRAK

Latar belakang: Pemacuan ventrikel kanan/ right ventricle (RV) yang berkepanjangan berhubungan dengan gangguan fungsi ventrikel kiri/ left ventricle (LV). Beberapa studi menunjukkan bahwa posisi lead, presentasi pemacuan dan durasi QRS saat pemacuan dapat mempengaruhi fungsi sistolik LV. Gangguan LV subklinikal bahkan dapat timbul lebih dini setelah pemasangan permanent pacemaker (PPM). Studi ini bertujuan untuk mendeteksi gangguan awal sistolik LV subklinikal yang diukur dengan global longitudinal strain (GLS) dengan menggunakan speckle tracking echocardiography (STE) 1 bulan paska pemasangan PPM. **Metode:** Studi single center, metode kohort prospektif dengan kriteria inklusi pada penelitian ini adalah semua pasien dengan indikasi implantasi PPM dengan fungsi sistolik LV awal normal. Variabel pada penelitian ini adalah posisi sadapan (apeks RV dibandingkan dengan right ventricular outflow tract (RVOT), persentase pemacuan ($\leq 40\%$ vs $> 40\%$) dan durasi QRS selama pemacuan ($\leq 150\text{ms}$ dan $> 150\text{ms}$). Perubahan (delta) GLS diukur sebelum dan satu bulan setelah implantasi PPM. **Hasil:** Pada 37 pasien dalam penelitian ini, terdapat perbedaan yang signifikan antara GLS sebelum (-20,30 SB 3,38) dan setelah (-16,93 SB 3,47) implantasi PPM ($p < 0,001$). Tidak terdapat perbedaan yang signifikan pada delta GLS baik antara pasien dengan posisi sadapan pada apeks RV vs RVOT ((2,30 (0,00-10,50) vs 2,95(0,10-8,30), $p = 0,648$) ataupun antara pasien dengan durasi QRS selama pemacuan $\leq 150\text{ms}$ vs $> 150\text{ms}$ ((1,70 (0,30-8,30) vs 3,45(0,0-10,5)), $p = 0,266$). Sementara itu, terdapat perbedaan delta GLS yang signifikan antara pasien dengan persentase pemacuan $\leq 40\%$ vs $> 40\%$ ((Rata-rata 1,92 SB 1,37 vs 3,98 SB 3,04)), $p = 0,007$). Analisis lebih lanjut menunjukkan bahwa persentase pemacuan hanya akan mempengaruhi delta GLS pada kelompok dengan pemacuan apeks RV ($\leq 40\%$ (1,58 SB 0,59) vs $> 40\%$ (4,67 SB 3,47), $p = 0,008$) dan tidak mempengaruhi delta GLS pada kelompok dengan pemacuan RVOT ($\leq 40\%$ (2,32 SB 1,98) vs $> 40\%$ (3,29 SB 2,48)), $p = 0,446$). **Kesimpulan:** Disfungsi sistolik LV subklinis menurun secara signifikan satu bulan setelah implantasi alat pacu jantung yang ditunjukkan oleh penurunan GLS. Persentase pemacuan $> 40\%$ merupakan faktor yang berhubungan dengan penurunan GLS satu bulan setelah implantasi PPM, terutama ketika sadapan ditempatkan pada apeks RV.

Kata kunci: Right ventricular pacing, global longitudinal strain, lead position, pacing percentage, QRS duration during pacing.

ABSTRACT

Background: Prolonged pacing of the right ventricle (RV) is associated with left ventricular (LV) systolic dysfunction. Several studies have shown that the RV pacing location, pacing burden (percentage), and paced QRS duration may affect LV systolic function. Subclinical LV dysfunction may occur early after implantation of a permanent pacemaker (PPM). Therefore, this study aims to detect early subclinical LV systolic dysfunction measured by global longitudinal strain (GLS) using speckle tracking echocardiography (STE) at one month after PPM implantation. **Methods:** A single-center, prospective cohort study was conducted, and all patients indicated for PPM implantation with preserved LV systolic function were included. Data of RV pacing location (RV apical vs right ventricular outflow tract (RVOT)), pacing burden (percentage) ($\leq 40\%$ vs $> 40\%$), and paced QRS duration (≤ 150 ms and > 150 ms) were obtained. The change of GLS was also measured before and one month after PPM implantation (delta GLS). **Results:** 37 patients were enrolled in this study, which demonstrated significant difference between GLS before (-20.30 SD 3.38) and after (-16.93 SD 3.47) PPM implantation ($p < 0.001$). There were no significant difference in delta GLS either between patients with RV pacing location on RV apical vs RVOT ((2.30 (0.00–10.50) vs 2.95 (0.10–8.30), $p = 0.648$) or between patient with paced QRS duration ≤ 150 ms vs > 150 ms ((1.70 (0.30–8.30) vs 3.45 (0.0–10.5)), $p = 0.266$). Meanwhile, there was a significant difference of delta GLS between patients with pacing burden $\leq 40\%$ vs $> 40\%$ (Mean 1.92 SD 1.37 vs 3.98 SD 3.04), $p = 0.007$). Further analysis found that pacing burden only affected the delta GLS in group with apical RV pacing ($\leq 40\%$ (1.58 SD 0.59) vs $> 40\%$ (4.67 SD 3.47), $p = 0.008$) and did not affect the delta GLS in group with RVOT pacing ($\leq 40\%$ (2.32 SD 1.98) vs $> 40\%$ (3.29 SD 2.48), $p = 0.446$). **Conclusion:** The pacing parameter, particularly pacing burden $> 40\%$ may induce the subclinical LV systolic dysfunction after one month of pacemaker implantation as shown by decline of GLS, especially when the RV pacing location was placed on apical.

Keywords: Right ventricular pacing location, global longitudinal strain, pacing burden, paced QRS duration.

INTRODUCTION

Pacemaker implantation is the mainstay treatment for symptomatic bradycardia.¹ However, prolonged right ventricular (RV) pacing has been reported to be associated with left ventricular (LV) systolic dysfunction. The reported incidence of heart failure after RV pacing varied between 3-31% after 1-3 years of PPM implantation.²⁻⁶ Also, right ventricular pacing creates a non-physiological activation sequence, and the impulse conduction velocity is slower compared to normal conduction, leading to electrical and mechanical dyssynchrony in the LV.⁷⁻⁹ Some studies showed that RV pacing position (apical vs right ventricular outflow tract (RVOT)), pacing burden (percentage) and paced QRS duration may lead to LV systolic dysfunction.^{2, 10-15}

Echocardiography is the most widely used non-invasive modality to evaluate LV function because of its portability, low risk, and

comparatively high temporal resolution.^{16, 17} Furthermore, global longitudinal strain (GLS) using 2D echocardiography has high sensitivity (92%) and specificity (89%) for detecting early-stage of LV systolic dysfunction, therefore it can be used to detect subclinical LV systolic dysfunction.¹⁸ In this study, GLS was assessed before and one month after PPM implantation. In addition, the effect of RV pacing position, pacing burden, and paced QRS duration on the changes of GLS within 1 month was also assessed.

METHODS

This is a prospective cohort study, and a total of 50 patients who underwent pacemaker implantation for symptomatic bradycardia at Hasan Sadikin Hospital were prospectively enrolled between May 1, 2018 and January 31, 2019. The patient's inclusion criteria were > 18 years, have preserved LV ejection fraction ($EF \geq 50\%$), and have normal QRS duration (≤ 120

ms) before implantation PPM. This study has been approved by the Ethical Committee of Hasan Sadikin Hospital, Bandung (Ref. no. LB.02.01/X.6.5/67/2019).

Meanwhile, those with history of acute coronary syndrome, valvular heart disease, congenital heart disease, and congenital AV block before implantation and during follow-up were excluded. Also, patients with sinus node disease who implanted AAI were excluded. Those with poor echocardiography window and insufficient image tracking in more than one segment during GLS measurement were also excluded. In addition, GLS was measured within one week before implantation (as baseline GLS) and one month after implantation. The delta GLS is defined as a change of GLS value before and after implantation. The LV systolic dysfunction is defined as the significance of decline GLS (delta GLS) before and one month after implantation that measured by the statistic analyzed (Paired T-test).

ECHOCARDIOGRAPHY AND GLOBAL LONGITUDINAL STRAIN

A detailed echocardiographic evaluation was performed before and after one month of pacemaker implantation using Vivid S6 and Vivid

7 (GE Vingmed Ultrasound AS, Horten, Norway). Echocardiographic calculations of chamber quantification were quantified in accordance with The American Society of Echocardiography 2015 recommendations.¹⁹ In addition, cardiac anatomy and valvular were defined through standard transthoracic study, and the biplane Simpson's rule was used to calculate LV ejection fraction (LVEF).

Cardiac images were obtained at a rate of 50-70 frames per second (fps) from apical long-axis, apical two and four chambers view (16 segment AHA/ASE model). All raw data were stored and transferred for offline analysis to a workstation with EchoPAC 201 Version software. A region-of-interest was semi-manually traced and the software calculated the mean global strain values for all predetermined LV segments (**Figure 1**). The GLS measurement was taken during paced rhythm beat. Also, all echocardiographic imaging and strain values measurements were carried out by a single cardiologist specialized in echocardiography who was blinded to pacing interrogation results.

PACEMAKER IMPLANTATION AND ELECTROCARDIOGRAPHY

In sinus node disease patients, (Atrio-ventricular) AV node function was tested before

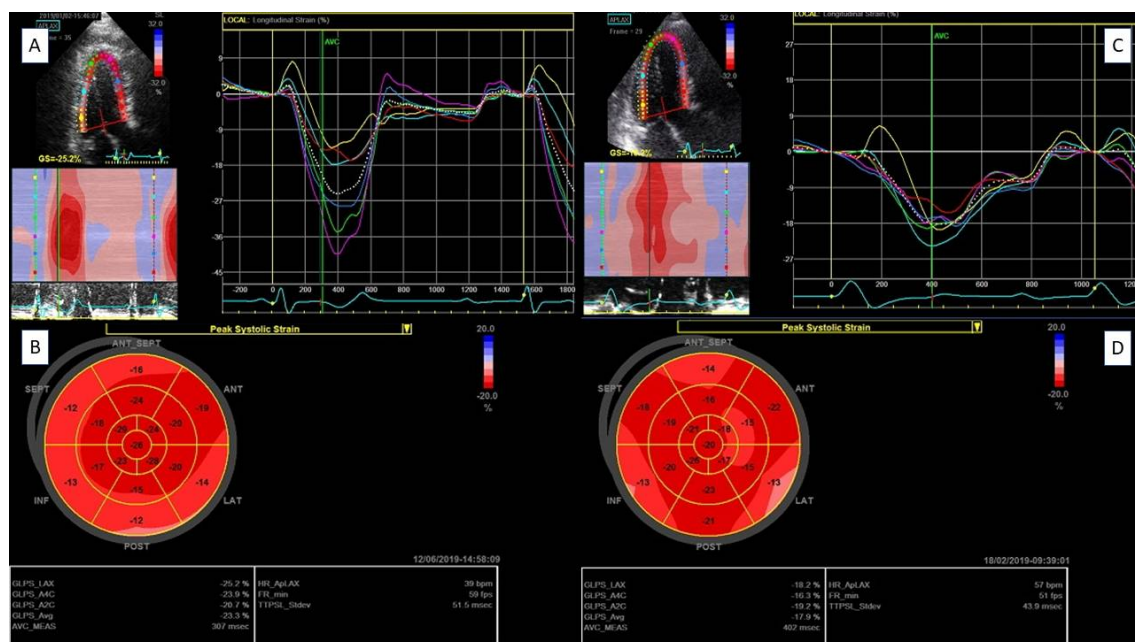


Figure 1. Global longitudinal strain at one week before pacemaker implantation (A and B), and one month after pacemaker implantation (C and D), corresponding strain curves are shown (A and C). The average GLS value at one week before pacemaker implantation is -23.3% (B), and the value is significantly decreased to -17.9% at one month after pacemaker implantation (D).

pacemaker implantation. Incremental atrial pacing was used to determine whether the patient had subclinical/impending AV block or AV node disease. When the atrial incremental atrial pacing showed AV 1:1 conduction in rate pacing >130 beats per mins, AAI was implanted. AV 1:1 conduction means that each capture atrial pacing was conducted to the ventricle. We excluded a patient who implants with AAI. In case of no 1:1 AV conduction with incremental atrial pacing in rate <130 beats per mins, it may suggest that patient had subclinical/ impending AV block or AV node disease. In such patient, VVI or DDI was advised over the AAI. The lower rate of VVI was determined according to baseline patient heart rate. For example, when the patient had a sinus node disease (with intermittent sinus pause), and the baseline heart rate was about 55-60 BPM, the lower rate was set at 50 BPM. This setting was important to avoid unnecessary RV pacing. In dual-chamber pacemaker, the AV interval was set up in the default setting (paced AV interval was 150 ms and sensed AV interval 120 ms). Meanwhile, in case of the prolonged PR interval, AV interval was set up (max of 250 ms) according to the physician's decision to reduced unnecessary RV pacing.

All pacemaker implantation was carried out by an experienced operator. Either RV apical or right ventricular outflow tract (RVOT) pacing

was achieved with an active fixation lead. Furthermore, the appropriate positioning of the lead was fluoroscopically confirmed at the time of implantation and by the QRS axis (during pacing) from ECG recorded after implantation (**Figure 2**). Right ventricle apical (RV apical) positions were considered when the QRS axis in inferior leads are predominantly negative, and RVOT positions were considered when QRS axis in inferior leads of ECG are predominantly positive. The pacing burden (percentage pacing) and other pacemaker parameters were determined from stored data after one month of follow-up. A standard 12 lead surface ECG was obtained at 25 mm/s. The paced QRS duration was defined as the length of time from the beginning of the pacing artifact (spike) to the end of the QRS complex. The QRS duration was measured using a digital caliper (KW06-351, Krisbow). The recorder QRS duration was the longest measured from all 12 leads of ECG. The pacing burden (percentage) was categorized into 2 groups with a cutoff of 40%. The paced QRS duration was categorized into 2 groups with a cutoff of 150 ms.

STATISTICAL ANALYSIS

The Shapiro-Wilk test was used to assess all the data distribution (normality data). Continuous variables were expressed as means (standard deviations), or median (range) when data were

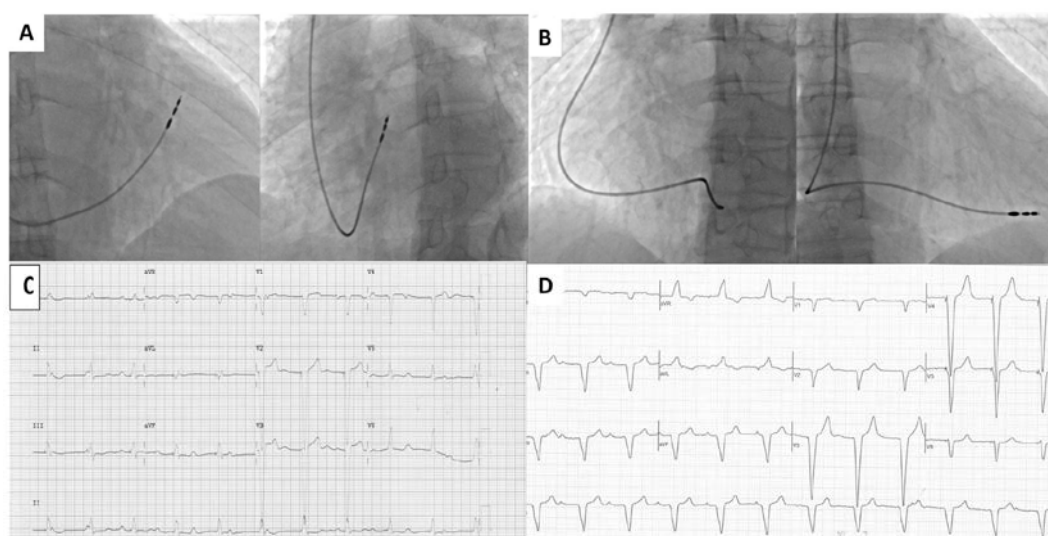


Figure 2. RAO 35° and LAO 40° position showed lead position on RVOT (A), confirmed by predominant positive axis of QRS in inferior leads, with the QRS duration 140 ms (B). RAO 35° and LAO 40° position showed lead position on RV apical (C), confirmed by predominant negative axis of QRS in inferior leads, with the QRS duration 166 ms (D). RAO : right anterior oblique, LAO: left anterior oblique.

not normally distributed. Categorical data were summarized as frequencies and percentages. Student's t-test or Mann-Whitney U test were the statistical methods used for paired or unpaired data depend on the normality data. Also, all probability values were deemed statistically significant at a level of ≤ 0.05 . Statistical analyses were performed with SPSS, version 25.0 (SPSS, Inc, Chicago, IL, USA).

RESULTS

Patient Characteristics

50 consecutive patients were enrolled, and a total of 13 were excluded due to poor echocardiography window (10 patients) and lost to follow-up (3 patients). Finally, 37 patients were studied; 13 male and 24 female with averages ages of 66 (SD 12) years. The majority of the population was female (64.9%), and hypertension was the most common comorbidity. The most frequent indication for pacing was total AV block (62.2%). The lead distributions were placed 19 patients at RV apical and 18 patients at RVOT. Almost all the implanted pacemakers were single-chambered VVI (83.8%). Patient characteristics are shown in **Table 1**.

Left ventricle systolic function and chamber remodelling

Neither LVEF and left ventricular internal diameter at systolic (LVVIDd) showed significant changes statistically before and after implantation ($p=0.23$ and $p=0.35$, respectively). Furthermore, GLS showed significant changes before and after one month of implantation (-20.30 SD 3.38 vs -16.93 SD 3.47, $p<0.001$) (**Table 2**).

The effect of RV pacing position, pacing burden, and paced QRS duration on global longitudinal strain

Several parameters that may affect GLS at one month were examined, including RV pacing location, pacing burden, and paced QRS duration. The value of delta GLS (before and after implantation) between RV pacing location of RV apical vs RVOT ($(2.30 (0.00-10.50)$ vs $2.95 (0.10-8.30)$, $p=0.648$) and paced QRS duration of <150 ms vs >150 ms ($(1.70 (0.30-8.30)$ vs $3.45 (0.0-10.5)$), $p=0.266$) did not significantly different. However, there was a significant difference of delta

Table 1. Baseline characteristics.

Characteristics	n=37
Age (y.o), mean (SD)	66 (12)
Sex, n %	
- Male	13 (35.1)
- Female	24 (64.9)
Diagnosis, n (%)	
- Sinus node disease (SND)	14 (37.8)
- Total AV block	23 (62.2)
Comorbid, n (%)	
- Hypertension	28 (75.7)
- Diabetes Mellitus	3 (8.1)
- Dyslipidemia	1 (2.7)
- Coronary artery disease	4 (10.8)
- Smoker	3 (8.1)
Medication used, n (%)	
- Calcium channel blocker	16 (43.2)
- Statin	2 (5.4)
- Angiotensin Converting Enzym inhibitor (ACE-I)	6 (16.2)
- Angiotensin Receptor Blocker (ARB)	6 (16.2)
- Antiplatelet	4 (10.8)
- Diuretic	2 (5.4)
Pacemaker mode, n (%)	
- VVI	31 (83.8)
- DDD	6 (16.2)
RV pacing location, n (%)	
- RV apical	19 (51.4)
- RVOT	18 (48.6)
QRS duration (ms)	
- Baseline QRS duration, mean (SD)	104 (19)
- Paced QRS duration	151 (128-189)
Pacing burden (Cumulative pacing rate %)	78.7 (5.7-100)

GLS: Global longitudinal strain; RV: Right ventricle; RVOT: Right ventricular outflow tract

GLS between patient with pacing burden $<40\%$ vs $>40\%$ (1.92 SD 1.37 vs 3.98 SD 3.04, $p=0.007$). Whereas patients with pacing burden $>40\%$ have worsened GLS (**Table 3**).

This finding was further analyzed to determine the effect of pacing burden on GLS based on RV pacing location. It was found that the pacing burden only affected delta GLS when it was $>40\%$ when RV pacing was placed on apical ($\leq 40\%$ (1.58 SD 0.59) vs $>40\%$ (4.67 SD 3.47), $p=0.008$), and did not affect the delta GLS in group with RVOT pacing ($\leq 40\%$ (2.32 SD 1.98) vs $>40\%$ (3.29 SD 2.48), $p=0.446$) (**Table 4**).

DISCUSSION

This is the first study in Indonesia that describes GLS in the population of patients who

Table 2. Systolic function and chamber remodeling.

Variables	Baseline n=37 mean (SD)	1 month after implantation n=37 mean (SD)	p-value [#]
LVEF	67.46 (4.71)	66.27 (6.96)	0.230
GLS	- 20.30 (3.38)	-16.93 (3.47)	<0.001
LVIDd	46.20 (5.52)	46.71 (4.66)	0.350
LVIDs	28.3 (5)	29.08 (5.2)	0.141
LV mass index	126.7 (41.1)	120.9 (39.2)	0.266
LV volume index	67.9 (21.09)	63.1 (16.88)	0.058
LA dimension	36.9 (7.21)	37.7 (5.98)	0.298

Paired T-test; LVEF: Left ventricle ejection fraction; GLS: Global longitudinal strain; LVIDd: Left ventricle internal diameter at diastolic; LVIDs: left ventricle internal diameter at systolic; LV: left ventricle; LA : left atrial

Table 3. Effect of several parameters on delta GLS.

Variables	Delta GLS	p-value
Lead placement-median		
RV apical (n = 19)	2.3 (0.00 – 10.50)	0.648 [†]
RVOT (n =18)	2.95 (0.10 – 8.30)	
Paced QRS duration-median		
≤150 ms (n = 17)	1.70 (0.30 – 8.30)	0.266 [†]
>150 ms (n = 20)	3.45 (0.0 – 10.5)	
Pacing percentage-mean (SD)		
≤40 % (n = 11)	1.92 (1.37)	0.007 ^{††}
>40 % (n = 26)	3.98 (3.04)	

[†]Mann-U Whitney test, ^{††}Unpaired t-test.

GLS: Global longitudinal strain; RV: Right ventricle; RVOT: Right ventricular outflow tract.

Note: delta GLS was a changed of GLS that measured before and after 1 month of implantation

Table 4. Effect of pacing percentage and lead position on delta GLS.

Variable	Pacing percentage (%)		p-value ^{††}
	≤40% n=11	>40% n=26	
Delta GLS, mean (SD)			
RV apical	1.58 (0.59)	4.67 (3.47)	0.008
RVOT	2.32 (1.98)	3.29 (2.48)	0.446

^{††}Unpaired t-test; GLS: Global longitudinal strain; RV: Right ventricle; RVOT: Right ventricular outflow tract. Note: delta GLS was a changed of GLS that measured before and after 1 month of implantation.

underwent PPM implantation. This study showed that LVEF and LVIDd did not show significant changes one month after implantation (**Table 3**). This finding may suggest that the macroscopic anatomical remodeling may not be seen within one month after implantation. The previous

study also has a similar finding that there was no significant difference of LVEF within one month after implantation, and also the changing of LVID may be seen later in longer follow-up^{20,21}

However, subclinical LV systolic dysfunction can already be found using GLS, one month after implantation. Furthermore, this study showed that subclinical LV systolic dysfunction already occurred as early as one month after implantation. Meanwhile, previous studies showed consistent results. Ahmed et al.²⁰ found that a decrease in GLS at one month correlated with the incidence of pacemaker induced cardiomyopathy (PICM) after 1 year. However, the difference compared to this study was that they only included patients with RV apical pacing. Also, RV pacing on RVOT and several parameters were not analyzed in that study (pacing percentage and QRS duration during pacing). Meanwhile, Algazaar et al. has shown worse GLS in patients with RV apical pacing compared with septum after six months of follow up. The RVOT pacing site in this study was also slightly higher than the septum.⁹

The vector of the QRS axis in normal condition was superior to inferior, made predominant positive QRS in inferior leads. Therefore, RVOT pacing location might be “more” similar to the physiologic condition in terms of a vector of QRS, compared to RV apical pacing. However, our study showed no difference between RVOT pacing and RV apical pacing (p = 0.648). The effect of RV pacing location is only significant if combined with the pacing burden (**Table 4**). The explanations of this finding probably due to; (1). The RV pacing location may affect the GLS depend on the time burden and duration. The more frequent RV apical pacing

occurs, the more non-physiologic axis that may induce LV systolic dysfunction, (2). It was still possible that RV pacing location might be the independent factor to predict the decline of GLS in long-term follow-up.

Our study showed significant changes of GLS related to pacing burden $\geq 40\%$ in the apical RV pacing group, which occurred within one month after implantation. In contrast, Dudoniene et al.²² did not find any difference in GLS between apical RV and septal pacing during three months follow-up period. Although both groups have shown significantly worse GLS compared to baseline. Also, the difference of pacing site between mid septum and more physiological pacing site (RVOT) used in this study may be the reason for this discrepancy. Thus far, studies regarding the effect of pacing location on LV performance showed conflicting results. Therefore, further studies are needed to answer this problem. Previous studies only included patients with dependent RV pacing ($>90\%$ pacing) because they aimed to study the effect of pacing location but not burden on GLS.^{9,20} Meanwhile, other studies addressing the effect of pacing burden on GLS were not found. Subsequently, it was assumed that pacing burden $>40\%$ means that the incidence of non-physiological pacing will be more frequent and worsen the intra-ventricular and inter-ventricular dyssynchrony. Our study used the cut-off value pacing burden of 40% , following the results from the previous study^{2,24}. Also, ventricular activation by pacemaker results in heterogenic electrical activation through each myocardial segment. In addition, frequent pacing has been shown to increase the risk of heart failure and hospitalization.²³

Our study showed that the greater GLS changes were seen in the group with paced QRS duration > 150 ms, although not statistically significant. Previous studies showed consistent results regarding paced QRS duration. They found that wider paced QRS duration correlated with worse LV systolic function that measured by LVEF, in long-term follow up (one year) and increase the risk of heart failure.^{24,25} Also, Khursid et al. found that pacing burden $>20\%$ and paced QRS duration ≥ 150 ms increased

the risk of PICM. This study also correlated the paced QRS duration in long-term LVEF (mean follow-up 2.5 years).²⁶ To the best of our knowledge, our study is the first study that examined the effect of paced QRS duration on early changes of GLS. Our study showed that the correlation between delta GLS and paced QRS duration is not statistically significant, even if the trend of correlation might be seen. This result is probably due to the shorter follow-up duration compared to the previous studies. It may be speculated that paced QRS duration may affect the delta GLS in longer follow-up. Therefore, a longer follow was needed to confirm this finding.

Data showed that subclinical LV dysfunction occurred as early as one month after pacemaker implantation which could be detected by GLS. In fact, worse GLS after pacemaker implantation has been associated with PICM in the future.²⁰ However, to the best of our knowledge, there is no data or consensus to state the absolute delta GLS in patients before and after pacemaker implantation. The previous study used the delta GLS to state the significance of GLS changes. The study by Xu et al.²⁷ showed that the significant delta GLS before and after implantation (-16.6 ± 1.2 to -14.9 ± 1.8) within one month would predict cardiomyopathy later. Ahmed et al.²⁰ also found a similar finding that the significant delta GLS (-16.3 ± 0.5 to -12.6 ± 0.5) within one month will predict LV systolic dysfunction later that showed by reduced EF. Our study showed that decline delta GLS was comparable (or even greater) (**Table 2**) compared to the previous study. Therefore, we assumed that based on our result and also previous study^{20,27}, the delta GLS >2 (become less negative within one month) will be useful for early detection of LV systolic dysfunction. Therefore, GLS needed to be evaluated before and after implantation, especially in the patient with a pacing burden $> 40\%$ and lead paced on RV apical, as showed by our study. However, the current practice does not incorporate GLS as a consideration either to upgrade the pacemaker into cardiac resynchronization therapy (CRT) or predict which patient will develop heart failure. Therefore, GLS inclusion

can be argued in future practice to predict clinical LV systolic dysfunction. The significant decline of GLS as showed by delta GLS in our study may be an important finding in clinical practice. It may be beneficial that a patient with implanted PPM to check and reprogram the PPM more frequently, especially in a patient who had significant delta GLS in a short term period. The patient should be warned of the clinical heart failure symptoms in the future, and if so, upgrading the PPM to CRT may be considered. Furthermore, the physician may set up the pacemaker to avoid unnecessary RV pacing (eg. set up appropriate lower rate limit of VVI pacemaker or appropriate setting of AV delay in DDD pacemaker) or even to do more physiological pacing, such as “bundle of his” pacing in purpose to prevent or delay PICM.

Study Limitation

This study has several limitations. First, a multivariable analysis could not be conducted due to a limited number of samples. Second, it only examined the short-term effect of pacemaker implantation. Third, a Cardiac CT examination to determine the precise RV pacing location was not performed. The decision of which patients were included in RV apical or RVOT group was only based on fluoroscopy image and confirmed by QRS axis in inferior leads. Fourth, the pacemaker majority was VVI. Therefore, it could not be analyzed whether maintaining AV synchrony (in DDD) may affect GLS or not. However, the burden of VVI >80% made the population in our study more homogeneous. Furthermore, this is the first study to analyze the effect of implantation parameters on early changes of early subclinical LV systolic dysfunction that measured by GLS.

CONCLUSION

The pacing parameter, particularly pacing burden >40% may induce the subclinical LV systolic dysfunction after one month of pacemaker implantation as shown by decline of GLS, especially when the RV pacing location was placed on apical.

CONFLICT OF INTEREST

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

REFERENCES

1. Brignole M, Auricchio A, Baron-Esquivias G, et al. ESC Guidelines on cardiac pacing and cardiac resynchronization therapy: the Task Force on cardiac pacing and resynchronization therapy of the European Society of Cardiology (ESC). *Eur Heart J*. 2013;34(29):2281-329.
2. Sweeney MO, Hellkamp AS, Ellenbogen KA, et al. Adverse effect of ventricular pacing on heart failure and atrial fibrillation among patients with normal baseline QRS duration in a clinical trial of pacemaker therapy for sinus node dysfunction. *Circulation*. 2003;107:2932-7.
3. Nahlawi M, Waligora M, Spies SM, et al. Left ventricular function during and after right ventricular pacing. *J Am Coll Cardiol*. 2004;44(9):1883-8.
4. Dreger H, Maethner K, Bondke H, et al. Pacing-induced cardiomyopathy in patients with right ventricular stimulation for 15 years. *EP Eur*. 2012;14(2):238-42.
5. Thackray SDR, Witte KK, Nikitin N, et al. The prevalence of heart failure and asymptomatic left ventricular systolic dysfunction in a typical regional pacemaker population. *Eur Heart J*. 2003;24(12):1143-52.
6. Zhang X H, Chen H, Siu C W, et al. New-onset heart failure after permanent right ventricular apical pacing in patients with acquired high-grade atrioventricular block and normal left ventricular function. *J Cardiovasc Electrophysiol*. 2008;19(2):136-41.
7. Prinzen FW, Vernoooy K, Lumen J, et al. Physiology of cardiac pacing and resynchronization. In: Kenneth A Ellenbogen GNK, Chu Pak Lau, Bruce L Wilkoff, Angelo Aurichio, eds. *Clinical cardiac pacing, Defibrillator and resynchronization therapy*. 5th ed. Philadelphia: Elsevier; 2017. p. 213-46.
8. Inoue K, Okayama H, Nishimura K, et al. Right ventricular septal pacing preserves global left ventricular longitudinal function in comparison with apical pacing. *Circ J*. 2011;75(7):1609-15.
9. Algazzar AS, Katta AA, Ahmed KS, et al. Changes in left ventricular global and regional longitudinal strain during right ventricular pacing. *Cardiol Res*. 2016;7(1):17-24.
10. Kiehl EL, Makki T, Kumar R, et al. Incidence and predictors of right ventricular pacing-induced cardiomyopathy in patients with complete atrioventricular block and preserved left ventricular systolic function. *Heart Rhythm*. 2016;13:2272-8.
11. Andersen HR, Nielsen JC, Thomsen PEB, et al. Long-term follow-up of patients from a randomised trial of atrial versus ventricular pacing for sick-sinus syndrome. *Lancet*. 1997;350(9086):1210-6.

12. Tse H-F, Yu C, Wong K-K, et al. Functional abnormalities in patients with permanent right ventricular pacing : The effect of sites of electrical stimulation. *J Am Coll Cardiol.* 2002;40(8):1451-8.
13. Shimony A, Eisenberg MJ, Filion KB, et al. Beneficial effects of right ventricular non-apical vs. Apical pacing: A systematic review and meta-analysis of randomized-controlled trials. *Europace.* 2012;14(1):81-91.
14. Kikuchi M, Tanno K, Miyoshi F, et al. Long-term effectiveness of right septal pacing vs. Right apical pacing in patients with atrioventricular block. *J Arrhythmia.* 2012;28(4):214-8.
15. Chen S, Yin Y, Lan X, et al. Paced QRS duration as a predictor for clinical heart failure events during right ventricular apical pacing in patients with idiopathic complete atrioventricular block: results from an observational cohort study (PREDICT-HF). *Eur J Heart Fail.* 2013;15:352-9.
16. Salm LP, Schuijff JD, de Roos A, et al. Global and regional left ventricular function assessment with 16-detector row CT: Comparison with echocardiography and cardiovascular magnetic resonance. *Eur J Echocardiogr.* 2006;7(4):308-14.
17. III JG, Tanaka H. Echocardiographic assessment of myocardial strain. *J Am Coll Cardiol.* 2011;58(14):1401-13.
18. Reisner SA, Lysyansky P, Agmon Y, et al. Global longitudinal strain: a novel index of left ventricular systolic function. *J Am Soc Echocardiogr.* 2004;17(6):630-3.
19. Lang RM, Badano LP, Mor-Avi V, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr.* 2015;16(3):233-71.
20. Ahmed FZ, Motwani M, Cunningham C, et al. One-month global longitudinal strain identifies patients who will develop pacing-induced left ventricular dysfunction over time: The pacing and ventricular dysfunction (PAVD) study. *PLOS ONE.* 2017;12(1):e0162072.
21. Silva RT, Martinelli Filho M, de Oliveira JC, et al. Ventricular remodeling in right ventricular apical pacing. *Arq Bras Cardiol.* 2007;88(2):152-8.
22. Baronaite-Dudoniene K, Vaskelyte J, Puodziukynas A, et al. Evaluation of left ventricular longitudinal function and synchrony after dual chamber pacemaker implantation. *Medicina.* 2014;50(6):340-4.
23. Murakami Y, Tsuboi N, Inden Y, et al. Difference in percentage of ventricular pacing between two algorithms for minimizing ventricular pacing: results of the IDEAL RVP (Identify the Best Algorithm for Reducing Unnecessary Right Ventricular Pacing) study. *Europace.* 2010;12(1):96-102.
24. Sharma G, Shetkar SS, Patel CD, et al. Paced QRS duration predicts left ventricular function in patients with permanent pacemakers e One-year follow-up study using equilibrium radionuclide angiography (ERNA). *Indian Pacing Electrophysiol J.* 2015;15(2):90-5.
25. Nakamura H, Mine T, Kanemori T, et al. Effect of right ventricular pacing site on QRS Width. *Asian Cardiovascular and Thoracic Annals.* 2011;19(5):339-45.
26. Khurshid S, Liang JJ, Owens A, et al. Longer paced QRS duration is associated with increased prevalence of right ventricular pacing-induced cardiomyopathy. *J Cardiovasc Electrophysiol.* 2016;27(10):1174-9.
27. Xu H, Li J, Bao Z, Xu C, Zhang Y, Liu H, Yang J. Early change in global longitudinal strain is an independent predictor of left ventricular adverse remodelling in patients with right ventricular apical pacing. *Heart Lung Circ.* 2019;28(12):1780-7.

Correlation between Interstitial Lung Disease Morphology Scores Based on High-resolution Computed Tomography Chest and Skin Fibrosis Degree Based on Modified Rodnan's Skin Score on Systemic Sclerosis

Irma Hassan Hikmat¹, Suci S. Ramdhini¹, Hari Soekersi¹, Sumartini Dewi^{2,3}

¹ Department of Radiology, Faculty of Medicine, Universitas Padjadjaran, Hasan Sadikin Hospital, Bandung, Indonesia.

² Division of Rheumatology, Department of Internal Medicine, Hasan Sadikin Hospital, Bandung, Indonesia.

³ Immunology Study Center, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia.

Corresponding Author:

Sumartini Dewi, MD. Division of Rheumatology, Department of Internal Medicine, Immunology Study Center, Faculty of Medicine, Universitas Padjadjaran. Jl. Eijkman No. 38, Bandung 40161, Indonesia.

Email: sumartini.dewi@unpad.ac.id

ABSTRAK

Latar belakang: Sklerosis sistemik (SSc) merupakan penyakit autoimun sistemik yang mengenai jaringan ikat multisistem dan salah satu penyebab terjadinya interstitial lung disease (ILD). Modified Rodnan's Skin Score (MRSS) merupakan pemeriksaan standar baku emas yang bersifat semikuantitatif, non-invasif untuk mengukur fibrosis kulit pada SSc. Kelainan fibrosis paru pada SSc terutama berbentuk ILD merupakan penyebab mortalitas terbanyak dan seringkali terlambat dalam diagnosis. Pemeriksaan baku emas untuk menilai morfologi ILD adalah dengan High Resolution Computed Tomography (HRCT) scan thoraks, namun ketersediaannya masih sangat terbatas. Derajat fibrosis kulit berdasarkan MRSS pada SSc dapat memprediksi adanya ILD pada beberapa penelitian, namun belum banyak diteliti di Indonesia. Tujuan penelitian ini untuk mengetahui hubungan morfologi ILD berdasarkan HRCT scan thoraks dengan derajat fibrosis kulit berdasarkan MRSS pada sklerosis sistemik. **Metode:** Penelitian ini merupakan penelitian observasional analitik retrospektif dengan desain cross sectional. Subjek penelitian ini adalah pasien sklerosis sistemik yang memiliki data hasil pemeriksaan MRSS dan HRCT scan thoraks sejak Juli 2019 hingga Maret 2020. Analisis statistik yang menggunakan uji korelasi Spearman's. **Hasil:** Terdapat 42 subjek penelitian, terdiri dari 41 perempuan (97.6%) dan satu laki-laki (2.4%) dengan rerata usia 41.29 ± 12.045 tahun dengan rentang usia dari 19 tahun hingga 60 tahun. Hasil uji korelasi berdasarkan Spearman's terdapat korelasi sedang dengan $R = 0,429$ yang bermakna ($p = 0.005$) antara skor morfologi ILD dengan nilai MRSS. **Kesimpulan:** Terdapat korelasi sedang yang bermakna antara skor morfologi Interstitial Lung Disease berdasarkan HRCT thoraks dengan derajat fibrosis kulit berdasarkan Modified Rodnan's Skin Score pada sklerosis sistemik.

Kata kunci: high-resolution computed tomography chest, interstitial lung disease, modified Rodnan's skin score, sklerosis sistemik.

ABSTRACT

Background: Systemic sclerosis (SSc) is a systemic autoimmune disease multiorgan/multisystem involvement. Modified Rodnan's Skin Score (MRSS) is a gold standard for measuring skin fibrosis in

SSc. In SSc, lung fibrosis disorders, especially interstitial lung disease (ILD), are the leading cause of mortality and often late in diagnosis. High-Resolution Computed Tomography (HRCT) Chest scan is a gold standard for evaluating ILD morphology, but its availability is limited. The degree of skin fibrosis based on MRSS in SSc can predict the presence of ILD in several studies but has not been widely studied in Indonesia. This study aimed to determine the relationship of the ILD morphology based on thoracic HRCT scan with the degree of skin fibrosis based on MRSS in SSc. **Methods:** This study is a retrospective analytic observational study with a cross-sectional design. The subjects of this study are SSc patients who had data of MRSS and HRCT chest scan from July 2019 to March 2020. Statistical analysis uses Spearman's correlation test. **Results:** There were 42 study subjects, consisting of 41 women (97.6%) and one man (2.4%) with an average age of 39.50 years old (age range of 19 years to 60 years old). Correlation test results based on Spearman's show a moderate correlation between the morphological score of ILD with MRSS with $R = 0.429$, which is significant ($p = 0.005$). **Conclusion:** There is a significant moderate correlation between the morphological scores of ILD based on HRCT chest and the degree of skin fibrosis based on MRSS in SSc.

Keywords: High-resolution computed tomography chest, interstitial lung disease, modified Rodnan's skin score, systemic sclerosis.

INTRODUCTION

Systemic sclerosis (SSc) is a systemic autoimmune disease affecting multisystem connective tissue and one of the causes of interstitial lung disease (ILD).^{1,2} The number of patients with systemic sclerosis continues to increase and has the potential for increased morbidity and mortality due to visceral organ fibrosis.^{3,4} Data on the incidence and prevalence of systemic sclerosis in Indonesia are not yet available. However, the number continues to increase and has the potential to become a severe and life-threatening disease. Systemic sclerosis is the third most common disease in Rheumatology Polyclinic of RSUP Dr. Hasan Sadikin Bandung, after systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). In 2019, there were 100 systemic sclerosis patients (7%) of the total patients visiting the rheumatology polyclinic. The survey was conducted in 2015 - 2016 by the rheumatology division, Department of Internal Medicine, Faculty of Medicine, Padjadjaran University (UNPAD) Bandung at Dr. Hasan Sadikin Bandung, and there were 24 (42.1%) patients who had involvement of pulmonary fibrosis.^{1,3}

In SSc, lung involvement may include pulmonary fibrosis or a ground-glass opacity image on a high-resolution computed tomography (HRCT) chest scan with a forced vital capacity

(FVC) value of less than 80% and a forced expiratory volume/FVC ratio of more than 80%.^{5,6} ILD is the leading cause of morbidity and mortality in systemic sclerosis patients up to 40% within ten years after disease onset.⁷

Matsuda *et al.*⁵ in 2019 stated that there is an association between MRSS and organ involvement; higher MRSS significantly increased the prevalence of SSc-related ILD. A study by Deepa *et al.*⁶ in India in 2016 revealed that Rodnan's score was significantly associated with severe lung involvement. Likewise, a study by Cottrell *et al.*⁷ in 2014 mentioned that the incidence of moderate to severe restrictive lung disease (RLD) was associated with an increase in skin sclerosis scores. These evidences refer that MRSS (Modified Rodnan's skin score) can be used as an alternative marker of lung involvement in systemic sclerosis.

MRSS is a semi-quantitative, non-invasive gold standard examination to measure skin fibrosis in SSc. In SSc, pulmonary fibrosis disorder, especially ILD, is the most common cause of mortality and lattermost diagnosed. Chest X-ray is not specific for ILD. The gold standard examination for assessing ILD morphology is the High-resolution Computed Tomography (HRCT) chest scan, but its availability is still very limited. The degree of skin fibrosis based on MRSS on SSc can predict the presence of ILD shown in

several studies, but it has not been widely studied in Indonesia. Research on the morphological relationship between ILD and MRSS has never been conducted in Indonesia. It is expected that this study would increase the evidence regarding MRSS scoring to predict the presence of ILD; hence the management of complications of systemic sclerosis will be more effective.

METHODS

This research is an observational analytic correlation study with a cross-sectional model, using retrospective data. This study measures the relationship between risk factors and their outcome. The risk factors and outcomes were observed once and at the same time. The subjects of this study were systemic sclerosis patients who met the 2013 ACR/EULAR criteria and visited the Rheumatology Department of Internal Medicine at Dr. Hasan Sadikin Bandung from July 2019 to March 2020.

This study has received ethical approval from the Research Ethics Committee of the Faculty of Medicine, Padjadjaran University, and Dr. Hasan Sadikin Bandung with a letter of ethics number LB.02.01/X.65/73/2020.

Subjects

The inclusion criteria for the subjects of this study were subjects aged 18-60 years old diagnosed with systemic sclerosis, who have completed data on the degree of skin fibrosis based on MRSS and spirometry results of restrictive lung abnormalities. Subjects with restrictive lung disease due to autoimmune diseases other than systemic sclerosis and subject with tuberculosis and malignancy were excluded from our study.

MRSS and ILD Evaluation

The range of MRSS values is 0 - 51. The standard value is achieved by transforming MRSS values into an interval scale with the following formula: Transformation value $100 = (\text{Actual value} - \text{lowest actual value}) / \text{Value range} \times 100$.

The HRCT chest examinations utilize the Multidetector CT/MDCT 128-Slice (Hitachi SCENARIA SE-128 –slice, Hitachi Healthcare), which produce digital Imaging and Communication in Medicine (DICOM) data.

The ILD morphological score was obtained from the analysis of HRCT imaging based on the Warrick semi-quantitative method. The total score based on the parenchymal lesion pathology and the extent of the lesion ranged from 0 to 30.45.54. Degree of severity include Mild (<8), Moderate (8-15), and Severe (> 15).

Statistical Analysis

Statistical analysis was conducted to assess the correlation between the ILD morphological score based on chest HRCT and the pulmonary fibrosis score based on MRSS on systemic sclerosis patients. Spearman correlation was used to determine the correlation between numerical and ordinal data. Interpretation of hypothesis test results was based on correlation strength, correlation direction, and p-value: Correlation strength (r) based on Guilford's criterion (1956): 0.0 - <0.2 = very weak; 0.2 - <0.4 = weak; 0.4 - <0.7 = moderate; 0.7 - <0.9 = strong; 0.9 - 1.0 = very strong.

RESULTS

The study subjects were 42 patients who met the inclusion and exclusion criteria as described in **Figure 1**.

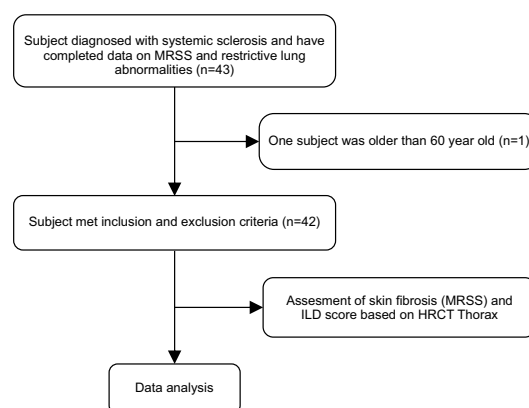


Figure 1. Patient flow chart.

Table 1 shows a total of 42 study subjects consisting of 41 women (97.6%) and one male (2.4%) with an average age of 39.5 years old and age range of 19 to 60 years old. The median duration of disease was three years with the range from 1 to 13 years. Most initial symptoms were presented as skin fibrosis in

Table 1. Subject Characteristic.

Variables	N=42
Sex, n (%)	
Male	1(2.4)
Female	41(97.6)
Age	
Median	39.50
Range (min-max)	19.00-60.00
Duration of disease (years)	
Median	3.00
Range (min-max)	1.00-13.00
Initial symptoms, n (%)	
Skin Fibrosis	38 (90.5)
Raynaud's Phenomenon	4 (9.5)
Chest X-ray, n (%)	
Normal	23 (54.8)
Bronchopneumonia	9 (21.4)
Bronchitis	3 (7.1)
Pneumonia	2 (4.8)
Inactive pulmonary TB	2 (4.8)
Pleura thickening	1 (2.4)
Active pulmonary TB	1 (2.4)
Interstitial lung disease	1 (2.4)
Morphology of ILD, n (%)	
Ground glass opacities	42 (100)
Irregular pleural margin	32 (76.2)
Septal or subpleural lines	40 (95.2)
Honeycombing	23 (54.8)
Subpleural cyst	19 (45.2)

Note: Categorical data are presented with number/frequency and percentage, while numeric data are presented with mean, median, standard deviation, and range.

38 (90.5%) subjects. Previous chest X-ray results were normal in 23 (55%) subjects, bronchopneumonia in 9 (21.4%) subjects, and bronchitis in 3 (7.1%) subjects, respectively. Only one (2.4%) patient was described as ILD.

After HRCT chest was performed, the ground glass opacities were found in all study subjects, septal or subpleural lines in 40 (95.2%) subjects, irregular pleural margins in 32 (76.2%) subjects, honeycombing in 23 (54.8%) subjects, and subpleural cysts in 19 (45.2%) subjects.

Table 2 shows an overview of the ILD morphological score, ILD, and MRSS morphological categories. The ILD morphological score has a median of 18.00 with a range of 6.00-27.00, consisting of 4 (9.5%) mild subjects, 11 (26.2%) moderate subjects and 27 (64.3%) severe subjects. MRSS has a mean of 18.93 (SD 8.247).

Table 2. ILD Morphological Score, ILD and MRSS Morphological Categories.

Variables	N=42
ILD Morphological Score	
Median	18.00
Range (min-max)	6.00-27.00
ILD Morphological Category, n (%)	
Mild	4 (9.5)
Moderate	11 (26.2)
Severe	27 (64.3)
MRSS	
Mean (SD)	18.93 (8.247)

Note: Categorical data are presented with number/frequency and percentage, while numeric data are presented with mean, median, standard deviation, and range.

Table 3. Analysis of the correlation between ILD Morphological Score and MRSS

Variables	Correlation	R	p-value
Correlation between Spearman Skor Morphological ILD score and MRSS value		0.429	0.005**

Note: p-value significance <0.05. Sign ** indicates statistically significant or significant a. r: correlation coefficient.

Table 3 shows the Spearman's correlation test between the ILD morphological score and MRSS obtaining a p-value of 0.005 (p-value <0.05) and an R-value of 0.429 (Guilford's criteria). R-value ≥ 0.40 (<0.70) indicates a moderate correlation between the ILD morphological score and the MRSS score.

MRSS value towards ILD morphological score shows a positive trend (**Figure 2**), which is explained by an increase in the MRSS value yet followed by an increase of ILD morphological score.

DISCUSSION

The results of this study show that the female distribution was more than male, i.e. 41 (97.6%) women and one (2.4%) man, with the median age of 39.5 years old. This is consistent with research by Vinent et al, Denton et al. and Budiman et al. stating that systemic sclerosis patients are dominated by women compared to male patients, with the most frequent range in patients at age 25-55 years.¹⁻³

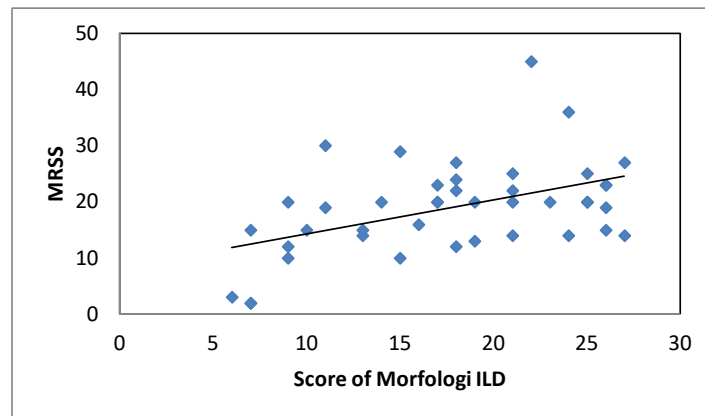


Figure 2. Correlation between ILD Morphological Score and MRSS Value.

Chest x-ray examination obtained normal results in 23 (55%) study subjects, whereas the others (45%) suggested bronchopneumonia disorders, bronchitis features, pneumonia, old pulmonary tuberculosis, pleural thickening, active pulmonary tuberculosis, and ILD. In SSc, patients with early symptoms of pulmonary involvement may have normal chest x-rays, but this does not eliminate the possibility of ILD. The ground-glass image is predominantly found in the basal lung area and can only be assessed in 25-44% of SSc patients.^{8,9} This chest x-rays imaging was described previously as bronchopneumonia, bronchitis, pneumonia, and pulmonary tuberculosis.

In this study, all subjects with the mild to severe category had ground-glass opacities morphology because of increased lung attenuation. This is consistent with research by Deepa *et al.* in 2016 in the Italian population, explaining that the initial changes of the ILD process were alveolitis which featured ground-glass opacity, septal/subpleural lines, and irregular pleural margins as a result of the thickening of the interstitial intralobular tissue. The subsequent process is fibrosis which gives a honeycombing and subpleural cyst morphology. Histologically, ILD in SSc includes non-specific interstitial pneumonia (NSIP) with more inflammatory features and less fibrosis, usual interstitial pneumonia (UIP) characterized by fibrosis and scarring, as well as a combination of both.^{6,10,11,12} In addition, **Table 4** explains the value of the MRSS with a mean of 18.93

(SD 8.247), where is different from the research by Matsuda *et al.*⁵ with a mean of 9.9 (SD 8.9) and Deepa *et al.*⁶ with a mean of 29.9 (SD 7.13). Correlation between ILD morphological score and MRSS value.

Our study revealed that there was a moderately significant correlation between ILD morphological scores based on chest HRCT and the degree of skin fibrosis based on MRSS. Our results are in line with Matsuda *et al.*⁵ in 2019 in the Japanese population that stated higher MRSS was associated with the presence of ILD in SSc ($p < 0.05$). Deepa *et al.*⁶ in 2016 in the Indian population stated that Rodnan's score was significantly associated with severe lung involvement ($p = 0.031$). Cottrell *et al.*⁷ in 2014 showed that in the United States population patients with higher skin fibrosis scores may develop to moderate to severe restrictive pulmonary involvement ($p < 0.001$).

The strong correlation between skin fibrosis and pulmonary fibrosis as ILD in SSc patients may be affected by similarities in the pathophysiology of skin and lung involvement in SSc, namely the presence of inflammatory cell invasion in the early stages and proliferation accompanied by degeneration of collagen fibers in the late stages of the disease.⁸

A similar study by Wu *et al.*¹³ in 2018 used a large European Scleroderma Trial and Research (EUSTAR) database, multicenter and prospective study to assess the association of increased skin fibrosis with organ involvement and increased mortality in diffuse SSc patients.

Out of 1021 subjects, 78 subjects had increased skin fibrosis within one year of observation. The study found that the increase in skin fibrosis in one year was associated with decreased lung function ($p = 0.004$) and a worse survival rate ($p = 0.063$); hence it can be confirmed that MRSS is an alternative marker for diffuse SSc.¹³

A study by Wangkaew *et al.*¹⁴ in 2016 in the Thai population showed a correlation between changes in HRCT and changes in clinical variables such as FVC, MRSS, erythrocyte sedimentation rate (ESR), and changes & SpO₂ in the early phase of SSc patients. On the other hand, a study by Yani *et al.*¹⁵ in 2019 concluded there is no correlation between serum Krebs von den Lungen (KL-6) levels with FVC and MRSS value of subjects with both restrictive lung disease and diffuse type systemic sclerosis. The KL-6 is a serum biomarker that IS produced by alveolar pneumocytes WHENEVER there is a fibrogenesis activity in the lungs. This study used the cohort method involving 31 subjects who underwent HRCT chest examination at the beginning and observation for the next 12 months, then calculated the HRCT score based on ILD morphology. The HRCT score was found to be a useful and sensitive method for assessing disease progression in SSc-related ILDs ($r = -0.38$, $P < 0.05$).

However, our study did not reflect the initial incidence of ILD in SSc patients. SSc patients who were sent for HRCT scan with suspected ILD were already undergoing modification therapy for Anti Rheumatic Drugs (DMARD)/conventional immunosuppressants with different doses and duration of therapy. There was no initial data on the chest HRCT examination when the patient was diagnosed with SSc; therefore, the chest HRCT examination during the study did not reflect the changes that occurred. Further cohort research with the completed HRCT dan MRSS baseline data is required in SSc patients for early detection of ILD and evaluating changes over a period of time.

CONCLUSION

There was a moderately significant correlation between the ILD morphological score based on HRCT chest and the degree of

skin fibrosis based on modified Rodnan's skin score on systemic sclerosis.

ACKNOWLEDGMENTS

IH, SS, HS, and SD conceived and designed the study. SS acquired the data. All authors contributed to the writing of the manuscript. This study was funded by Universitas Padjadjaran Internal Grant.

REFERENCES

1. Vincent V, Dewi S, Wachjudi R. Correlation Between Serum Procollagen Type 1 N-Terminal Propeptide Level With Modified Rodnan's Skin Score In Systemic Sclerosis Patients. Indonesian Journal of Rheumatology. 2018;9.
2. Denton C, Khanna D. Systemic sclerosis. www.thelancet.com. 2017;390.
3. Budiman A, Dewi S, Prananta M. Clinical Manifestation and Laboratory Finding of Sclerosis Systemic Patient in Dr. Hasan Sadikin General Hospital Bandung - A Descriptive Quantitative Study. Indonesian Journal of Rheumatology. 2018;10(1).
4. Domsic RT, Rodriguez-Reyna T, Lucas M, Fertig N, Medsger TA, Jr. Skin thickness progression rate: a predictor of mortality and early internal organ involvement in diffuse scleroderma. Ann Rheum Dis. 2011 Jan;70(1):104-9.
5. Matsuda KM, Yoshizaki A, Kuzumi A, Fukasawa T, Ebata S, Miura S, et al. Skin thickness score as a surrogate marker of organ involvements in systemic sclerosis: a retrospective observational study. Arthritis Res Ther. 2019 May 28;21(1):129.
6. Deepa AS, Rachel RP, Ramchandran P, Devaraj U, Arnold SA, Shobha V, et al. Pulmonary involvement in systemic sclerosis: A clinical profile. Lung India. 2016 Mar-Apr;33(2):144-7.
7. Cottrell T, Robert A Wise, Wigley FrM, Boin3 F. The degree of skin involvement identifies distinct lung disease outcomes and survival in systemic sclerosis. Ann Rheum Dis 2014. 2014;73:1060-1066.
8. Peroš-Golubičić T. Scleroderma and Lung. Interstitial Lung Disease (Clinical Focus). Jaypee Brothers Medical Publishers; 2011
9. Strollo D, Goldin J. Imaging lung disease in systemic sclerosis. Current rheumatology reports. 2010;12(2):156-61.
10. Caron M, Hoa S, Hudson M, Schwartzman K, Steele R. Pulmonary function tests as outcomes for systemic sclerosis interstitial lung disease. European Respiratory Review. 2018;27(148):170102.
11. Veraldi KL, Hsu E, Feghali-Bostwick CA. Pathogenesis of pulmonary fibrosis in systemic sclerosis: lessons from interstitial lung disease. Current rheumatology reports. 2010;12(1):19-25.

12. Hussein K, Shaaban LH, Mohamed E. Correlation of high-resolution CT patterns with pulmonary function tests in patients with interstitial lung diseases. *Egyptian Journal of Chest Diseases and Tuberculosis*. 2016;65(3):681-8.
13. Wu W, Jordan S, Graf N, de Oliveira Pena J, Curran J, Allano Y, et al. Progressive skin fibrosis is associated with a decline in lung function and worse survival in patients with diffuse cutaneous systemic sclerosis in the European Scleroderma Trials and Research (EUSTAR) cohort. *Ann Rheum Dis*. 2019 May;78(5):648-56.
14. Wangkaew S, Euathrongchit J, Wattanawittawas P, Kasitanon N. Correlation of delta high-resolution computed tomography (HRCT) score with delta clinical variables in early systemic sclerosis (SSc) patients. *Quant Imaging Med Surg*. 2016 Aug;6(4):381-90.
15. Yani H, Dewi S, Rahmadi AR. Correlation between Serum Krebs von den Lungen-6 Levels with Forced Vital Capacity and Modified Rodnan Skin Score of Patients with Restrictive Lung Disease in Diffuse-Type Systemic Sclerosis. *Indonesioan Journal of Rheumatology*. 2019;11(2):145-17.

Association of Polymorphisms in C-Reactive Protein (CRP) Promoter -821 A>G, -390 C>A/T, and Plasma Interferon- α (IFN- α) with Plasma CRP Level in Javanese Systemic Lupus Erythematosus (SLE) Patients

Awalia¹, Harianto Notopuro², Joewono Soeroso¹

¹ Division of Rheumatology, Department of Internal Medicine, Faculty of Medicine, Universitas Airlangga - Dr. Soetomo Academic General Hospital, Surabaya, Indonesia.

² Department of Biochemistry, Faculty of Medicine, Universitas Airlangga Surabaya, Indonesia.

Corresponding Author:

Awalia, MD. Division of Rheumatology, Department of Internal Medicine, Faculty of Medicine, Universitas Airlangga - dr. Soetomo Academic General Hospital, Surabaya, Indonesia. Jl. Prof. Dr. Moestopo 47 Surabaya, 60132, Indonesia. Email: awalia_nov74@yahoo.com.

ABSTRAK

Latar belakang: Sebagai reaktan fase akut, CRP diperlukan untuk membersihkan sel apoptosis dan kompleks imun pada SLE. CRP yang tidak responsif ini mungkin disebabkan oleh variasi genetik dan IFN- α yang melimpah yang dapat menghambat sekresi CRP. Penelitian ini bertujuan untuk menganalisis hubungan polimorfisme nukleotida tunggal (SNP) pada promotor CRP dan IFN- α plasma dengan kadar CRP pada pasien SLE di Jawa. Kami juga menganalisis hubungan SNP ini dengan SLE. **Metode:** Empat puluh SLE dan 40 pasien spondyloarthritis (sebagai kontrol) dimasukkan. Subjek SLE menjalani pemeriksaan laboratorium rutin, kadar CRP, IFN- α serum, dan sekuensing DNA untuk mendeteksi SNP pada promotor CRP. Kelompok kontrol hanya menjalani sekuensing DNA. **Hasil:** Median usia pasien SLE adalah 31,5 tahun. Skor rata-rata SLAM adalah 8,5. Usia rata-rata kelompok kontrol adalah 39 tahun. CRP rata-rata 5,19 SB 2,69 mg / L, IFN-plasma median adalah 46,02 pg/ml. Tidak ada perbedaan signifikan SNP di CRP -821 (rs2794521) atau -390 (rs3091244) antara SLE dan kontrol. SNP baru ditemukan pada CRP -456 A>G pada 5 pasien SLE, tetapi tidak ada pada kontrol. SNP ini akan meningkatkan risiko SLE 2.143 kali lipat. Ada korelasi negatif sedang antara tingkat IFN- α dan CRP plasma. Regresi linier hanya menunjukkan tingkat IFN- α (tidak juga dengan SNP) berkorelasi dengan CRP serum. **Kesimpulan:** IFN- α plasma berhubungan dengan kadar CRP. Tidak ada hubungan SNP di CRP -821, -390, dan -456 dengan level CRP. SNP CRP -456 A>G akan meningkatkan risiko SLE dengan rasio odds 2.143.

Kata kunci: promotor CRP, interferon- α , kadar CRP, lupus eritematosus sistemik, manusia, penyakit.

ABSTRACT

Background: As an acute-phase reactant, CRP is needed to clear apoptotic cells and immune complexes in SLE. This unresponsive CRP may be caused by genetic variation and abundant IFN- α that might inhibit CRP secretion. This study aims to analyze the association of single nucleotide polymorphisms (SNP) in CRP promoter and plasma IFN- α with CRP level in Javanese SLE patients. We also analyzed the association of these SNPs with SLE. **Methods:** Forty SLE and 40 spondyloarthritis (as control) patients were included. SLE subjects underwent routine laboratory test, CRP level, serum IFN- α , and DNA sequencing to detect SNPs in CRP promoter. The control group only underwent DNA sequencing. **Results:** The median age of SLE patients was 31.5 years. The median SLAM score was 8.5. The median age of the control group was 39 years. The average CRP was 5.19 SD

2.69 mg/L, median plasma IFN- α was 46.02 pg/ml. There was no significant difference of SNPs in CRP -821 (rs2794521) or -390 (rs3091244) between SLE and control. New SNP was found in CRP -456 A>G in 5 SLE patients, but none in controls. This SNP would increase SLE risk 2.143 times. There was a moderate negative correlation between IFN- α level and plasma CRP. Linear regression only showed IFN- α level (not either SNP) correlated with serum CRP. **Conclusion:** Plasma IFN- α correlates with CRP level. There was no association of SNPs in CRP -821, -390, and -456 with CRP level. SNP CRP -456 A>G would increase the risk of SLE with an odds ratio of 2.143.

Keywords: CRP promoter, interferon- α , CRP level, systemic lupus erythematosus, human, health.

INTRODUCTION

C-reactive protein (CRP) is an acute-phase reactant that increases during inflammation due to IL-6 stimulation. Its function is to clear apoptotic cells, increase phagocytosis, and release inflammatory cytokines. Therefore, it is used as a biomarker for infection, atherosclerosis, inflammation, and autoimmune process. Unlike other autoimmune diseases, CRP level in SLE is normal or slightly elevated despite high disease activity and abundant IL-6 unless there is a bacterial infection. Low CRP level may cause inadequate apoptotic cells clearance, and this uneliminated product will induce immune reaction by producing autoantibodies, forming immune complex deposited in tissues and cause further inflammation cascade. Indeed, protective effects of CRP in the disease process have been demonstrated in animal models of lupus.¹⁻³ There are three hypotheses as to why CRP is unresponsive in SLE: variation of CRP gene, the anti-CRP antibody that binds CRP, and IFN, inhibiting CRP hepatocytes' CRP secretion. There are currently no studies elucidating which factor was more dominant.^{1,4}

There are several studies in various population showing CRP gene polymorphisms related to SLE and CRP level. SNPs in promoter CRP -390 (or 1440, rs3091244) and -821 (or 1009, rs2794521) are those showing correlation with SLE and CRP level in Korean, Filipino, and Afro-American population.⁵⁻⁷ These SNPs have never been studied in our population (Javanese ethnic in the Indonesian population).

The anti-CRP antibody is often found in SLE, but its relation with plasma CRP level is doubted lately. It is said that anti-CRP correlates with SLE activity but not with CRP level. When produced

by hepatocytes, CRP is in pentamer form (native-CRP). In a special condition like inflammation, it will dissociate into monomer CRP (mCRP) in the tissues that is more functional biologically. Serum anti-CRP is an antibody against mCRP. On the other hand, measured plasma CRP is pentameric CRP.^{4,8,9} In viral infection and SLE, we find a high level of IFN- α so it is presumed that IFN- α may inhibit CRP production by hepatocytes.^{2,4,10} This study aims to analyze the association of SNPs in promoter CRP and plasma IFN- α level with CRP level in Javanese SLE patients.

METHODS

The sample size was determined using the formula for linear regression study. Previous study in the Philippines showed β -coefficient = 0.45 for SNPs correlation with plasma CRP level. Therefore, we included 40 SLE patients as sample group, and 40 spondyloarthritis (SpA) patients as control. SpA patients were used as control because like other autoinflammatory disorders, in severe SpA patients we generally would see an increase of plasma CRP level.

All SLE patients came to either the rheumatology outpatient clinic for routine control or admitted to the hospital ward due to SLE flares. We included all SpA patients from the rheumatology outpatient clinic. All of the patients were from dr. Soetomo Academic General Hospital Surabaya, Indonesia. Diagnosis of SLE was made using ACR classification criteria for SLE 2019.^{11,12} Demographic data and clinical manifestation were obtained from history taking, physical examination, and medical record. SLAM (Systemic Lupus Activity Measurement) and SLEDAI (SLE Disease Activity Index) were

used to measure the SLE disease activity score.

Blood Samples

Blood samples were taken from SLE subjects to check CBC, ESR, CRP, complement C3, C4 level and other routine laboratory tests for SLE. All of the examinations were done at Clinical Pathology Laboratory at dr. Soetomo Academic General Hospital Surabaya. CRP level was measured using the immunoturbidimetry method with an upper normal limit level was 5 mg/L. Complement C3 and C4 level were measured using radial immunodiffusion technique, and the normal limit for C3 was 90-180 mg/dL and for C4 was 9-36 mg/dL. Plasma samples were stored at -800C to measure IFN- α level until ELISA was performed. ELISA kit was Human IFN alpha ELISA Kit Invitrogen BMS216/BMS216TEN. Upon completing a sample assay using the kit protocol, absorbance was determined at 450 nm on Microplate reader: iMark (BioRad). Both SLE subjects and control underwent DNA sequencing to detect SNPs in CRP promoter through DNA isolation from peripheral blood mononuclear cells (PBMC). PCR machine was Perkin Elmer PJ 2000, QIA quick gel extraction kit (Qiagen, cat.no 28704) was used for DNA purification. The forward and reverse primer is shown in **Figure 1**. Numbering system of SNPs in this study was based on *GenBank accession number AF449713*.¹³

Ethics

This study had received approval from the Ethics Committee of dr. Soetomo Academic

General Hospital with Ethical Clearance Number 1014/KEPK/III/2019 dated March 8, 2019.

Statistical Analysis

SPSS.21 was used for data analysis. Firstly, data distribution was tested. If homogenous, Pearson correlation was used, and if not, the Spearman correlation test was used to analyze the association between SNPs CRP -390, -821, plasma IFN- α level and plasma CRP level. Genotypes of promoter CRP were also compared between SLE and the control group using the Mann-Whitney test. Then multivariate analysis using linear regression was done to analyze the association of some independent variables (SNPs, IFN- α) with plasma CRP level.

RESULTS

Forty Javanese SLE patients (26 from the rheumatology outpatient clinic, 14 patients from the internal medicine ward) and 40 Javanese SpA patients from the outpatient clinic were included in this study from August 2019 to February 2020. Diagnosis of SLE was made based on SLE classification criteria from ACR 2019, and diagnosis of SpA was based on ASAS classification criteria 2010 [11,12]. All patients with median age were 31,5 years old (range 18-59) for the SLE group and 39 years old for the control group (range 21-60). Clinical characteristic of SLE subjects is shown in **Table 1**. Laboratory result of SLE patients is revealed in **Table 2**.

Positive PCR result as CRP gene amplification with suitable primer on several SLE subjects can

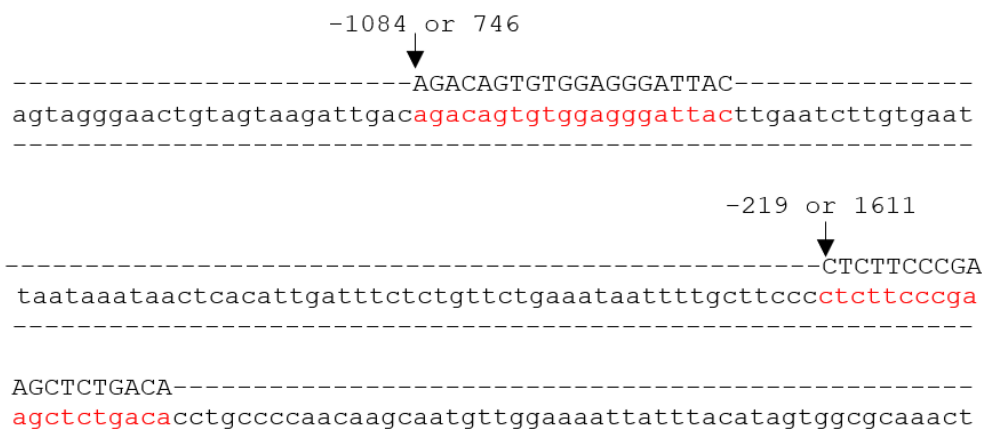
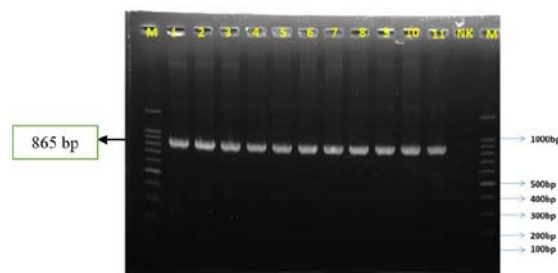


Figure 1. Forward and reverse primer.

Table 1. Clinical characteristic of subjects

Clinical manifestation	Median	Number (%)
Duration of illness (months)	23	
Body mass index	23.19	
Underweight		4 (10)
Normal		22 (55)
Overweight		14 (35)
Skin and mucosa manifestation		
Malar rash		9 (22,5)
Discoid rash		6 (15)
Oral ulcer		4 (10)
Hair loss		17 (42.5)
Photosensitivity		1 (2.5)
Musculoskeletal manifestation		
Arthritis		22(55)
Muscle pain		2 (5)
Fatigue		2 (5)
Panniculitis		2 (5)
Lung manifestation		2 (5)
Heart manifestation		
Heart failure		2 (5)
Hypertension		2 (5)
Serositis		5 (12.5)
Nephritis		13 (32.5)
Neuropsychiatric		2 (5)
SLAM score	8.5 (0-26)	
Mild (<7)		16 (40)
Moderate (7-20)		21 (52.5)
Severe (20)		3 (7.5)
SLEDAI	2 (0-28)	
Mild (1-5)		28 (70)
Moderate (6-10)		3 (7.5)
Severe (>10)		9 (22.5)

be seen in **Figure 2**. It shows DNA ribbon with 865 bp nucleotides.

**Figure 2.** CRP gene electrophoresis showing DNA ribbon 865 bp.

During DNA sequencing, we found a new SNP in promoter CRP -456 A>G (nucleotide 1374 from the front). To our knowledge, this SNP has never been reported in any publication before. Five SLE patients had the SNP, but none in the control group. This SNP would increase SLE risk 2.143 times compared to wildtype. Genotype and allele distribution of CRP gene in both groups are shown in **Table 3** and **Table 4**.

Since Shapiro-Wilk test showed $p < 0.05$ for IFN- α level, Spearman correlation test was used. It revealed a significant association between plasma IFN- α and CRP level ($p = 0.003$ and $r = -0.455$ (moderate negative correlation)). Spearman test also showed a moderate positive correlation between plasma IFN- α level and SLE disease activity (SLAM score) with $p = 0.001$ and $r = 0.568$ and with SLEDAI $p = 0.004$ and $r = 0.440$. There was no significant association between

Table 2. Laboratory characteristic of subjects.

Laboratory examination	Mean (SD)	Median	Number (%)
Positive anti ds-DNA			13 (32,5)
Hematology			
Hb (g/dl)	10.78 (2.92)		
Anemia			22 (55%)
Leukocytes ($\times 10^3$ /ml)	8.92 (4.68)		
Leukopenia			5 (12.5)
Lymphocyte		1715	
Lymphopenia			17 (42.5)
Thrombocyte	252,150 (144.462)		
Thrombocytopenia			11 (27.5)
Serum complement			
C3	88.41 (44.28)		
low (<90 mg/dL)			16 (40)
C4	20.76 (12.22)		
low (<9 mg/dL)			7 (17.5)
ESR (mm/h)		24 (6-155)	
High (>20mm/h)			26 (65)
C-reactive protein (mg/L)	5.19 \pm 2.69 (0.5-9.8)		
IFN- α (pg/ml)		46.02 (16.43-177.96)	

Table 3. Genotype distribution of CRP gene in SLE group and control

Location	Genotype	SLE group (%)	Control (%)	p
-821	AA (wildtype)	22 (55)	21 (52.5)	p = 0.996 *
	AG	15 (37.5)	18 (45)	
	GG	3 (7.5)	1 (2.5)	
-390	CC (wildtype)	26 (65)	29 (72.5)	p = 0.387 *
	CA	7 (17.5)	6 (15)	
	CT	5 (12.5)	5 (12.5)	
	AA	2 (5)	0	
-456	AA (wildtype)	35 (87.5)	40 (100)	p = 0.027 ** (OR 2.143)
	GG	5 (12.5)	0	

* Not significant with Mann-Whitney Test (p>0.05)

** Significant difference with Fisher Exact test (p<0.05)

Table 4. Allele distribution of CRP gene in SLE group and control.

Location	Allele	SLE group (%)	Control (%)	p
-821	A	59 (73.75)	60 (75)	p=0.856*
	G	21 (26.25)	20 (25)	
-390	C	64 (80)	69 (86.3)	p=0.436**
	T	5 (6.3)	5 (6.3)	
	A	11 (13.8)	6 (7.5)	
-456	A	70 (87.5)	80 (100)	p = 0.001***
	G	10 (12.5)	0	

* Not significant with chi-square test (p>0.05)

** Not significant with Mann-Whitney test (p>0.05)

*** Significant difference with Fisher Exact test (p<0.05)

CRP level and SLE disease activity (p=0.903). Neither of the SNPs showed a significant correlation with plasma CRP level (p>0.05). All of the variables showed in **Table 5** related to plasma CRP level.

Linear regression study also revealed only IFN- α correlated with plasma CRP level multivariately, as shown in **Table 6**, with mathematic model was $CRP = 6.910 - 0.025 IFN\alpha$.

Table 5. Correlation of each variable with plasma CRP level.

Variables	Plasma CRP level
SNP at promoter CRP -821	p=0.546*
SNP at promoter CRP -390	p=0.711*
SNP promoter CRP -456	p=0.161*
Plasma interferon- α	p=0.003** r= -0.455

* Not significant with Spearman test

** Significant Spearman test

Table 6. Linear regression analysis for this study.

Variables	β	b	p
IFN- α	-0.025	-0.421	0.005
SNP -456	-0.956	-0.119	0.439
Constanta	6.910		0.001

DISCUSSION

Baseline characteristics of the subjects involved in this study were slightly different than the South Korean and Phillipine cohort, but based on the sample size formula for a minimal patient number for linear regression study, 40 subjects were sufficient. Other studies also used normal subjects as control, but in this study, we used another autoinflammatory rheumatic disease as the control because there was increasing CRP in high disease activity SpA patients.^{5,7} The mean CRP level in this study was 5.19 (SD 2.69) mg/L (range 0.5-9.8 mg/L), a little higher than other studies such as in Mexico with a mean CRP was 1.46 mg/L (0.5-4.77 mg/L) and the Philippines with 1.5 mg/L.^{7,14} This might be because SLE subjects were from the outpatient clinic and those admitted at the hospital due to flare. All the patients from other study were from the outpatient clinic only (a stable condition with remission or low disease activity).

There was no association found in this study between SNPs at -821 nor -390 with SLE or plasma CRP level. Kim et al.⁵ showed a significant difference of SNP -390 between

the SLE group and control ($p=0.033$), but not SNP -821. A study by Kim et al. also revealed a correlation between SNP -390 (not for SNP -821) with plasma CRP level ($p=0.03$). A more extensive study in the Philippines showed a moderate correlation between SNP -390 with plasma CRP level (β -coefficient = 0.45), but not for SNP promoter CRP -821.^{5,7} A USA study involving the Caucasian race only showed a significant correlation between SNP -390 promoter CRP with plasma CRP level ($p=0.012$), but not for SNP -821. Other studies in the USA also showed a significant difference between the SLE group and control for SNP -390 with OR 1.43 ($p=0.001$).^{6,15} Studies in China and Taiwan involving healthy subjects showed a correlation between SNP promoter CRP -821 (not CRP -390) with plasma basal CRP level.¹⁶ New SNP on promoter CRP -456 (1374) was found in this study, and none of those studies reported this SNP before. SNP promoter CRP -456 A>G had a significant difference between the SLE group and controls with OR 2.143, but it did not correlate with plasma CRP level.

Hepatocytes synthesize CRP molecules as a response to IL-6 during inflammation. In viral infection and SLE flare, plasma CRP is normal though there is increasing in IL-6. It is caused by abundant IFN- α during SLE flare and viral infection. All subtypes of IFN- α can inhibit CRP promoter gene activity. This inhibition depends on the dose and is mediated by type-I IFN receptors. The IFN- α -dependent inhibition of CRP promoter activity was confirmed by studies of CRP secretion in primary human hepatocytes. IL-1 β -induced CRP secretion was inhibited by 49.2%, and IL-6-induced secretion was inhibited by 51.5%, whereas the inhibition induced by IL-1 β plus IL-6 was moderate (21.1%). After preincubation of IFN- α for 6 hours, there is suppression of promoter activity despite stimulation of IL-6 and IL-1 β . CRP itself also may inhibit the production of IFN- α by pDC induced by the immune complex. As of writing, there is no study directly compares serum IFN- α and CRP level. A study by Enocsson et al.² showed inhibition of the transcription process in CRP promoter during CRP synthesis by hepatocytes.^{2,4,8} This study directly correlated

plasma IFN- α level with plasma CRP level, and it showed moderate correlation in both bivariate and multivariate analysis.

In a longitudinal study, it is reported that there is increasing of IFN- α in active SLE patient serum. Plasma IFN- α level also correlates with SLEDAI score. There is also increasing IFN- α gene expression in active SLE patients, and there is a significant difference in SLEDAI scores between patients with high and low plasma IFN- α level ($p=0.0038$). Direct measurement of plasma IFN- α has shown more accurate and specific than IFN- α signature.¹⁷⁻¹⁹ All type I IFN will increase during SLE flare related to higher disease activity. IFN signature gene expression is also high in severe organ disturbance in lupus-like nephritis or neuropsychiatry.^{20,21} Our study also showed a moderate positive correlation between IFN- α and disease activity in SLE patients.

CONCLUSION

This study showed the correlation between plasma IFN- α with CRP level in both bivariate and multivariate analysis. There was a new SNP found in this study at CRP promoter -456 A>G. There was no association of SNPs in promoter CRP -821, -390, and -456 with CRP level, but SNP promoter CRP -456 A>G would increase the risk of SLE (OR 2.143).

ACKNOWLEDGMENTS

We thank all the SLE patients at Dr. Soetomo Academic General Hospital Surabaya who had participated in this study.

CONFLICT OF INTEREST

There is no conflict of interest in this study.

REFERENCES

1. Batuca J, Delgado Alves J. C-reactive protein in systemic lupus erythematosus. *Autoimmunity*. 2009;42(4):282-5.
2. Enocsson H, Gullstrand B, Eloranta ML, et al. C-reactive protein levels in systemic lupus erythematosus are modulated by the interferon gene signature and CRP gene polymorphism rs1205. *Front Immunol*. 2021;11:1-6.
3. S C. C - reactive protein: An inflammatory marker with

- specific role in physiology, pathology, and diagnosis. *Internet J Rheumatol Clin Immunol*. 2014;2(S1).
4. Enocsson H, Sjöwall C, Skogh T, Eloranta ML, Rönnblom L, Wetterö J. Interferon- α mediates suppression of C-reactive protein explanation for muted C-reactive protein response in lupus flares? *Arthritis Rheum*. 2009;60(12):3755–60.
 5. Kim HA, Chun HY, Kim SH, Park HS, Suh CH. C-reactive protein gene polymorphisms in disease susceptibility and clinical manifestations of Korean systemic lupus erythematosus. *J Rheumatol*. 2009;36(10):2238–43.
 6. Edberg JC, Wu J, Langefeld CD, et al. Genetic variation in the CRP promoter: Association with systemic lupus erythematosus. *Hum Mol Genet*. 2008;17(8):1147–55.
 7. Rhodes B, Wong A, Navarra S V., Villamin C, Vyse TJ. Genetic determinants of basal C-reactive protein expression in Filipino systemic lupus erythematosus families. *Genes Immun*. 2008;9(2):153–60.
 8. Enocsson H, Sjöwall C, Kastbom A, et al. Association of serum c-reactive protein levels with lupus disease activity in the absence of measurable interferon- α and a c-reactive protein gene variant. *Arthritis Rheumatol*. 2014;66(6):1568–73.
 9. Li QY, Li HY, Fu G, Yu F, Wu Y, Zhao MH. Autoantibodies against C-reactive protein influence complement activation and clinical course in lupus nephritis. *J Am Soc Nephrol*. 2017;28(10):3044–54.
 10. Mahajan A, Herrmann M, Muñoz LE. Clearance deficiency and cell death pathways: A model for the pathogenesis of SLE. *Front Immunol*. 2016;7:1–12.
 11. Fanouriakis A, Tziolos N, Bertsias G, Boumpas DT. Update in the diagnosis and management of systemic lupus erythematosus. *Ann Rheum Dis*. 2021;80(1):14–25.
 12. Aringer M, Costenbader K, Daikh D, et al. 2019 European League Against Rheumatism/American College of Rheumatology Classification Criteria for Systemic Lupus Erythematosus. *Arthritis Rheumatol*. 2019;71(9):1400–12.
 13. Carlson CS, Aldred SF, Lee PK, et al. Polymorphisms within the C-reactive protein (CRP) promoter region are associated with plasma CRP levels. *Am J Hum Genet*. 2005;77(1):64–77.
 14. Atisha-Fregoso Y, Lima G, Carrillo-Maravilla E, et al. C-reactive protein (CRP) polymorphisms and haplotypes are associated with SLE susceptibility and activity but not with serum CRP levels in Mexican population. *Clin Rheumatol*. 2018;37(7):1817–24.
 15. Shih PB, Manzi S, Shaw P, et al. Genetic variation in C-reactive protein (CRP) gene may be associated with risk of systemic lupus erythematosus and CRP concentrations. *J Rheumatol*. 2008;35(11):2171–8.
 16. Sheu WHH, Chen YDI, Yu CY, et al. C-reactive protein gene polymorphism 1009A>G is associated with serum CRP levels in Chinese men: A TCVGHAGE study. *Clin Chim Acta*. 2007;382(1–2):117–23.
 17. Bengtsson AA, Sturfelt G, Truedsson L, et al. Activation of type I interferon system in systemic lupus erythematosus correlates with disease activity but not with antiretroviral antibodies. *Lupus*. 2000;9(9):664–71.
 18. Baechler EC, Batliwalla FM, Karypis G, et al. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. *Proc Natl Acad Sci U S A*. 2003;100(5):2610–5.
 19. Kirou KA, Gkrouzman E. Anti-interferon alpha treatment in SLE. *Clin Immunol [Internet]*. 2013;148(3):303–12. Available from: <http://dx.doi.org/10.1016/j.clim.2013.02.013>
 20. Tanaka A, Ito T, Kibata K, et al. Serum high-mobility group box 1 is correlated with interferon- α and may predict disease activity in patients with systemic lupus erythematosus. *Lupus*. 2019;28(9):1120–7.
 21. Oke V, Gunnarsson I, Dorschner J, et al. High levels of circulating interferons type I, type II and type III associate with distinct clinical features of active systemic lupus erythematosus. *Arthritis Res Ther*. 2019;21(1):1–11.

Wound Healing in Diabetic Foot Ulcer Patients Using Combined Use of Platelet Rich Fibrin and Hyaluronic Acid, Platelet Rich Fibrin and Placebo: An Open Label, Randomized Controlled Trial

Ronald W. Kartika^{1*}, Idrus Alwi², Franciscus D. Suyatna³, Em Yunir², Sarwono Waspadji², Suzana Immanuel⁴, Saptawati Bardosono⁵, Saleha Sungkar⁶, Jusuf Rachmat⁷, Mirta Hedyati⁸, Todung Silalahi⁹

¹ Program Doctoral, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia.

² Department of Internal Medicine, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

³ Department of Clinical Pharmacology, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia.

⁴ Department of Clinical Pathology, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

⁵ Department of Nutrition, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia.

⁶ Department of Clinical Parasitology, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia.

⁷ Department of Thoracic Cardiac and Vascular Surgery, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia.

⁸ Department of ENT, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia.

⁹ Department of Internal Medicine, Krida Wacana Christian University, Jakarta, Indonesia.

Corresponding Author:

Prof. Idrus Alwi, MD., PhD. Division of Cardiology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo Hospital. Jl. Diponegoro no. 71, Jakarta 10430, Indonesia. Email: idrus_a@hotmail.com; ronaldkartika@gmail.com.

ABSTRAK

Latar belakang: Manajemen perawatan standar luka kaki diabetes (LKD) terdiri atas debridemen, kompres NaCl, kontrol infeksi dan kontrol glikemik yang ketat. Saat ini penggunaan autologous platelet-rich fibrin (PRF) dianggap sebagai metode tambahan untuk mengobati LKD. Tujuan penelitian ini untuk mengevaluasi peran kombinasi A-PRF + hyaluronic acid (HA) dalam pengobatan LKD. **Metode:** Desain penelitian adalah RCT open label yang dilakukan di RSUD Koja dan RS Gatot Soebroto pada Juli 2019 - April 2020. Pasien LKD dengan durasi luka tiga bulan, Wagner-2 dengan ukuran ulkus <40 cm² diikutsertakan dalam penelitian ini. Subjek dibagi secara acak menjadi tiga kelompok, yaitu kelompok A-PRF+AH, A-PRF dan NaCl 0,9% sebagai kontrol masing-masing 10 subjek. Biomarker VEGF dan IL-6 diambil dari LKD dengan usap lidi kapas dan dianalisis menggunakan ELISA. Pemeriksaan biomarker dan indeks granulasi pada hari ke-0, ke-3 dan ke-7. Data dianalisis menggunakan SPSS versi 20 dan diuji dengan Anova dan Kruskal Wallis untuk membandingkan pengaruh angiogenesis dan inflamasi pada ketiga kelompok sedangkan indeks granulasi diukur dengan ImageJ. **Hasil:** Pada kelompok A-PRF+AH terjadi peningkatan signifikan VEGF pada hari ke-0 (232,8 pg/mg protein) hingga ke-7 (544,5 pg/mg protein) dibandingkan A-PRF pada hari ke-0 (185,7 pg/mg protein) sampai ke-7 (272,8 pg/mg protein), dan kontrol pada hari ke-0 (183,7 pg/mg protein) sampai ke-7 (167,4 pg/mg protein). Didapatkan peningkatan VEGF pada kelompok A-PRF-HA dibandingkan kelompok A-PRF dan kontrol NaCl pada hari ke-3 ($p = 0,022$) dan ke 7 ($p = 0,001$) IL-6 menurun signifikan pada kelompok A-PRF+AH dari hari ke-0 (106,4 pg/

mg protein) sampai ke-7 (88,7 pg/mg protein) dibandingkan PRF dari hari ke-0 (91,9 pg/mg protein) sampai ke-7 (48,8 pg/mg protein) dan IL-6 meningkat pada kelompok NaCl hari ke-0 (125,3 pg/mg protein) sampai ke-7 (167,9 pg/mg protein). Didapatkan penurunan IL-6 pada kelompok A-PRF-HA dibandingkan kelompok A-PRF dan kontrol NaCl pada hari ke-7 ($p = 0,041$). **Kesimpulan:** Kombinasi A-PRF+HA dapat meningkatkan angiogenesis dan mengurangi inflamasi pada LKD sehingga dapat menjadi metode terapi baru pada LKD.

Kata kunci: ulkus kaki diabetik, fibrin kaya trombosit, asam hialuronat.

ABSTRACT

Background: Autologous platelet-rich fibrin (A-PRF) is an adjunctive method for diabetic foot ulcer (DFU) in addition to glycaemic control and debridement. This study aimed to evaluate the role of A-PRF + hyaluronic acid (HA), A-PRF and sodium chloride 0.9% (control) in DFU wound healing. Nowadays, the use of PRF autologous consider as adjuvant therapy in DFU treatment. **Methods:** This open-label randomized controlled trial was conducted at Koja District Hospital and Gatot Soebroto Hospital from July 2019 to April 2020. DFU patients with wound duration of three months, Wagner-2, and ulcer size $< 40 \text{ cm}^2$ were recruited and randomly assigned into A-PRF + AH, A-PRF and control group. On day-0, day-3 and day 7, samples and photographs were taken. Samples were analysed with ELISA and photographs were analysed with ImageJ to calculate granulation index (GI). Statistical analysis was performed using SPSS version 20. **Results:** Topical therapy with A-PRF + AH was associated with a significant increase in VEGF from day 0 (232.8 pg/mg) vs day 7 (544.5 pg/mg) compared to A-PRF on day 0 (185.7 pg/mg) vs day 7 (272.8 pg/mg), and the controls on day 0 (183.7 pg/mg) vs day 7 (167.4 pg/mg). On evaluation of VEGF swab, there is increasing significantly in A-PRF+HA group compare others group in day 3 ($p=0.022$) and day 7 ($p= 0.001$). In the A-PRF + AH group, there was a significant decrease in IL-6 from day 0 (106.4 pg/mg) vs day 7 (88.7 pg/mg) compared with PRF on day 0 (91.9 pg/mg) vs day 7 (48,8 pg/mg). IL-6 was increased in the control group from day 0 (125.3 pg/mg) vs day 7 (167.9 pg/mg). On evaluation of IL-6 swab, there is decreasing significantly in A-PRF+HA group compare others group in day 7 ($p= 0.041$). **Conclusion:** The PRF + HA combination increased angiogenesis and reduced inflammation in DFUs and may represent a new DFU therapy.

Keywords: diabetic foot ulcer; platelet-rich fibrin; hyaluronic acid.

INTRODUCTION

Diabetic foot ulcers (DFUs) are challenging to health care professionals due to limited availability of effective topical therapeutic interventions. Growth factor treatment has shown to be beneficial for healing DFUs in conjunction with extensive surgical debridement.¹ Neuropathy and peripheral vascular disease are two major causal factors of foot ulcers in patients with diabetes.²

The major problem with DFUs is the length of time needed to heal. There is chronic inflammation that show elevated levels of matrix metalloproteinase (MMPs) and tissue inhibitor of metalloproteinase (TIMP), which significantly contribute to delayed healing.³ Inflammatory cells release cytokines, including interleukins (e.g., IL-1 and IL-6) and tumour necrosis factor- α (TNF- α).⁴ Neuro-ischemic disease in

DFUs limits oxygen and nutrients supply to the wound, preventing wound healing. Due to the lack of oxygen and nutrients, epithelial cells at the wound site are unable to express essential factors for healing, such as vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF).⁵ The decreased levels of growth factor will delay the healing process.⁶ As an adjunct to topical DFU therapy, a dressing is needed that can contain growth factors and reduce inflammation.

Platelet rich plasma (PRP) has been used for wound healing, but it requires a fairly complicated process and there may be an allergic effect from CaCl₂ which is needed to activate plasma platelets. Thus, the second generation of PRP, namely platelet rich fibrin (PRF) have been developed. It has been proposed as an

adjunct for the treatment of DFUs and other chronic and acute wounds.⁷ The alpha granules of platelets contain growth factors, including PDGF, VEGF, and transforming growth factor- β (TGF- β), which stimulates cell proliferation and differentiation that results in new tissue formation.⁸ For over 20 years, PRF gel has been used to stimulate wound healing.⁶ Autologous PRF gel consists of cytokines, growth factors, chemokines, and a fibrin scaffold derived from the patient's blood.^{7,8} Advanced-PRF (A-PRF) contains additional growth factors that results in faster and better wound healing.⁹ Since patients with DFUs have low growth factors and prolonged inflammation, hyaluronic acid (HA) is added to A-PRF to optimize growth factor release and control inflammation.¹⁰ HA is a glycosaminoglycan that can enhance angiogenesis, wound healing, as well as reduce chronic inflammation in DFUs¹¹

The combination of A-PRF + HA has not been used for the treatment of DFUs. Therefore, this study is required to identify the effect of combined A-PRF and HA for the treatment of DFUs.

METHODS

We conducted an open-label randomized controlled trial from July 2019 to April 2020. Informed consent was obtained from patients, including the use of photographs (ImageJ). This study was approved by The Ethics Committee of the Faculty of Medicine Universitas, Indonesia (ID 0855/UN2.F1/ETIK/2018).

The study was conducted at Koja District Hospital and Gatot Soebroto Hospital, Jakarta. Diabetic patients with chronic DFUs with an average wound duration of three months, categorized as Wagner 2, ulcer < 40 cm² were included in the study. All patients had their blood sugar under control with an oral antidiabetic in the range of 150 – 200 mg/dl. The HbA1c range was 6 – 7.5 mg/dl. Patients exhibiting the following criteria were excluded: platelet dysfunction syndrome, critical thrombocytopenia, unstable hemodynamics, and pregnancy. Patients who failed to complete follow up visits were dropped out.

A-PRF + HA Preparation

A-PRF was used in the form of a gel. To make the A-PRF gel, 20 – 40 mL venous blood was centrifuged at 200 \times G for 8 minutes to obtain the fibrin, buffy coat, and erythrocyte layers.

The erythrocyte layer was carefully removed from the buffy coat with sterile scissors and transferred to a new sterile tube. A-PRF was formed by the fibrin and buffy coat, in which platelets (rich growth factor and cytokine) were entrapped in the fibrin.

To make homogenous A-PRF + HA gel, 1 mL A-PRF was mixed with 0.6 mL 0.2% HA using a vortex machine for approximately 20 seconds.

Wound Treatment and Monitoring

In this study, the subjects were divided into three groups: 1) A-PRF + HA; 2) A-PRF; and 3) 0.9% sodium chloride (NaCl) as a control.

A-PRF + HA was used as a single application to the surface of the wound and covered with a protective bandage. Similar procedures were performed in other groups. The wounds were photographed before and after treatment using a digital camera. Images were uploaded and analysed using ImageJ. Granulation Index (GI) was calculated using red-yellow-black analysis.

Biomarkers of Wound Healing

To identify the process of angiogenesis and inflammatory conditions during DFU healing, samples were collected using a cotton swab from the DFU surface before topical therapy. The level of VEGF and IL-6 was measured using ELISA.

The data were analysed using SPSS version 20. The association between the variables was analysed using an ANOVA and Kruskal Wallis test.

RESULTS

We recruited 36 DFU patients in Metabolic Endocrine Internal Medicine Out Patient Discharge (OPD). From the 36 subjects, only 30 subjects satisfied the inclusion criteria and has completed follow-up. The subjects, consisted of 12 men and 18 women. The median age was 64 years in women and 61 years in men. The subjects were randomly divided into three groups (A-PRF + HA, A-PRF, and control) comprised of 10 subjects per group.

Table 1. Subjects baseline characteristic.

Characteristic	A-PRF + HA (n = 10)	A-PRF (n = 10)	Control (n = 10)
Age (year) ^a	59.8 (SD 12.7)	64.7 (SD 12.0)	66 (36–71)
Sex n (%)			
Male	5/10	4 (40)	3 (30)
Female	5/10	6 (60)	7 (70)
BMI ^a	28.9 (SD 2.7)	27.3 (SD 2.08)	28.4 (SD 2.5)
Haemoglobin (g/dL)	12.7 (27.4–39.0)	13.1 (SD 1.3)	12.05 (10.1–16.5)
Haematocrit e (%)	36.3 (29.2–42.9)	35.6 (SD 4.6)	33.8 (24.4–40.8)
Leucocyte (10 ³ /μL) ^a	13.30 (SD 1.08)*	11.08 (SD 1.33)	9.23 (SD 1.66)
Platelet (10 ³ /μL) ^a			
Random Blood	354.9 (SD 167.5)	338.8 (SD 164.5)	319.9 (SD 128.4)
Glucose mg/dL ^b	286.0 (170–390)	243.8 (SD 47.4)	254.7 (SD 58.6)
HbA1C (%) ^a	11.34 (SD 1.30)	9.0 (SD 0.68)	8.5 (SD 0.72)
Cholesterol total (mg/dL)	214.5 (SD 16.9)	249.3 (SD 16.1)	202.3 (SD 38.6)
Albumin (mg/dL)	3.3 (2.8–4.2)	3.1 (2.8–4.2)	3.2 (SD 0.39)

^amean (SD), ANOVA test^bmedian (min-max), Kruskal Wallis test

Biomarker Output

Table 2 shows the level of VEGF collected from the DFU swabs. At baseline, the VEGF levels were not significantly different among the three groups, meaning that the data was homogenous. The evaluation on day three and day 7 showed a significant increase in the VEGF levels in the A-PRF + HA group compared with the A-PRF and control groups.

The VEGF levels were increased in the A-PRF + HA group from day-0 to day 3 (232.8 pg/mg to 320.6 pg/mg) and to day 7 (232.8 pg/mg to 544.5 pg/mg). In the A-PRF group, there was a decrease in the VEGF levels on day 3 (185.7 pg/mg to 180.4 pg/mg) but an increase on day 7 (185.7 pg/mg to 272.8 pg/mg). In the control group, there was also a decrease in the VEGF levels on day 3 (183.7 pg/mg to 144.8 pg/mg) and day 7 (183.7 pg/mg to 167.4 pg/mg). Table 2 shows an evaluation of VEGF swab. There is a significant increase in the A-PRF+HA group compared to others groups in day 3 (p=0.022) and day 7 (p= 0.001). In the subgroup

analysis using Post-hoc ANOVA test, in day 3, A-PRF+HA increase significantly compare with A-PRF (p=0.014) and control (p=0.003). In day 7, A-PRF+HA increase significantly compare with A-PRF (p=0.002) and control (p<0.001). Meanwhile increases in the A-PRF group was no significant compared with controls on day 3 (p=0.612) and day 7 (p=0.186)

Another method of evaluating inflammation is through detecting base VEGF increase before therapy. **Figure 1** shows the delta of the VEGF based on each intervention. The delta of the VEGF levels in the A-PRF + HA group increased: Δ 0–3 (43.1 pg/mg) and Δ 0–7 (275.8 pg/mg). In the A-PRF group, there was an increase in Δ0–3 (1.8 pg/mg) and 0–7 (104.7 pg/mg). In the control group, there was a decrease in Δ 0–3 (4.9 pg/mg) and an increase Δ 0–7 (28.3 pg/mg). In the A-PRF + HA group, there was an increase in ΔVEGF compared to the A-PRF and control groups on day 3 (p = 0.003) and day 7 (p < 0.001).

Following the intervention (**Table 3**), there was a decrease in IL-6 in the A-PRF + HA group

Table 2. Mean VEGF in different treatment groups

Intervention	A-PRF + HA Mean (SD)	A-PRF Mean (SD)	Control Mean (SD)	P
Day 0	232.8 (125.7)	185.7 (100.8)	183.7 (127.2)	0.568
Day 3	320.6 (165.8)	180.4 (87.4)	144.8 (87.7)	0.022
Day 7	544.5 (266.8)	272.8 (97.7)	167.4 (98.8)	0.001

mean (SD) , anova test

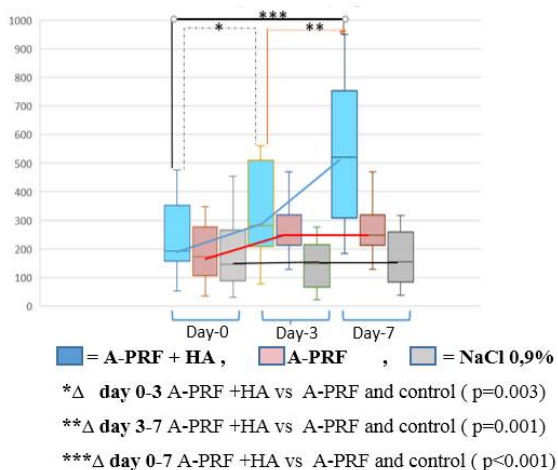


Figure 1. ΔVEGF based on intervention group.

on day 3 (106.4 pg/mg to 99.5 pg/mg) and day 7 (106.4 pg/mg to 88.7 pg/mg). In the PRF group, the IL-6 levels decreased on day 3 (91.9 pg/mg to 72.8 pg/mg) and day 7 (91.9 pg/mg to 4 pg/mg). In the control group, the IL-6 levels increased on day 3 (125.3 pg/mg to 131.1 pg/mg) and day 7 (125.3 pg/mg to 167.9 pg/mg). IL-6 was significantly decreased in the A-PRF + HA group compared with the A-PRF and control groups on day 7 (p = 0.041). In the subgroup analysis using Post-hoc ANOVA test, in day 3, there is a significant decrease in the A-PRF+HA group compared to controls (p=0.049). In day 7, A-PRF+HA group also had a significant decrease compared to A-PRF group (p=0.023) and controls (p=0.041).

Another method of evaluating inflammation is through detecting base VEGF increase before therapy. Figure 2 shows the ΔIL-6 each

intervention based on a baseline. In the A-PRF + HA group, there was a decrease in Δ 0–3 (10.9 pg/mg), and Δ 0–7 (18.3 pg/mg). In the A-PRF group, there was a decrease in Δ 0–3 (3.7 pg/mg), and Δ 0–7 (7.8 pg/mg). In the control group, there was a decrease in Δ 0–3 (4.3 pg/mg) and Δ 0–7 (35.5 pg/mg).

In the A-PRF + HA group, there was a decrease in ΔIL-6 compared with the PRF and control groups on day 3 (p = 0.018) and day 7 (p = 0.015).

Clinical Outcomes

Table 4 shows that the granulation index increased significantly in the A-PRF + HA group compared with the A-PRF and control groups on day 3 and day 7.

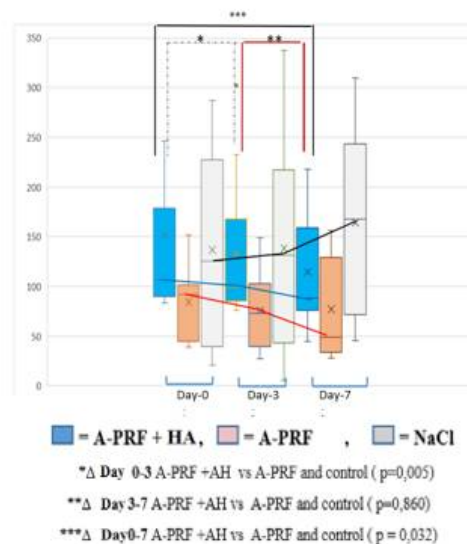


Figure 2. Δ IL-6 based on intervention group.

Table 3. Mean level of IL-6 from DFU swab following intervention.

Intervention	A-PRF + HA median (min-max)	A-PRF median (min-max)	Control median (min-max)	P
Day 0	106.4 (83.1–407.6)	91.9 (38.6 –151.6)	125.3 (20.3–287.0)	P=0.287
Day 3	99.5 (76.3–302.2)	72.8 (27.1–148.9)	131.1 (5.3–337.5)	p=0.141
Day 7	88.7 (44.3–217.9)	48.8 (27.7–156.2)	167.9 (27.7–156.2)	p=0.041*

(min-max), Kruskal Wallis test

Table 4. Mean of Granulation Index Based on Intervention

Intervention	A-PRF + AH	A-PRF	Control	p
Day 0	42.1 (18.4–57.8)	33.6 (14.1–58.9)	42.6 (19.6–52.6)	0.921
Day 3	83.1 (58.9–101.9)	68.3 (28.083.19)	59.7 (37.2–75.7)	0.004
Day 7	97.4 (94.7–99.8)	81.9 (48.4–96.8)	66.0 (47.7–86.4)	< 0.001

*Data median (min-max), Kruskal Wallis

DISCUSSION

The use of topical A-PRF + HA induced the formation of healthy granulation tissue and allowed successful granulation of the wound area. Improvement of the granulation tissue was observed because A-PRF works synergistically with HA to increase the release of growth factors and to trigger angiogenesis. In addition, the combination of A-PRF + HA decreases inflammation as shown by the decrease in IL-6.¹²

Growth Factor Release from A-PRF + HA

Type 2 Diabetes Mellitus is associated with chronic hyperglycaemia and can often cause tissue injury. Dysregulation of growth factors in DM occurs through biochemical and hemodynamic pathways. In some tissues affected by DM, excessive growth factors are induced, whereas a relative deficit of growth factors occurs in other sites. The changes in growth factors contribute to tissue pathology, including fibrosis, persistent inflammation, or a combination of the two.¹³

In this study, there is significant increase of VEGF in the swab sample in A-PRF+HA group compare with A-PRF and control NaCl on day-3 ($p=0.022$) and day 7 ($p=0.001$).

The increase of VEGF level in A-PRF+HA cause granula- α of platelets trapped in HA and slow release growth factors. In an *in vitro* study, some growth factors (PDGF, VEGF and TGF- β) contained in PRF incubated in Dulbecco's modified Eagle's Medium (DMEM) were able to survive over 14 days of observation, with a peak on day 7.¹⁷ The addition of HA to PRF increases the release rate of VEGF because VEGF released from PRF will adhere to the HA and be slowly distributed onto the DFU surface. Adding VEGF to the DFU will stimulate endothelial cells to undergo angiogenesis and stimulate the formation of granulation tissue.¹⁸ Furthermore, it was observed that the new formulation of A-PRF released significantly higher total quantities of growth factors when compared to traditional PRF. In A-PRF, using low time (only 10 minutes) and low speed (200G) settings on the centrifuge prevents platelet breakdown. The addition of HA will induce the release of growth factors from A- so that it can work more effectively.¹⁹

In the present study, adding HA to the PRF increased the concentration of the released growth factors.^{19,20} This study showed that VEGF levels would increase on day 3 and day 7, evidently filling the literature gap of the effect of combined use of PRF and HA.

The addition of HA to PRF can increase the selective permeability of activated platelet α granules. This causes changes in inflammation, angiogenesis, and immunity. Therefore, HA administration can also inhibit platelet aggregation and affect the release of growth factors from platelet concentrate.²⁰

The Combination of A-PRF + HA Reduces Inflammation

In this study, effect combination HA to A-PRF also decreases the inflammatory progression in DFU, as shown by decreased levels of IL-6 on day 7 ($p=0.041$) compare with A-PRF and NaCl. These findings conclude that stimulatory effect of HA on growth factor also decreases the inflammatory progression in DFU. Both PRF and HA injections are therapeutic options for osteoarthritis and chronic tendinopathy. Although several studies on PRF and HA have been published, the effects of mixing PRP and HA are not fully understood. PRF can stimulate the healing process of different tissues by delivering various growth factors and cytokines that are released by platelets. Ilio et al.¹⁹ reported that the expression of P selectin was dramatically increased after PRF interacted with a bio macromolecule complex film (HA collagen (I)/chitosan). HA engagement of CD44 leads to increased MAP kinase-dependent trafficking of TGF- β receptors to lipid raft-associated pools, which facilitates increased receptor turnover and attenuation of TGF- β 1-dependent alteration in proximal tubular cell function. Further investigation is needed to elucidate the associated mechanism on growth factor delivery.²¹

A-PRF + HA also increases M1 to M2 polarization as an anti-inflammatory effect. The topical application of A-PRF + HA exerts a protective effect by decreasing the release of cytokines (IL-1 β , IL-6 and TNF- α) during initial inflammatory process. This effect allows for the acceleration of the inflammatory phase, in the otherwise prolonged inflammation due to hyperglycaemia in DFU.²¹

The topical application of A-PRF + HA will bind to the ICAM-1 and VCAM-1 receptors, which will inhibit the prolonged response of leukocytes (lymphocytes and monocytes) to prevent chronic inflammation.²² An in vitro study by Chen et al.²³ showed a synergistic effect of anabolic HA and PRP on cartilage regeneration in osteoarthritis. It was also reported that the combination of HA and PRP reduced pro-inflammatory cytokines and increased articular chondrocyte proliferation and chondrogenic differentiation via the Erk1/2 pathway in HA and the Smad2/3 pathway in PRP.²⁴ **Figure 3** shows the propose mechanism by which A-PRF + HA increases granulation in diabetic foot healing.

Propose Mechanism Combine of A-PRF+HA in DFU Healing

The pathomechanism of A-PRF + HA in increasing the granulation in diabetic foot healing is shown in **Figure 3**.

CONCLUSION

The combination of A-PRF + HA increased the release of VEGF and decreased IL-6 levels on day 3 and day 7. A-PRF + HA elevated granulation index through angiogenesis and anti-

inflammatory pathways. Thus, the combination of A-PRF and HA provides beneficial effect in DFU healing process.

ACKNOWLEDGMENTS

This study was a dissertation of the Doctoral Program of Medical Science Universitas Indonesia.

CONFLICT OF INTEREST

This study was funded by Universitas Indonesia, Jakarta.

REFERENCES

1. Carvajal Am, Gluud C, Nicola S, Racines DS, Reveiz L, Olivia P. Growth factors for treating diabetic foot ulcers. *Cochrane Database Syst Rev.* 2015;28(10): 548–55.
2. Amstrong DG, Cohen K, Courric S, Bharara M, Marston W. Diabetic foot ulcers and vascular insufficiency: our population has changed, but our methods have not. *J Diabetes Sci Technol.* 2011;5(6): 1591–5.
3. Jindatanmanusan P, Luanraksa S, Boonsiri T, Nimmanon T, Arnutti P. Wound fluid matrix metalloproteinase-9 as a potential predictive marker for the poor healing outcome in diabetic foot ulcers. *Pathol Res Int.* 2018;25(4):1–6.

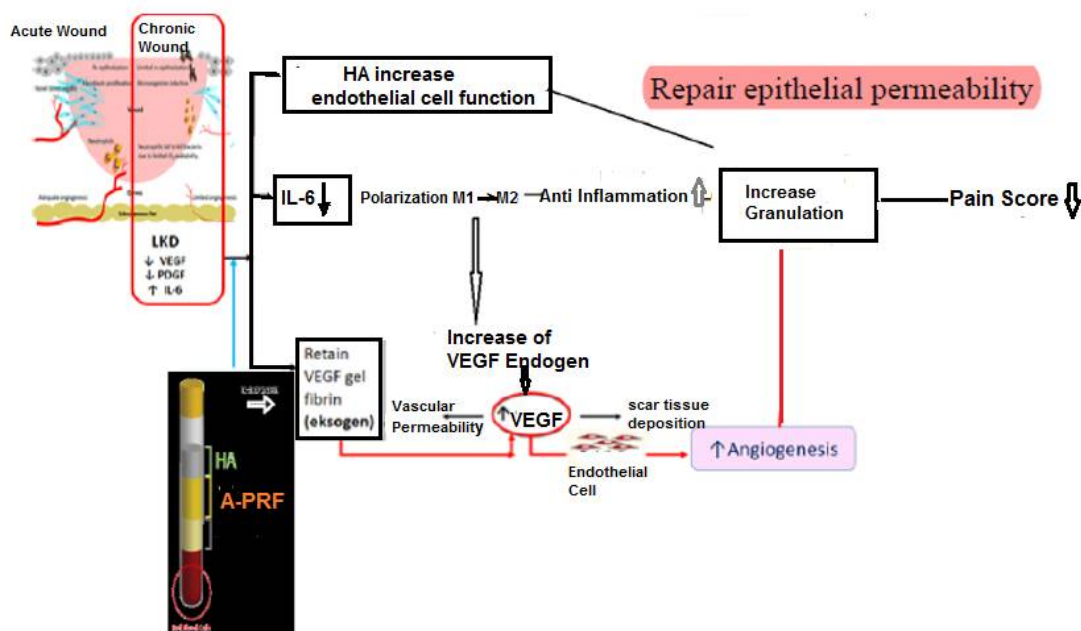


Figure 3. Proposed pathomechanism that allows A-PRF and HA to increase granulation in DFU healing.

4. Kany S, Tilman K, Vollrath JT, Relja B. Cytokines in inflammatory disease. *Int J Mol Sci.* 2019;20(23):6008.
5. Zubair M, Jamal A. Role of growth factors and cytokines in diabetic foot ulcer healing. *Rev Endocr Metab Disord.* 2019;20(8):1–11.
6. Tiaka EK, Papanas N, Mmanolakis N, Georgiadis G. Epidermal growth factor in the treatment of diabetic foot ulcers: an update. *Perspectives Vascular Surgery.* 2012;24(1):37-44.
7. Moraes VY, Lenza M, Tamaoki MJ, Faloppa F, Belloti JC. Platelet-rich therapies for musculoskeletal soft tissue injuries. *Cochrane Database Syst Rev.* 2014;29:7–19.
8. Evert P, Onichi K, Jayaram P, Lana JF, Meutner K. Platelet-rich plasma L new performance understandngs and therapeutic consideration in 2020. *Int J Mol Sci.* 2020;21(20):7794; <https://doi.org/10.3390/ijms21207794>.
9. Caruana A, Savina D, Macedo J, Soares S. From platelet-rich plasma to advanced-platelet-rich Fibrin: Biological achievements and clinical advances in modern surgery. *Eur J Dent.* 2019;13(2):280–6.
10. Raeissadat S, Rayegani S, Hassanabadi H. Knee osteoarthritis injection choices: platelet- rich plasma (PRP) versus hyaluronic acid (a one-year randomized clinical trial). *Clin Med Insights Arthritis Musculoskelet Disord.* 2015;8:1–8.
11. Ramos-Torrecillas J, García-Martínez O, De Luna-Bertos E, Oca~Na-Peinado Fm, Ruiz C. Effectiveness of platelet-rich plasma and hyaluronic acid for the treatment and care of pressure ulcers. *Biol Res Nurs.* 2015;17:152–8.
12. Anikumar TV, Muhamed J, Jose A, et al. Advantage of hyaluronic acid as a component of fibrin sheet for care of acute wound. *Biologicals.* 2011;39(2):81-8. doi 10.1016/j.biologicals.2011.01.003
13. Sridharan K, Sivaramakrishnan G. Growth factors for diabetic foot ulcers: mixed treatment comparison analysis of randomized clinical trials. *Br J Clin Pharmacol.* 2018;84(3):434–44. DOI: 10.1111/bcp.13470
14. Drela E, Kulwas A, Wieslaw J, Goralczyk B, Boinska J, Drewniak W. VEGF-A and PDGF-BB angiogenic factors and the stage of diabetic foot syndrome advancement. *Endocrinol.* 2014;65(4):306–12.
15. Amoli M, Ranjbar S, Roohipour N, Sayahpour F, Amiri P, Zahedi P. VEGF gene polymorphism association with diabetic foot ulcer. *Diabetes Res Clin Pract.* 2011;93:215–9.
16. Tahergorabi Z, Khazae M. Imbalance of angiogenesis in diabetic complications: the mechanisms. *Int J Prev Med.* 2012;3(12):827–38.
17. Perez AG, Rodrigues AA, Luzo AC, Lana JF. Fibrin network architectures in pure platelet-rich plasma characterized by fiber radius and correlated with clotting time. *J Mater Sci Mater Med.* 2014;25(8):1967-77. doi: 10.1007/s10856-014-5235-z.
18. Pardue E, Ibrahim S, Ramamurthi A. Role of hyaluronan in angiogenesis and its utility to angiogenic tissue engineering. *Organogenesis.* 2008;4(4):203–14.
19. Ilio K, FurukawaKI, Tsuda E, et.al. Hyaluronic acid induces the release of growth factors from platelet-rich plasma *Asia-Pacific Journal of Sports Medicine. Arthroscopy, Rehabilitation and Technology.* 2016;4:27–32.
20. Garbin LC, Olver CS. Platelet-rich products and their application to osteoarthritis. *J Equine Vet Sci.* 2020;86(10):10-18. doi: 10.1016/j.jevs.2019.102820.
21. Transforming Growth Factor- β 1 (TGF- β 1)-stimulated Fibroblast to Myofibroblast Differentiation Is Mediated by Hyaluronan (HA)-facilitated Epidermal Growth Factor Receptor (EGFR) and CD44 Co-localization in Lipid Rafts. *J. Bio Chemistry.* 2013;288(21):14824-38. doi10.1074/jbc.M113.451336
22. Yazdanpanah L, Nasiri M, Adarvishi S, Literature review on the management of diabetic foot ulcer, *World J Diabetes.* 2015;6(1):37-53. doi: [10.4239/wjd.v6.i1.37](https://doi.org/10.4239/wjd.v6.i1.37)
23. Chen WH, Lo WC, Hsu WC. Synergistic anabolic actions of hyaluronic acid and platelet-rich plasma on cartilage regeneration in osteoarthritis therapy. *Biomaterials.* 2014;35:9599–607.
24. Jayakumar R, Prabakaran M, Kumar S, Tamura H, Biomaterials based on chitin and chitosan in wound dressing applications. *Biotech Adv.* 2012(29):2322-337.

The Validity and Reliability of the MacNew Heart Disease Health Related Quality of Life Questionnaire: The Indonesian Version

Nur Basuki¹, Doa El-Ansary^{2,3}, Stefan Höfer⁴, Bambang Dwiputra⁵,
Nantinee Nualnim¹

¹ Faculty of Physical Therapy, Mahidol University, Thailand.

² Department of Nursing and Allied Health; Faculty of Health, Art and Design, Swinburne University of Technology, Melbourne, Victoria, Australia.

³ Department of Surgery, School of Medicine, University of Melbourne, Melbourne, Australia.

⁴ Department of Medical Psychology, Innsbruck Medical University, Innsbruck, Austria.

⁵ Department Prevention and Rehabilitation, national cardiovascular center "Harapan Kita" Jakarta, Indonesia.

Corresponding Author:

Nantinee Nualnim, Ph.D. Faculty of Physical Therapy, Mahidol University 999 Phuttamonthon 4 RD., Salaya, Phuttamonthon, Nakhonpathom, 73170, Thailand. Email: nantinee.nua@mahidol.edu.

ABSTRAK

Latar belakang: kuesener kualitas hidup terkait kesehatan penyakit jantung dari MacNew digunakan secara luas pada penderita jantung untuk mengevaluasi dampak penyakit dan intervensi termasuk rehabilitasi. Selain versi bahasa Inggris, tersedia juga beberapa bahasa lain kecuali bahasa Indonesia. Oleh karena itu, tujuan dari penelitian ini adalah 1) menerjemahkan kuesioner kualitas hidup terkait kesehatan jantung MacNew dari versi bahasa Inggris ke bahasa Indonesia dan 2) untuk memperkirakan validitas dan reliabilitas awal dari MacNew versi bahasa Indonesia untuk pasien penyakit arteri koroner. **Metode:** Prosedur penerjemahan forward dan backward digunakan untuk mengembangkan kuesener MacNew versi bahasa Indonesia. MacNew versi Bahasa Indonesia diberikan kepada 24 pasien satu minggu setelah operasi revaskularisasi. Reliabilitas dinilai dengan konsistensi internal dan reliabilitas tes-retes. Untuk mengevaluasi validitas sewaktu (concurrent validity), dilakukan penilaian terhadap korelasi domain yang kompatibel antara MacNew versi Indonesia dan SF-36. **Hasil:** Macnew versi Indonesia memiliki konsistensi internal yang bagus baik pada skala global maupun pada ketiga subskala nya dengan nilai Cronbach α melebihi 0,95. Hasil evaluasi terhadap reliabilitas tes ulang menunjukkan hasil yang dapat diterima dengan koefisien korelasi intra-kelas 0,66 untuk skor global. Selain itu, korelasi antara MacNew Indonesia dan SF-36 menunjukkan hasil yang signifikan dengan nilai korelasi Pearson berkisar antara 0.47 hingga 0.71. **Kesimpulan:** Hasil penelitian awal kuesener MacNew versi Bahasa Indonesia menunjukkan validitas dan reliabilitas yang dapat diterima sebagai alat ukur untuk menilai kualitas hidup terkait kesehatan pada dengan penyakit arteri koroner di Indonesia.

Kata kunci: Kualitas hidup, validitas, reliabilitas, MacNew versi Indonesia. penyakit arteri koroner.

ABSTRACT

Background: The MacNew heart disease health related quality of life tool is used widely in populations with cardiac comorbidity to evaluate the impact of disease and intervention including rehabilitation. In addition to the English version, it is also available for several other languages except the Indonesian language. Therefore, the

purpose of this study were 1) to translate the MacNew questionnaire from the English version to the Indonesian language and 2) to estimate the preliminary validity and reliability of the Indonesian MacNew for patients with coronary artery disease. **Methods:** Forward and backward translation procedure was used to develop the Indonesian MacNew. The Indonesian MacNew was administered to 24 patients after one week of revascularization surgery. Reliability was assessed by internal consistency and test-retest reliability. To evaluate concurrent validity, the correlation of the compatible domain of the Indonesian MacNew and SF-36 was assessed. **Results:** Internal consistency reliability of the Indonesian MacNew was confirmed with Cronbach's α of the global scale and all three subscales exceeding 0.95. Test-retest reliability was acceptable with intraclass correlation coefficient of 0.66 for the global score. Furthermore, an acceptable concurrent validity was established with statistically significant correlation between Indonesian MacNew and SF-36 (pearson correlation ranging from 0.47 to 0.71). **Conclusions:** The first results of the Indonesian MacNew indicate acceptable validity and reliability as a measurement tool to assess health related quality of life of Indonesian patients with coronary artery disease.

Keywords: Quality of life, validity, reliability, Indonesian MacNew, coronary artery disease.

INTRODUCTION

Currently, traditional clinical outcomes such as morbidity, mortality, and physiological responses to the treatment have been widely investigated, however it is shown to be not quite in line with the patient's perspective on their health status.^{1,2} Health related quality of life (HRQL) is recognized as a more comprehensive assessment of health status perceived by the patients on the impact of disease and medical intervention. HRQL is defined as how well a person functions in their life and his or her perceived wellbeing in physical, mental and social domains of health.³ There are two approaches of measuring HRQL, namely generic HRQL and disease specific HRQL. Generic HRQL is used to assess a wide range of health status. In Indonesia, for example the Short Form 36 (SF-36) questionnaire is a well-known generic instrument used in cardiac patients.⁴

Disease specific HRQL are more relevant and sensitive to change in HRQL than generic HRQL in reflecting the impact of cardiovascular disease and the health status changes during medical treatment and recovery. There are many tools for assessing HRQL in cardiac patients such as the Seattle Angina Questionnaire,^{5,6} Minnesota Living with Heart Failure,^{7,8} and the MacNew heart disease HRQL questionnaire. Globally, the MacNew heart disease questionnaire has been shown to better and more comprehensively reflect the experience of coronary artery disease (CAD) patients in all types of cardiac events

and its items covering the broad spectrum of health.^{1,9} The MacNew consists of 27 items divided into three domains: physical limitation, emotional function and social function which can be combined to give a global HRQL score.¹ This questionnaire has been reported as a valid, reliable and responsive to evaluate the impact of treatment, including cardiac rehabilitation for patients with myocardial infarction, angina pectoris, and heart failure in over 40 languages.^{1,9}

The original MacNew heart disease HRQL was developed in the English language and has been translated into over 40 languages except the Indonesian language. As such, the utility uptake of this tool within the Indonesian health care system to evaluate HRQL is poor. Therefore, the purpose of this study was to translate the MacNew heart disease HRQL from the original English version to the Indonesian language and to evaluate the validity and reliability of the Indonesian MacNew heart disease HRQL for patients with CAD in a domestic Indonesian population.

METHODS

This study was implemented as part of a longitudinal study that aimed to examine the changes in HRQL after cardiac rehabilitation program in CAD patients in Indonesia. Twenty-four adults aged above 18 years with CAD referred to phase II CR in longitudinal research study were recruited from the national cardiovascular center "Harapan Kita" in Jakarta, Indonesia.

The inclusion criteria were: 1) patients who have had myocardial infarction (MI) or who had undergone revascularization (PCI and CABG), 2) can speak, read and comprehend the Indonesian language. Patients with cognitive impairment, impaired vision that cannot be corrected with spectacles were excluded from the study. Ethics was obtained from the Committee of Institutional Review Board/ Health Research Ethics, National cardiac Center “Harapan Kita” Hospital (Ethics number: LB.02.01/VII/284/KEP.051/2018).

MacNew HRQL

The forward and backward translation guideline from macnew.org was used to develop the Indonesian MacNew heart disease HRQL questionnaire from the English version. Two people who were fully bilingual in Indonesian and English have been asked independently to translate the English MacNew to Indonesian, in which one of them is a health care professional and the other one is not health care professional. The translator should use the same questionnaire format as the original English MacNew. The results of the translation from English to Indonesian obtained from two translators were evaluated and cross checked by a third independent person for consistency to obtain one final version of the translation. A further two people were invited to do back-translation into English to attain concurrent validity. The first results of translation process were sent to macnew.org for evaluation of the accuracy of the translation. The questionnaire was then further revised in several iterations until linguistic equivalence was established.

Short Form-36 (SF-36)

The SF-36 consists of 36 items divided into eight domains: physical functioning (PF), role limitations due to physical health (RP), role limitations due to emotional problems (RE), vitality (VT), mental health (MH), social functioning (SF), bodily pain (BP), general health (GH). The SF-36 is widely used in patients with CAD and is available in Indonesian (4). The SF-36 was used to evaluate the validity of Indonesian MacNew HRQL in this study.

Outcome Measurements

All eligible subjects were asked to complete the Indonesian MacNew heart disease HRQL and SF-36 Indonesian version. The order of survey completion was determined by computer generated randomization procedure that was administered by an independent person of the study. Each subject was provided a 15-minutes rest period between the two questionnaires. The first measurement of both questionnaires was conducted one week after revascularization procedure, while the second measurement was conducted between 7-10 days thereafter.

Statistical Analysis

Frequencies and mean (SD) were used to describe subject characteristics. Cronbach's alpha was used to examine the internal consistency reliability, while intra-class correlation coefficient (ICC) was used to evaluate the test-retest reliability. Furthermore, Pearson correlation coefficient was used to assess concurrent validity of the Indonesian MacNew against the Indonesian SF-36 health survey.

RESULTS

Subject Characteristic

Subject characteristics were presented in **Table 1**. Twenty-two male (91.7%) and two female patients (8.3%) participated in this study. The subject age was 57.3 ± 6.8 years. All subjects were married and mostly graduated from university and only 4.2% had a primary level of education. More than half of the subjects had hypertension (54.2%), hypercholesterolemia (62.5%), sedentary (75%) and were current smoker (54.2%). Most of the subjects underwent coronary artery bypass graft (CABG) surgery.

Health Related Quality of Life Outcome Score

The score of Macnew HRQL questionnaire and SF-36 were presented in **Table 1**. The mean score of the Indonesian MacNew global and subscales ranged from 4.42 (SD 1.2) for the social subscale to 4.9 (SD 1.0) for the emotional subscale. The mean value of the physical component scale of the SF-36 was 57.6 (SD 16.8) and the value of the mental component scale of the SF-36 was 63.3 (SD 19.30).

Table 1. Subject characteristics.

Subject characteristics	Mean (SD)	N	%
Age (years)	57.3 (6.8)	24	
Sex			
Male		22	91.7
Female		2	8.3
Family status			
Single		0	0
Married		24	100
Type of work			
Working in sitting \geq 2 hours/day		13	54.2
Working need walking \geq 2 hours/day		11	45.8
Level of Education			
Basic school		1	4.2
Elementary School		0	0
High school		8	33.3
University		15	62.5
BMI	25 (3)	24	
History of exercise			
Regular		6	25
Not Regular		18	75
Smoking status			
Present Smoker		13	54.2
Never smoked		7	29.2
Ex-smoker		3	12.5
Diabetes		6	25
Hypertension		13	54.2
Hypercholesterolemia		15	62.5
Type of surgery			
PCI		1	4.2
CABG multiple vessels		23	95.8
Left Ventricular Ejection Fraction (%)	58.5 (13.2)	24	
MacNew			
Global	4.6 (1.0)		
Physical	4.4 (1.2)		
Emotional	4.9 (1.0)		
Social	4.4 (1.2)		
SF-36			
Physical Component Scale	57.6 (16.8)		
Mental Component Scale	63.3 (19.3)		

BMI, body mass index; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft; SF-36, short form-36

Table 2. Internal consistency (Cronbach's α), test-retest reliability (ICC 3,2), floor and ceiling effects of the Indonesian MacNew.

	MacNew			
	Global	Physical	Emotional	Social
% Floor	0%	0%	0%	0%
% Ceiling	0%	0%	0%	0%
Cronbach's α	0.95	0.98	0.97	0.98
ICC (3,2)	0.66	0.36	0.82	0.60

ICC, intra-class correlation coefficient

MacNew Psychometric Properties

The Psychometric properties of Indonesian version of MacNew HRQL questionnaire were presented in **Table 2**.

Floor and Ceiling Effects

There are no floor effects (score = 1) and ceiling effects (score = 7) of the MacNew global score as well as in all three domains.

Internal Consistency

The internal consistency of the Indonesian MacNew heart disease HRQL was analyzed separately for the global scale and for each domain. The Cronbach's α coefficient for the global scale and all three domains was high. The Cronbach's α coefficient for global scale was 0.95, while for each domain scale ranging from 0.96 – 0.98.

Test-retest Reliability

Test-retest reliability of the Indonesian version of MacNew heart disease HRQOL was found to be satisfactory. The ICC for the global score was 0.66 demonstrating good reliability. While the ICC score for the subscales were 0.82 for the emotional subscale indicating excellent reliability and 0.60 for the social subscale demonstrating good reliability. The physical subscale was associated with an ICC of 0.36 indicating a poor reliability.¹⁰

Validity

The concurrent validity of the Indonesia version of MacNew heart disease HRQL and SF-36 Indonesian version demonstrated moderate to strong correlation (**Table 3**). This result was interpreted as follows: small $r = 0.10 - 0.29$, medium $r = 0.30 - 0.49$, Large $r = 0.50 - 1.0$.¹¹ Moreover, most of these correlations were significant at 0.05 and some of them were significant at 0.01.

Table 3. Pearson correlation of the association between Indonesian MacNew and SF-36.

MacNew	SF-36	
	Physical Scale	Mental Scale
Global	0.50*	0.63**
Physical	0.47*	
Emotional		0.71**
Social		0.63**

* $p < 0.05$, ** $p < 0.01$

DISCUSSION

This study examined the reliability and validity of the Indonesian version of the MacNew heart disease HRQL questionnaire. The internal consistency of the three domains in the English version of the MacNew heart disease HRQL ranged from 0.93 to 0.95.¹² This compares well to the internal consistency for other language versions of the MacNew, which range from 0.78 to 0.97.^{1,13} Although the present study was limited by its small sample size, the results demonstrated satisfactory psychometric properties concurrent with the English version. Internal consistency reliability in the present study was ≥ 0.95 .

The test-retest reliability was 0.66 in global, 0.82 in emotional and 0.60 in social scale. However, the ICC for the physical subscale was lower (0.36) compared to other language versions. The low test-retest reliability value in physical domain may be due to the fact that we conducted baseline measurement within one week after surgery, while the second measurement conducted 7-10 days after baseline measurement. All patients followed an outpatient cardiac rehabilitation program, therefore their physical function was expected to improve, reflecting improvement in the HRQL. Our findings are similar to those reported in the Farsi version, where patients did not meet the criteria for clinical stability.¹⁴

The validity of the Indonesian MacNew was found to be acceptable with statistically significant Pearson correlation value in all domains ranging from 0.47 to 0.71. The value of the SF36 Mental Component Scale had the highest correlation with the MacNew Emotional sub-scale (0.71). These results are in line with the previous study in China mainland and Sri Lanka.^{15, 16} On the other hand, the correlation between Physical Component Scale of the SF-36 and MacNew physical subscale was lower than the previous reported study.¹⁵⁻¹⁷ Future studies are needed to assess the construct validity using larger sample sizes.

The limitation of this study is that the study was conducted one week after surgery. During this period, patients experienced pain and were still recovering post-operatively. It is suggested

that future studies should include patients at various stages of the post-operative recovery period. Another limitation of this study is that the patients were predominantly male (91.7%) and although this is representative of the population with CAD in that age range, results cannot inform findings for the female population. Furthermore, this study only evaluated concurrent validity and as such future studies should include an evaluation of construct validity. Construct validity will provide evidence to support or refute the theoretical framework behind the concept.

CONCLUSION

The current study gave first indications that the Indonesian MacNew translation has good reliability and acceptable validity as a measurement tool to assess disease specific HRQL of Indonesian patients with CAD. The Indonesian MacNew may be used as a disease-specific instrument to assess and evaluate HRQL in Indonesia.

CONFLICT OF INTEREST

Stefan Höfer is co-developer of the MacNew questionnaire, while the remaining authors declare no competing interest.

CONSENT TO PARTICIPATE

The written informed consent was obtained from all participants.

FUNDING

There was no funding in the present study.

ACKNOWLEDGMENTS

We would like to thank MacNew.org for permission to use the questionnaire and National Cardiovascular Center “Harapan Kita” in Jakarta for permission to collect the data and further thanks to the patients for their valuable contribution.

REFERENCES

1. Höfer S, Lim L, Guyatt G, et al. The MacNew heart disease health-related quality of life instrument: A summary. *Health and Quality of Life Outcomes*. 2004;2(1):3.

2. Chen T-H, Li L, Kochen MM. A systematic review: how to choose appropriate health-related quality of life (HRQOL) measures in routine general practice? *J Zhejiang Univ Sci B*. 2005;6(9):936-40.
3. Karimi M, Brazier J. Health, health-related quality of life, and quality of life: What is the difference? *Pharmaco Economics*. 2016;34(7):645-9.
4. Salim S, Yamin M, Alwi I, et al. Validity and reliability of the Indonesian version of SF-36 quality of life questionnaire on patients with permanent pacemakers. *Acta Med Indones*. 2017;49(1):10-6.
5. Spertus JA, Winder JA, Dewhurst TA, et al. Development and evaluation of the Seattle Angina Questionnaire: A new functional status measure for coronary artery disease. *J Am Coll Cardiol*. 1995;25.
6. Kimble LP, Dunbar SB, Weintraub WS, et al. The Seattle angina questionnaire: reliability and validity in women with chronic stable angina. *Heart Disease (Hagerstown, Md)*. 2002;4(4):206-11.
7. Heo S, Moser DK, Riegel B, et al. Testing the psychometric properties of the Minnesota Living with Heart Failure questionnaire. *Nursing Res*. 2005;54(4):265-72.
8. Munyombwe T, Hofer S, Fitzsimons D, et al. An evaluation of the Minnesota Living with Heart Failure Questionnaire using Rasch analysis. *Qual Life Res*. 2014;23(6):1753-65.
9. Höfer S, Saleem A, Stone J, et al. The MacNew heart disease health-related quality of life questionnaire in patients with angina and patients with ischemic heart failure. *Value in Health*. 2012;15(1):143-50.
10. Cicchetti DV. Guidelines, criteria, and rules of thumb for evaluating normed and standardized assessment instruments in psychology. *Psychological Assessment*. 1994;6(4):284-90.
11. Pallant J. *SPSS survival manual: a step by step guide to data analysis using SPSS for windows (Version 10)*. Buckingham: Open University Press; 2001.
12. Valenti L, Lim L, Heller RF. An improved questionnaire for assessing quality of life after myocardial infarction. *Qual Life Res*. 1996;5.
13. Lee WL, Chinna K, Bulgiba A, et al. Test-retest reliability of HeartQoL and its comparability to the MacNew heart disease health-related quality of life questionnaire. *Qual Life Res*. 2016;25(2):351-7.
14. Asadi-Lari M, Javadi HR, Melville M, et al. Adaptation of the MacNew quality of life questionnaire after myocardial infarction in an Iranian population. *Health and quality of life outcomes*. 2003;1:23-6.
15. Wang W, Lau Y, Palham S, et al. Psychometric testing of the Chinese Mandarin version of the MacNew Heart Disease Health-related Quality of Life questionnaire for patients with myocardial infarction in mainland China. *Int J Nursing Pract*. 2015;21(2):147-55.
16. Seneviwickrama KL, Samaranayake DB, Fonseka P, et al. Psychometric evaluation of the Sinhalese version of MacNew heart disease health related quality of life questionnaire in patients with stable angina. *Health and quality of life outcomes*. 2016;14:1-9.
17. Fattirolli F, Marchionni N, Hofer S, et al. The Italian MacNew heart disease health-related quality of life questionnaire: a validation study. *Intern Emerg Med*. 2015;10(3):359-68.

The Relationship between Folic Acid and Vitamin B12 Serum Levels with High Sensitivity C-reactive Protein and Homocysteine in Chronic Hemodialysis Patients: A Cross-sectional Study

Aida Lydia¹, Dimas Priantono², Kuntjoro Harimurti³, Idrus Alwi⁴

¹ Division of Nephrology and Hypertension, Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia - Cipto Mangunkusumo National Hospital, Jakarta, Indonesia.

² Department of Internal Medicine, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo National Hospital, Jakarta, Indonesia.

³ Clinical Epidemiology Unit, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo National Hospital, Jakarta, Indonesia.

⁴ Division of Cardiology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo National Hospital, Jakarta, Indonesia.

Corresponding author:

Aida Lydia, MD., PhD. Division of Nephrology and Hypertension, Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia - Cipto Mangunkusumo Hospital. Jl. Diponegoro no. 71, Jakarta 10430, Indonesia. Email: aidalydia@gmail.com.

ABSTRAK

Latar belakang: Pemberian suplementasi asam folat (AF) dan vitamin B12 pada pasien PGK, telah secara rutin diresepkan untuk menurunkan kadar homosistein dan peradangan pada pasien PGK. Akan tetapi, belum banyak penelitian mengenai hubungan kadar asam folat dan vitamin B12 pada pasien yang menjalani hemodialisis dua kali seminggu. Penelitian ini bertujuan untuk menilai kadar folat serum dan B12 pada pasien hemodialisis kronis dan hubungannya dengan kadar homosistein dan hsCRP dalam darah. **Metode:** Studi ini merupakan studi potong lintang yang mengikutsertakan seluruh pasien hemodialisis rutin dua kali seminggu di RS Cipto Mangunkusumo, Jakarta, Indonesia. Sampel untuk pengukuran kadar asam folat, vitamin B12, homosistein, dan hsCRP diambil dari sampel darah predialisis. Subjek yang memiliki kondisi medis yang mempengaruhi hasil pemeriksaan dikeluarkan dari penelitian ini. Korelasi antar variabel dianalisis menggunakan uji korelasi Spearman. **Hasil:** Delapan puluh subjek diikutsertakan dalam penelitian ini. Pada subjek yang tidak mendapatkan suplementasi asam folat [26 (32.5%)] dan suplementasi vitamin B12 [16 (20.0%)], hanya 3,85% subjek memiliki kadar asam folat yang rendah dan tidak ada subjek yang memiliki kadar vitamin B12 yang rendah. Didapatkan adanya korelasi negatif sedang antara asam folat serum dan kadar homosistein ($p \leq 0,001$; $r = -0,42$) dan korelasi lemah antara vitamin B12 serum dan kadar homosistein ($p = 0,009$; $r = -0,29$). Pada subjek dengan risiko kardiovaskular tinggi ($CRP > 3$, $n = 49$), terdapat korelasi negatif sedang antara asam folat serum dan kadar homosistein ($p \leq 0,001$; $r = -0,561$) dan korelasi negatif yang lemah antara vitamin B12 dan homosistein ($p = 0,018$; $r = -0,338$). **Kesimpulan:** Kadar vitamin B12 dan asam folat berhubungan negative dengan kadar homosistein, terutama pada kelompok dengan risiko kardiovaskular risiko tinggi.

Kata kunci: asam folat, vitamin B12, kardiovaskular, mortalitas, morbiditas, hsCRP, penyakit ginjal kronis, hemodialisis.

ABSTRACT

Background: Folic acid (FA) and vitamin B12 treatment have been routinely prescribed to lower serum homocysteine levels and to reduce inflammation. However, no study has been conducted to determine serum folic acid (SFA) and vitamin B12 (B12) levels in patients who have twice-weekly hemodialysis. The aim of our study was to assess serum folate and B12 levels in chronic hemodialysis patients and their relationship with hsCRP and homocysteine levels. **Methods:** Our study was a cross-sectional study involving patients who had twice-weekly hemodialysis in Dr Cipto Mangunkusumo National Hospital Jakarta, Indonesia. Predialysis blood samples were taken to measure SFA, B12, homocysteine and hsCRP levels. Patients with medical conditions affecting the assays were excluded. Spearman correlation was used to compare variables. **Results:** Eighty subjects were enrolled in this study. Among those without folic acid and vitamin B-12 supplementation, only 3.85% of subjects had low folic acid levels, and none had low vitamin B12 levels. A moderate negative correlation between serum folic acid and homocysteine level ($p \leq 0.001$; $r = -0.42$) and a weak correlation between serum vitamin B12 and homocysteine level ($p = 0.009$; $r = -0.29$) was found. Among the high-risk cardiovascular group ($CRP > 3$, $n = 49$), there is a moderate negative correlation between serum folic acid and homocysteine level ($p \leq 0.001$; $r = -0.561$) and a weak negative correlation between vitamin B12 and homocysteine level ($p = 0.018$; $r = -0.338$). **Conclusions:** There is a significant negative correlation between serum vitamin B12 and folic acid with homocysteine levels, especially in high-risk cardiovascular group.

Keywords: Folic acid, vitamin B12, cardiovascular, mortality, morbidity, hsCRP, CKD, chronic hemodialysis.

INTRODUCTION

Chronic kidney disease (CKD) is a resource-draining condition with poor outcomes.¹ The spectrum of CKD ranges from early to end-stage kidney disease (ESKD), which needs kidney replacement therapy (KRT). The Indonesian Renal Registry and Indonesian Ministry of Health data showed that 25 million Indonesians are affected by CKD, and hemodialysis is the most common KRT modality among this population.²⁻³ Chronic kidney disease patients have a 16 to 30-fold higher mortality compared to the general population.^{4,5} The most common cause of death in CKD patients is cardiovascular disease (CVD), which comprises 37% of all causes of mortality.⁶

Homocysteine is an independent risk factor for CVD. Studies have shown that homocysteine-lowering therapy could decrease cardiovascular mortality and morbidity in CKD patients.^{7,8} Folic acid has an essential role in homocysteine methylation, while vitamin B12 acts as a coenzyme in the process. Theoretically, supplementation of those two could lower the homocysteine level.^{9,10} C-reactive protein (CRP) is also a good predictor for mortality.¹¹ A small increase in serum CRP level could be detected by high-sensitivity CRP (hs-CRP) assays.¹²

High serum hsCRP has been correlated with inflammation and major adverse cardiovascular events (MACE).¹³

In chronic hemodialysis patients, folic acid and vitamin B12 are two of the most routinely prescribed drug “cocktails”; however, until now, there is unclear evidence for folic acid and vitamin B12 deficiency in chronic hemodialysis patients. This practice is based on the theory that folic acid and vitamin B12 deficiency will occur in CKD. Studies in predialysis patients (i.e., CKD stage 1-4) by Hassan et al. in 2015¹⁴ showed that folic acid deficiency increases along with kidney disease progression. In hemodialysis patients, the folic acid deficiency could be worse. Folic acid is a water-soluble nutrient with low molecular weight material. Therefore, it can be removed during the dialysis session and worsen the deficiency. Most of the published studies have demonstrated the relationship between folic acid level and all-cause mortality in hemodialysis population. However, those studies could not conclude whether supplementation of folic acid and vitamin B12 could decrease serum homocysteine level, hs-CRP, or cardiovascular events, and most of them observe the relationship in predialysis patients or thrice-weekly hemodialysis patients.^{7-10 15-23} There

has not been any study involving twice-weekly dialysis patients, which are the most common type of hemodialysis patients in Indonesia.

In Indonesia, the National Health Insurance only reimburses three-weekly dialysis for a highly selected group of patients. The differences in dialysis frequency, high-flux vs. low flux dialyzers, and patient characteristics creates a demand for this kind of study. This study aims to give insight into whether folic acid and vitamin b12, a routinely prescribed supplementation, had a significant relationship with hsCRP and homocysteine, markers for a poor outcome in a twice-weekly hemodialysis population. Our study aimed to assess the level of folic acid and vitamin B12 and their relationship to hsCRP and homocysteine levels in twice-weekly hemodialysis patients.

METHODS

We performed a cross-sectional study at the Hemodialysis Unit in Cipto Mangunkusumo National Hospital, Jakarta, Indonesia, involving adult patients aged 18 years or older. The patients had been diagnosed with CKD and had undergone twice-weekly chronic hemodialysis for at least three months. We excluded patients with severe acute illness, malignancies, autoimmune disease, tuberculosis infection, exposure to immunosuppressive treatments, and gastrointestinal tract surgery history that could impair folic acid/vitamin B12 absorption. Informed consent was requested from each eligible subject for the willingness to participate in the study. Using sample size formula for correlation analysis, with alpha 0.05 and power of 80%, the minimum sample size needed for this study was 62 subjects.²⁴ The ethical clearance was obtained from the Research Ethics Committee Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta.

Demographic and clinical data

Data collection was done from August 2019 to October 2019. The patients' demographic characteristics and clinical data were collected through interviews and examination, as well as from electronic or paper-based medical records

provided at the Hemodialysis Unit. Folic acid (ECLIA/ electrochemiluminescence immunoassay), vitamin B12 (CMIA/ Chemiluminescent Microparticle Immunoassay), and homocysteine (CMIA) assays were performed at the Prodia Laboratory, Jakarta, Indonesia (ISO 9001 and ISO 15189 certification). Serum hsCRP (Immunturbidimetry) and other laboratory assays were carried out at the Department of Clinical Pathology Laboratory, Cipto Mangunkusumo National Hospital.

Statistical Analysis

Patients' baseline data, such as demographic data, clinical data, and laboratory measurements, were summarized as numerical and nominal values accordingly. Mean and standard deviations or median and interquartile ranges were used appropriately for each parameter. Spearman correlation was used to evaluate the correlation between folic acid/B12 levels and homocysteine/hsCRP levels. A p-value of <0.05 was considered as statistically significant.

RESULTS

We screened 90 subjects. After applying inclusion and exclusion criteria, 80 subjects were included (**Figure 1**). **Table 1** summarized the baseline characteristics of the subjects. As summarized in **Table 1**, the mean age of subjects in our study was 52 (standard deviation 12.5) years. We had an almost equal number of male and female participants (38

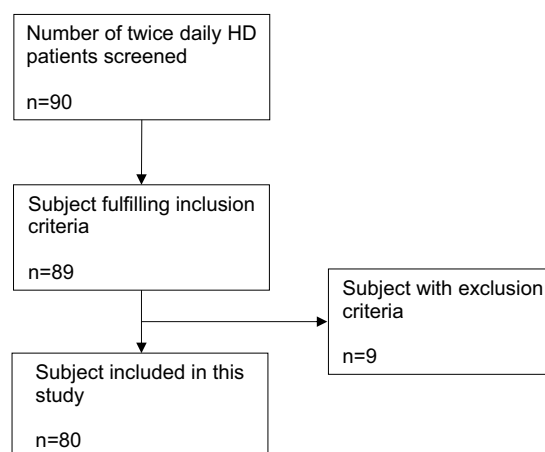


Figure 1. Flow diagram

and 42 subjects, respectively). The median duration of CKD diagnosis was 6.5 years, with a median duration of hemodialysis treatment of 5 years. The most prevalent cause of CKD was hypertension.

Results of folic acid, vitamin B12, homocysteine and hsCRp assays can be seen in **Table 2**. Normal-high B12 and folic acid levels show in 98.75% and 95% patients, respectively. Without folic acid supplementation, only 3.85% of subjects had low folic acid levels, and none

of the non-vitamin B12 supplemented group had low vitamin B12 levels. About 61.3% of the subjects had high hsCRP levels. Meanwhile, high homocysteine was found in 75% of subjects and in 93.75% of subjects who have not received vitamin B12 supplementation.

Among subjects given folic acid supplementations, high hsCRP was found in 61% of subjects, while 68.5% of subjects had high homocysteine levels. Meanwhile, in subjects who were not given folic acid supplementation,

Table 1. Characteristics of subjects

Variables (N=80)	Result
Age (years), mean (SD)	52.03 (SD 12.46)
Sex (n,%)	
- Male	38 (47.5)
- Female	42 (52.5)
Duration of CKD diagnosis (years), median (IQR)	6.5 (6.75)
Duration of hemodialysis treatment (years), median (IQR)	5 (6.75)
Duration of hemodialysis (hours/session), median (IQR)	5 (0)
Qb (ml/min), median (IQR)	280 (50)
Qd (ml/min)	500
Ultrafiltration volume (ml), median (IQR)	3,700 (1,500)
Kt/V, mean (SD)	1.81 (0.36)
Weekly folate supplementation (mg/week), median (IQR)	35 (105)
- No supplementation (n,%)	26 (32.5)
- 35 mg/week (n,%)	21 (26.3)
- 70 mg/week (n,%)	8 (10.0)
- 105 mg/week (n,%)	25 (31.3)
Weekly vitamin B12 supplementation (mcg/week), median (IQR)	1,050 (4,650)
- No supplementation (n,%)	17 (21.3)
- Oral (n,%)	42 (52.5)
- Parenteral (n,%)	9 (11.3)
- Oral+parenteral (n,%)	12 (15)
BMI (kg/m ²), mean (SD)	25.06 (4.68)
Cause of CKD (n,%)	
- Hypertension	31 (38.8)
- Hypertension and diabetes	22 (27.5)
- Glomerulonephritis	9 (11.3)
- Kidney stones	5 (6.3)
- Diabetes	3 (3.8)
- Polycystic kidney disease	3 (3.8)
- Preeclampsia	2 (2.5)
- Nephrotoxic agents	2 (2.5)
- Others	2 (2.5)
- Undetermined	1 (1.3)

SD, standard deviation; IQR, interquartile range; CKD, chronic kidney disease; BMI, body mass index

Table 2. Folic acid, Vitamin B12, Homocysteine and hs CRP Measurement Results

Variables (N=80)	Total	Folic acid Supplementation N (%)		p-value	Vitamin B12 Supplementation N (%)		p-value
		No 26 (32.5%)	Yes 54 (67.5%)		No 16 (20.0%)	Yes 64 (80.0%)	
Folic acid - ng/mL (median; IQR)	18.04 (36.89)	11.65 (6.71)	27.92 (56.36)	0.001*	12.18 (6.59)	24.61 (54.9)	0.006*
- Low (N,%)	4 (5)	1 (3.85)	3 (5.56)	0.001*	0 (0)	4 (6.25)	0.003*
- Normal (N,%)	35 (43.8)	22 (84.62)	13 (24.07)		14 (87.50)	21 (32.81)	
- High (N,%)	41 (51.2)	3 (11.54)	38 (70.37)		2 (12.50)	39 (60.94)	
Vitamin B12 - pg/mL (median; IQR)	1,695 (58,286.5)	1,149.5 (3,303)	1,817.5 (145,227)	0.049*	1,017.5 (1,224)	1,817.5 (130,152.5)	0.023*
- Low (N,%)	1 (1.25)	1 (3.85)	0 (0)	0.192	0 (0)	1 (1.56)	0.246
- Normal (N,%)	22 (27.5)	9 (34.62)	13 (24.07)		7 (43.75)	15 (23.44)	
- High (N,%)	57 (71.25)	16 (61.54)	41 (75.93)		9 (56.25)	48 (75.0)	
Homocysteine - μ mol/L (median; IQR)	20.85 (11.45)	22 (13.8)	20.35 (11.6)	0.122	22 (13.05)	20.7 (12.45)	0.324
- Low (N,%)	0 (0)	0 (0)	0 (0)	0.054	0 (0)	0 (0)	0.053
- Normal (N,%)	20 (25)	3 (11.54)	17 (31.48)		1 (6.25)	19 (29.69)	
- High (N,%)	60 (75)	23 (88.46)	37 (68.52)		15 (93.75)	45 (70.31)	
hsCRP - mg/L (median; IQR)	4.7 (12.63)	4.1 (7)	4.9 (13.7)	0.801	7.4 (11.85)	4.3 (12.2)	0.923
- <1,0 (N,%)	8 (10)	2 (7.69)	6 (11.11)	0.878	2 (12.50)	6 (9.38)	0.892
- 1,0-3,0 (N,%)	23 (28.7)	8 (30.77)	15 (27.78)		4 (25.0)	19 (29.69)	
- >3,0 (N,%)	49 (61.3)	16 (61.54)	33 (61.11)		10 (62.5)	39 (60.94)	

hsCRP, high-sensitivity C Reactive Protein (hsCRP); IQR, interquartile range; *) $p < 0.05$.

high hsCRP was found in 61% of subjects, and high homocysteine was found in 75% of subjects. Furthermore, in subjects given vitamin B12 supplementation, high hsCRP was found in 60.94% of subjects, while high homocysteine was found in 70.31%. In subjects who were not given vitamin B12 supplementation, high hsCRP was found in 62.5% of subjects, and high homocysteine was found in 93.8% of subjects.

We found a moderately negative correlation between serum folic acid and homocysteine level ($p \leq 0.001$; $r = -0.4228$) and a weak correlation between serum vitamin B12 and homocysteine level ($p = 0.009$; $r = -0.2905$) (Table 3); however, no correlation was found between folic acid and vitamin B12 with hsCRP levels.

We performed subgroup analysis according to the subject hsCRP level to determine the high-risk group's correlation (hsCRP > 3 mg/L, $n = 49$). There is no difference found in folic acid, vitamin B12 and homocysteine levels between group ($p > 0.05$) (Table 4). However, in high

Table 3. Correlation between folic acid and vitamin B12 to hsCRP and homocysteine level

Variables	r	p-value
Folic acid and hsCRP	-0.0535	0.637
Folic acid and homocysteine	-0.4228	0.001*
Vitamin B12 and hsCRP	-0.1443	0.202
Vitamin B12 dan homocysteine	-0.2905	0.009*

hsCRP, high-sensitivity C Reactive Protein; *) $p < 0.05$.

risk group, stronger correlations were observed both in serum folic acid ($r = -0.561$, $p \leq 0.001$) and vitamin B12 ($r = -0.338$, $p = 0.018$) with homocysteine level. But, no correlations were observed between vitamin B12 and folic acid with hsCRP levels (Table 5).

DISCUSSION

Most of our subjects had high folic acid and vitamin B12 levels. Even in those without supplementation, 96.16% of subjects had normal-high folic acid, and 100% had normal-high vitamin B12 levels. A study by Fehrman-

Table 4. Folic acid, vitamin B12 and homocysteine comparison between cardiovascular risk group.

Variables (N=80)	Total	Cardiovascular risk		p-value
		High 49 (6.13%)	Low 31 (38.7%)	
Folic acid - ng/mL (median; IQR)	18.04 (36.89)	17 (39.01)	18.85 (28.52)	0.976
Low (N,%)	4 (5%)	3 (75.00%)	1 (25.00%)	0.785
Normal (N,%)	35 (43.8%)	22 (62.86%)	13 (37.14%)	
High (N,%)	41 (51.2%)	24 (58.54%)	17 (41.46%)	
Vitamin B12 - pg/mL (median; IQR)	1,695 (58,286.5)	1,446 (50,495)	1,918 (168,218)	0.388
Low (N,%)	1 (1.25%)	0 (0%)	1 (100.00%)	0.352
Normal (N,%)	22 (27.5%)	15 (68.18%)	7 (31.82%)	
High (N,%)	57 (71.25%)	34 (59.65%)	23 (40.35%)	
Homocysteine - μ mol/L (median; IQR)	20.85 (11.45)	20.7 (10.5)	22.2 (11.3)	0.122
Low (N,%)	0 (0%)	0 (0%)	0 (0%)	0.895
Normal (N,%)	20 (25%)	12 (60.00%)	8 (40.00%)	
High (N,%)	60 (75%)	37 (61.67%)	23 (38.33%)	

IQR, interquartile range.

Table 5. Subgroup analysis in subjects according to high sensitivity C-reactive protein.

Variables	hsCRP<3.0 n=31 (38.75%)		hsCRP>3.0 n=49 (61.25%)	
	r	p-value	r	p-value
Folic acid and hsCRP	-0.141	0.439	-0,059	0,686
Folic acid and homocysteine	-0.250	0.175	-0,561	<0,001*
Vitamin B12 and hsCRP	-0.445	0.012*	0,014	0,925
Vitamin B12 dan homocysteine	-0.205	0.269	-0,338	0,018*

hsCRP, high-sensitivity C Reactive Protein; *) p<0.05.

Ekhol et al.²⁵ in 2008 also demonstrated a high level of vitamin B12 in the dialysis population. Many things can contribute to the elevated levels of vitamin B12 in hemodialysis patients. First, the high dose (5,000 mcg/session) of intradialytic parenteral vitamin B12 and oral vitamin B12 supplementation among the dialysis population can not be removed by dialysis. Vitamin B12 (C63H88CoN14O14P) has a molecular weight of 1,355 Daltons.²⁶⁻²⁷ This molecule is categorized as middle molecules (500-15,000 Daltons) alongside insulin and β -2 microglobulin and therefore, it can not be removed by dialyzer.²⁷

Second, folic acid and vitamin B12 could be deposited in the body for an extended period (1-1.5 years for folic acid and 3-5 years for vitamin B12). Third, in chronic kidney disease, the production of transcobalamin II is decreased. Transcobalamin II protein functions as a vitamin B12 transporter in the small intestine.²⁸

Therefore, vitamin B12 uptake by tissues is reduced.^{10,29}

Elevated levels of hsCRP in the majority (61.3%) of the subjects are closely related to a chronic inflammatory state. This marker was chosen because of its consistency with cardiovascular risk, comparable with systolic blood pressure and total cholesterol.³⁰ A study by Bazeley et al. in chronic kidney disease patients in 10 countries showed an increase in hsCRP related to increased mortality.¹¹ Large cardiovascular studies, such as the JUPITER study, have shown that hsCRP >2mg/L is a strong indication of therapy, and a marked decrease in cardiovascular risk was observed after therapy.³⁰ We also found that hyperhomocysteinemia is prevalent in most hemodialysis patients (75%). Hyperhomocysteinemia is proven to be related to arterial thrombosis and atherosclerosis. A study by Pastore et al.³¹ showed that hyperhomocysteinemia occurs in more than

90% of dialysis patients.

We found that folic acid and vitamin B12 level was negatively correlated with homocysteine level, which is consistent with the results of a study conducted by Nand et al., who had such evaluation in the non-hemodialysis CKD population.⁷ In the subgroup analysis, we found a moderate correlation of folic acid and homocysteine in the group of patients with hsCRP levels of > 3.0 mg/dL. We also found a mild correlation between vitamin B12 and homocysteine levels. This phenomenon could describe the protective effect that could only be seen in high-risk patients.

Our study also showed that vitamin B12 level and hsCRP was negatively correlated in low-risk patients. There is speculation that some microelements decrease during inflammation. However, a previous study reported that inflammation has no impact on the level of vitamin B12.³¹ There is also inconsistency in the results between the two groups. In the low-risk group, there was negative correlation, while the high-risk group, had positive correlation even though not statistically significant.

Selection bias in this study is unlikely to occur because the inclusion and exclusion criteria were appropriate and consistently applied in this study. Likewise, with information bias and measurement bias, those biases are unlikely to occur in this study because the data and parameters studied were measured directly and obtained from medical records.

Theoretically, we did not find potential confounders in the relationship between the variables studied. Therefore, we did not perform multivariate analysis. However, we identified a modifier effect in cardiovascular risk, which we then performed subgroup analysis. There is a possibility that our findings were found by chance. This may be due to the relatively small sample size.

Moreover, we did not measure the duration of folic acid and vitamin B12 supplementation and the variation in folic acid and vitamin B12 supplementation and intake among patients. Further research is needed to confirm these findings, and a randomized controlled trial study is recommended to see biological plausibility and

time relationship.

Despite the above limitations, our study is the first study in Indonesia assessing the serum folic acid and vitamin b12 levels in the CKD population and their relationship with homocysteine and hsCRP levels. We had an 88.88% response rate which means our study can be implemented to our eligible population. However, generalization to all CKD patients needs cautions since we only included patients with stable conditions and had no comorbidity nor other conditions that can affect folic acid and B12 absorptions. In addition, we studied patients with CKD who underwent twice-weekly hemodialysis while previous studies were mostly done in thrice-weekly hemodialysis patients. We suggest conducting randomized, double-blind clinical trials to compare the benefits of each treatment.

CONCLUSION

In conclusion, there is a negative correlation between serum vitamin B12 and folic acid with homocysteine levels. Moreover, in a high cardiovascular risk subject, we found stronger correlations between folic acid and homocysteine levels and also vitamin B12 and homocysteine levels. Our findings suggest that supplementation of FA and vitamin B12 might more give benefit in high risk cardiovascular (hsCRP>3) hemodialysis patients

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper

FUNDING STATEMENT

Universitas Indonesia funded this study for the research grant under the PITTA (Internationally Indexed Publication for UI Student's Thesis) program.

REFERENCES

1. Xu X, Qin X, Li Y, et al. Efficacy of folic acid therapy on the progression of chronic kidney disease: the renal substudy of the china stroke primary prevention trial. *JAMA Intern Med* 2016;176(10):1443-50. doi: 10.1001/jamainternmed.2016.4687
2. Azalea M, Andayani TM, Satibi. Analisis biaya

- pengobatan penyakit ginjal kronis rawat inap dengan hemodialisis di rumah sakit. *Jurnal Manajemen dan Pelayanan Farmasi* 2016;6(2):141-50.
3. Suhardjono. Hemodialisis: prinsip dasar dan pemakaian kliniknya. In: Setiati S, Alwi I, Sudoyo AW, et al., eds. *Buku Ajar Ilmu Penyakit Dalam*. Jakarta: Interna Publishing 2014:2192-96.
 4. Jha V, Garcia-Garcia G, Iseki K, et al. Chronic kidney disease: global dimension and perspectives. *The Lancet* 2013;382(9888):260-72. doi: 10.1016/s0140-6736(13)60687-x
 5. Parfrey PS, Foley RN. The clinical epidemiology of cardiac disease in chronic renal failure. *J Am Soc Nephrol* 1999;10:1606-15.
 6. Team IRR. 10th report of Indonesian renal registry. Jakarta: Indonesian Renal Registry, 2018.
 7. Nand M, Sharma M, Mittal N. Prevalence of hyperhomocysteinaemia in chronic kidney disease and effect of supplementation of folic acid and vitamin B12 on cardiovascular mortality. *JACM* 2013;14(1):33-36.
 8. Tamadon MR, Jamshidi L, Soliemani A, et al. Effect of different doses of folic acid on serum homocysteine level in patients on hemodialysis. *Iran J Kidney Dis* 2011;5(2):93-96.
 9. Qin X, Huo Y, Langman CB, et al. Folic acid therapy and cardiovascular disease in ESRD or advanced chronic kidney disease: a meta-analysis. *Clin J Am Soc Nephrol* 2011;6(3):482-8. doi: 10.2215/CJN.05310610
 10. Amini M, Khosravi M, Baradaran HR, et al. Vitamin B12 supplementation in end stage renal disease: a systematic review. *MJIRI* 2015;29(167):1-8.
 11. Bazeley J, Bieber B, Li Y, et al. C-reactive protein and prediction of 1-year mortality in prevalent hemodialysis patients. *Clin J Am Soc Nephrol* 2011;6(10):2452-61. doi: 10.2215/CJN.00710111
 12. Li Y, Zhong X, Cheng G, et al. Hs-CRP and all-cause, cardiovascular, and cancer mortality risk: A meta-analysis. *Atherosclerosis* 2017;259:75-82. doi: 10.1016/j.atherosclerosis.2017.02.003
 13. Hwang YC, Morrow DA, Cannon CP, et al. High-sensitivity C-reactive protein, low-density lipoprotein cholesterol and cardiovascular outcomes in patients with type 2 diabetes in the EXAMINE (Examination of Cardiovascular Outcomes with Alogliptin versus Standard of Care) trial. *Diabetes Obes Metab* 2018;20(3):654-59. doi: 10.1111/dom.13136
 14. Hassan K. Association of low potassium diet and folic acid deficiency in patients with CKD. *Ther Clin Risk Manag* 2015;11:821-7. doi: 10.2147/TCRM.S83751
 15. Jamison RL, Hartigan P, Kaufman JS, et al. Effect of homocysteine lowering on mortality and vascular disease in advanced chronic kidney disease and end-stage renal disease: A randomized controlled trial. *JAMA* 2007;298(10):1163-70. doi: 10.1001/jama.298.10.1163
 16. Jardine MJ, Kang A, Zoungas S, et al. The effect of folic acid based homocysteine lowering on cardiovascular events in people with kidney disease: systematic review and meta-analysis. *BMJ* 2012;344:e3533. doi: 10.1136/bmj.e3533
 17. Wrono EM. Randomized Trial of Folic Acid for Prevention of Cardiovascular Events in End-Stage Renal Disease. *Journal of the American Society of Nephrology* 2004;15(2):420-26. doi: 10.1097/01.asn.0000110181.64655.6c
 18. Chang TY, Chou KJ, Tseng CF, et al. Effects of folic acid and vitamin B complex on serum C-reactive protein and albumin levels in stable hemodialysis patients. *Curr Med Res Opin* 2007;23(8):1879-86. doi: 10.1185/030079907X218077
 19. Heinz J, Kropf S, Domrose U, et al. B vitamins and the risk of total mortality and cardiovascular disease in end-stage renal disease: results of a randomized controlled trial. *Circulation* 2010;121(12):1432-8. doi: 10.1161/CIRCULATIONAHA.109.904672
 20. Righetti M, Serbelloni P, Milani S, et al. Homocysteine-lowering vitamin B treatment decreases cardiovascular events in hemodialysis patients. *Blood Purif* 2006;24(4):379-86. doi: 10.1159/000093680
 21. Righetti M, Ferrario G, Milani S, et al. Effects of folic acid treatment on homocysteine levels and vascular disease in hemodialysis patients. *Med Sci Monit* 2003;9(4):37-42.
 22. Vianna ACA, Mocelin AJ, Matsuo T, et al. Uremic hyperhomocysteinemia: A randomized trial of folate treatment for the prevention of cardiovascular events. *Hemodial Int* 2007;11(2):210-16. doi: 10.1111/j.1542-4758.2007.00171.x
 23. Trimarchi H, Schiel A, Freixas E, et al. Randomized trial of methylcobalamin and folate effects on homocysteine in hemodialysis patients. *Nephron* 2002;91:58-63.
 24. Hulley SB, Cummings SR, Browner WS, Grady D, Newman TB. *Designing clinical research : an epidemiologic approach*. 4th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2013. Appendix 6C, page 79
 25. Fehrman-Ekholm I, Lotsander A, Logan K, et al. Concentrations of vitamin C, vitamin B12 and folic acid in patients treated with hemodialysis and on-line hemodiafiltration or hemofiltration. *Scand J Urol Nephrol* 2008;42(1):74-80. doi: 10.1080/00365590701514266
 26. Information NCfB. Vitamin B12, CID=25102581. PubChem Database, 2019.
 27. Azar A, Canaud B. Hemodialysis system. In: Azar AT, ed. *Modelling and control of dialysis systems*. Berlin: Springer-Verlag 2013:99-166.
 28. Clase CM, Ki V, Holden RM. Water-soluble vitamins in people with low glomerular filtration rate or on dialysis: a review. *Semin Dial* 2013;26(5):546-67. doi: 10.1111/sdi.12099
 29. Soohoo M, Ahmadi SF, Qader H, et al. Association of serum vitamin B12 and folate with mortality in incident

- hemodialysis patients. *Nephrol Dial Transplant* 2017;32(6):1024-32. doi: 10.1093/ndt/gfw090
30. Yousuf O, Mohanty BD, Martin SS, et al. High-sensitivity C-reactive protein and cardiovascular disease: a resolute belief or an elusive link? *J Am Coll Cardiol* 2013;62(5):397-408. doi: 10.1016/j.jacc.2013.05.016
 31. Pastore A, Angelis SD, Casciani S, et al. Effects of folic acid before and after vitamin B12 on plasma homocysteine concentrations in hemodialysis patients with known MTHFR genotypes. *Clin Chem* 2006;52(1):145-48.

Correlation of Serum Ferritin and Cardiac Iron Toxicity with Cardiac Function in Transfusion Dependent Beta-Thalassemia Major Patients

Tubagus Djumhana Atmakusuma¹, Rajesh Kalwani², Sally Aman Nasution³, Cleopas Martin Rumende⁴

¹Division of Hematology-Medical Oncology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

²Department of Internal Medicine, Pantai Indah Kapuk Hospital, Jakarta, Indonesia.

³Division of Cardiology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia.

⁴Division of Respiriology and Critical Care, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia.

Corresponding Author:

Tubagus Djumhana Atmakusuma, MD, PhD. Division of Hematology-Medical Oncology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Dr. Cipto Mangunkusumo Hospital. Jalan Diponegoro no. 71, Jakarta 10430, Indonesia. Email: hom_fkui@yahoo.com.

ABSTRAK

Latar Belakang: Toksisitas besi pada jantung merupakan penyebab utama kelainan jantung pada pasien-pasien thalassemia. Modalitas utama untuk mendeteksi toksisitas besi pada jantung adalah dengan magnetic resonance imaging (MRI) T2* yang ketersediaannya terbatas. Penelitian ini bertujuan untuk mengetahui korelasi antara serum ferritin dan toksisitas besi pada jantung dengan fungsi jantung (fraksi ejeksi dan rasio E/A) pada penderita thalassemia beta bergantung transfusi. **Metode:** Penelitian potong lintang dilakukan di Poliklinik Thalassemia Dewasa Rumah Sakit Cipto Mangunkusumo, Indonesia dari Desember 2017 hingga Maret 2018. Analisis statistik menggunakan uji korelasi Pearson atau Spearman antara nilai MRI T2* dengan fraksi ejeksi dan rasio E/A. **Hasil:** Median dari rerata 4 bulan kadar ferritin serum adalah 5130 ng/mL. Rerata nilai MRI T2* jantung adalah 24,96 ms. Hemosiderosis jantung berat terjadi pada 11,3 % subjek. Kadar ferritin serum berkorelasi lemah dengan toksisitas besi pada jantung ($r=-0,272$, $p=0,032$) dan fraksi ejeksi ($r=-0,283$, $p=0,026$), tetapi toksisitas besi pada jantung tidak berkorelasi dengan fraksi ejeksi dan rasio E/A. **Kesimpulan:** Kadar ferritin serum berkorelasi lemah dengan toksisitas besi dan fungsi sistolik jantung, tetapi toksisitas besi pada jantung tidak berkorelasi dengan fungsi sistolik dan diastolik jantung.

Kata kunci: Toksisitas besi pada jantung, ferritin serum, fungsi sistolik, fungsi diastolik, MRI T2* jantung.

ABSTRACT

Background: Cardiac iron toxicity is a major cause of mortality in transfusion-dependent beta-thalassemia major patients. The main modality for detecting cardiac iron toxicity is MRI T2* with limited availability. This study aims to obtain iron toxicity profiles in transfusion-dependent beta-thalassemia patients; to see a correlation between iron toxicity and cardiac function. **Methods:** We conducted a cross-sectional study at the Adult Thalassemia Polyclinic of Cipto Mangunkusumo Hospital, Indonesia from December 2017 to March 2018. We performed the statistical analysis using Pearson/Spearman Test comparing MRI T2* values with ejection

fraction and E/A ratio. Results: The median of 4-months mean of ferritin levels was 5130 ng/mL. The mean for cardiac T2* was 24.96 ms. Severe cardiac hemosiderosis (mean cardiac MRI T2* < 10 ms) occurred in 11.3% of the subjects. There was weak correlation between serum ferritin with cardiac iron toxicity ($r=-0.272$, $p=0.032$) and ejection fraction ($r=-0.281$, $p=0.013$). But, there was no correlation between cardiac iron toxicity with ejection fraction and E/A ratio. **Conclusion:** Serum ferritin correlated weakly with cardiac iron toxicity and cardiac systolic function. Meanwhile, there was no correlation between cardiac iron toxicity with cardiac systolic and diastolic function.

Keywords: cardiac iron toxicity, serum ferritin, systolic function, diastolic function, cardiac MRI T2*.

INTRODUCTION

Thalassemia is one of the most common genetic disorders, affecting 4.83% of the population worldwide.¹ Advances in the treatment of thalassemia major has resulted in better prognoses of the patients. Therefore, those with thalassemia major could survive up to third or fourth decade of life. A long-term study reported that 68% of patients with thalassemia major could survive to their mid-30s.^{2,3} Severe anaemia in thalassemia major requires regular transfusion since early age.⁴ Regular transfusion leads to an improvement in the quality of life; however, it results in new problems; these include iron overload, which may cause cardiac disease, endocrinopathy, and liver disease.⁵

Cardiac disease could be manifested by cardiomyopathy. Cardiomyopathy due to iron overload is the leading cause of death in thalassemia major patients. In several countries, this mortality may be up to 70%.⁵ Cardiac siderosis usually progresses without symptoms for several years. The onset of symptoms of heart failure is associated with a high likelihood of mortality. The time between the onset of symptoms and death is usually quite short and ranges from 6 - 12 months. If patients with cardiac iron accumulation can be identified early, this would be very helpful because chelation therapy could be started aggressively.^{6,7} Several international publications showed that cardiac iron toxicity in various countries vary greatly, with a range of 30-85%.⁸ During the Coronavirus 2019 (COVID-19) pandemic, people with haemoglobin disorders, including thalassemia are considered high risk for severe disease.⁹ Mortality in COVID-19 is also linked to cardiovascular disease. Therefore, assessment

of cardiac function is critical in determining the prognosis of such patients.

Cardiac magnetic resonance imaging (MRI) T2* is the recommended modality for detecting iron toxicity and is the only non-invasive way to identify the severity of iron deposition in the myocardium. The cardiac T2* is the time taken for the signal decay from the myocardium and is expressed in milliseconds. The more iron deposits in the myocardium the lower the value of T2*. Thus, the more severe the cardiac iron toxicity the lower the cardiac T2* value. The values used as a reference are <10ms as severe hemosiderosis, 10 - 20ms as mild to moderate hemosiderosis, and normal if T2*>20ms. In addition to diagnosing iron toxicity, cardiac MRI T2* is also used to determine and monitor iron chelation therapy. Cardiac MRI T2* can also be used to predict the occurrence of heart failure. A value of less than 10 ms predicts heart failure, while a value of more than 20 ms predicts that heart failure will not occur for the following year. A Cardiac MRI T2* is recommended once a year to assess iron toxicity and effectiveness of iron chelation.^{10,11} However, in Indonesia, the MRI T2* examination is only available in four centres, i.e. Aceh, Jakarta, Bandung and Surabaya (three of them in the Java Island). MRI is not available in the central and eastern region of Indonesia. Therefore, some centres utilize serum transferrin saturation and ferritin as surrogate markers of iron overload.

Cardiac iron toxicity is associated with cardiac dysfunction. Echocardiography can give a picture of the patient's systolic and diastolic function. The systolic function is assessed from the left ventricular ejection fraction. An ejection fraction below 50% indicates impaired

ventricular contractility. Damage caused by iron mediated generation of free radicals is one of the main mechanism of cardiomyocyte damage.¹² The higher the cardiac iron toxicity, the lower the ejection fraction or left ventricular systolic function and the higher the risk of heart failure.^{11,13} Some echocardiographic parameters can be used to assess diastolic function. One of them is the blood flow through the mitral valve, which is the ratio of the blood flow velocity in the early diastolic phase to the advanced diastolic phase or known as the E / A ratio.¹⁴ According to several studies, diastolic dysfunction occurs earlier than systolic dysfunction in adult transfusion dependent thalassemia patients.¹⁴ Echocardiography is a simple bedside diagnostic procedure which is available in every regional hospital. The device is portable, lightweight, and can be operated even in remote healthcare facilities. In addition, echocardiography can detect diastolic dysfunction, which is known to be related to cardiac siderosis.¹⁵

Limited availability of MRI T2* inspired to study correlation of serum ferritin and cardiac iron toxicity with cardiac function; systolic and diastolic in adults with transfusion-dependent beta thalassemia major. This study describes data of Thalassemia's cardiac function patients in Indonesia because differences in transfusion practices such as inadequate transfusion due to donor shortage might interfere with cardiac function by causing anemic heart disease.

METHODS

This was a cross-sectional study of 62 patients with transfusion-dependent beta thalassema major, conducted by evaluating the medical records of beta-thalassemia patients ≥ 18 years of age who were transfusion dependent and who came for a regular check at the Adult Thalassemia Polyclinic, Department of Internal Medicine, Cipto Mangunkusumo Hospital, Jakarta, Indonesia. The study was conducted from December 2017 to March 2018.

The diagnosis of thalassemia had been made with high-performance liquid chromatography / HPLC or microcapillary method included in this study. We excluded patients who had acute inflammation (temperature $> 38^{\circ}\text{C}$), congenital

heart abnormalities, acute heart failure, and claustrophobia.

The parameters that were used to determine ferritin level, the iron toxicity, systolic function, and diastolic function were cardiac MRI T2*, ejection fraction and E/A ratio. The mean ferritin serum was obtained from medical records for four months and categorized into values of $<1000\text{ng / mL}$, $1000\text{-}2500\text{ ng / mL}$, and $>2500\text{ ng / mL}$. MRI T2* values were grouped as severe hemosiderosis (T2* $<10\text{ ms}$), mild-moderate hemosiderosis (T2* $10\text{-}20\text{ ms}$) and normal (T2* $>20\text{ ms}$). The E/A ratio was categorized as mild, moderate and severe diastolic dysfunction (E / A <1 ; $1\text{-}2$; and >2).

Data collection was processed by using the SPSS 20 program for Windows. The analysis was performed by testing the Pearson conversion coefficient (r) for normal distribution data or Spearman conversion coefficient (rho) for abnormal data distribution. All patients were given an oral and written explanation of the objectives, benefits, and research procedures. All patients agreed and gave their informed consent. This study was approved by the Ethical Committee of Faculty of Medicine, Universitas Indonesia.

RESULTS

Sixty-two patients who had undergone MRI and echocardiography were willing to take part in the study, 26 (41.6%) were females and 36 (58.1%) were male. The median age was 22 (18-39) years. The number of patients with beta-thalassemia was 34 (54.8%) and beta-thalassemia HbE was 28 (45.2%). The median of 4-months mean of ferritin level was 5130 ng/mL (741-18605). The number of patients with serum ferritin above 2500 ng / mL was 52 (83.9%), 1000-2500 ng / mL was 9 (14.5%), and under 1000ng / mL was 1 (1, 6%). Iron overload (Transferin saturation $\geq 50\%$) was found in 59 patients (95.2%). Twenty-nine patients used Deferiprone; five used Deferasirox, five used Deferoxamine and 23 used combination iron chelators. (Table 1)

The mean value of cardiac MRI T2* was 24.96 ms. Cardiac iron toxicity (Cardiac MRI T2* $< 10\text{ ms}$ and $10\text{-}20\text{ ms}$) was seen in 38.7%

patients. Mild-moderate hemosiderosis (Cardiac MRI T2* 10-20 ms) was found in 27.4% patients. Severe hemosiderosis (Cardiac MRI T2* < 10 ms) was found in 11.3% patients. The mean ejection fraction was 67.55 %. Mean Diastolic dysfunction E/A 1-2 and >2 was seen in all patients, 57 (91,9%) patients with moderate diastolic dysfunction and five patients with severe diastolic dysfunction E/A > 2 (**Table 1**).

Correlation Between Cardiac Iron Toxicity with Cardiac Function

There was no correlation between cardiac T2* and ejection fraction ($r = -0.076$, $p = 0.556$). There was no correlation between cardiac T2* and E/A ratio ($r = -0.136$, $p = 0.292$). Thus, there was no correlation between cardiac iron toxicity with cardiac function (**Figure 1**).

Correlation Between Iron Overload with Iron Toxicity, Systolic Function, and Diastolic Function

There was a weak negative correlation between serum ferritin and MRI T2* ($r = -0.272$, $p = 0.032$). However, no correlation was found between transferrin saturation and MRI T2* values ($r = -0.084$, $p = 0.514$). (**Figure 2**)

This study also found weak negative correlation between ferritin and ejection fraction ($r = -0.283$, $p = 0.026$). There was no correlation between transferrin saturation and ejection fraction ($r = 0.097$, $p = 0.453$). (**Figure 3**) Also, no correlation was found between ferritin and transferrin saturation with diastolic function ($r = 0.073$, $p = 0.575$ and $r = 0.009$, $p = 0.946$, respectively). (**Figure 4**)

Table 1. Clinical, biochemical and cardiac characteristics of the patients of Thalassemia β -dependent transfusion.

Characteristics	N= 62
Sex, n (%)	
- Male	36 (58.1)
- Female	26 (41.6)
Age (years), median (min-max)	22 (18-39)
Genotype diagnosis, n (%)	
- Beta-thalassemia	34 (54.8)
- Thalassemia beta/HbE	28 (45.2)
Pre-transfusion haemoglobin, Mean (SD)	8.51 (1.23)
Transferrin saturation (%), median (min-max)	100 (34-107)
- $\geq 50\%$, n (%)	59 (95.2)
- $< 50\%$, n (%)	3 (4.8)
4 Months-mean of Ferritin serum,(ng/mL), median (min-max)	5130 (741-18605)
- < 1000 ng/mL, n (%)	1 (1.6)
- 1000-2500 ng/mL, n (%)	9 (14.5)
- > 2500 ng/mL, n (%)	52 (83.9)
Iron chelation, n (%)	
- Deferiprone	29 (46.8)
- Deferasirox	5 (8.1)
- Deferoxamine	5 (8.1)
- Combination	23 (37.1)
Cardiac MRI T2*, mean (SD)	24.96 (12.13)
- < 10 ms	7 (11.3)
- 10-20 ms	17 (27.4)
- > 20 ms	38 (61.8)
Ejection fraction, mean (SD)	67.55 (6.50)
E/A ratio	1.51 (1.10-2.70)
E/A category, n (%)	
- < 1	0 (0)
- 1-2	57 (91.9)
- > 2	5 (8.1)

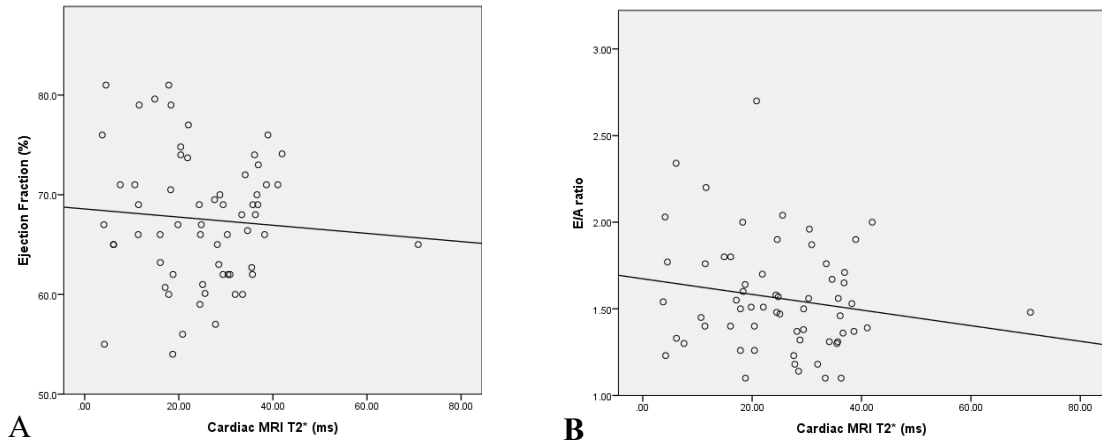


Figure 1. Correlation Between Cardiac Iron Toxicity with Ejection Fraction (A) and E/A ratio (B)

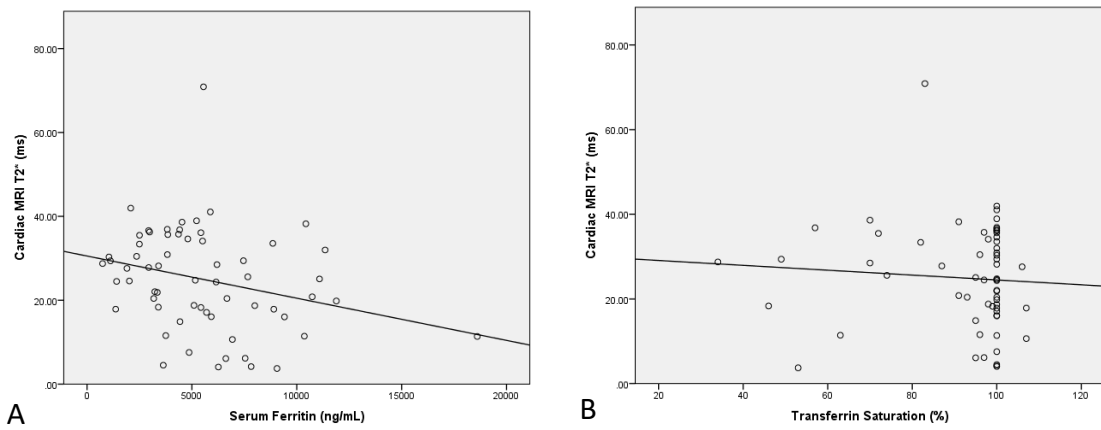


Figure 2. Correlation Between serum ferritin with MRI T2* (A) and transferrin saturation with MRI T2* values (B)

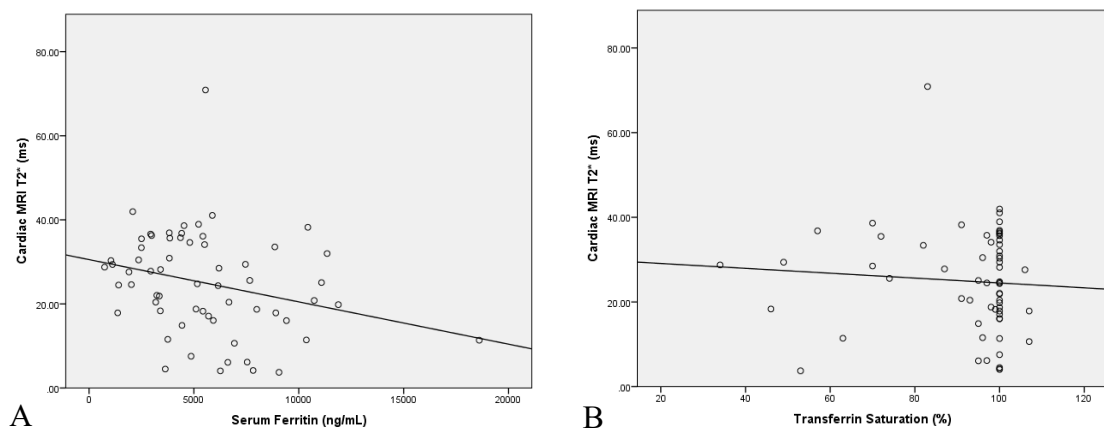


Figure 3. Correlation Between serum ferritin with ejection fraction (A) and transferrin saturation with ejection fraction (B)

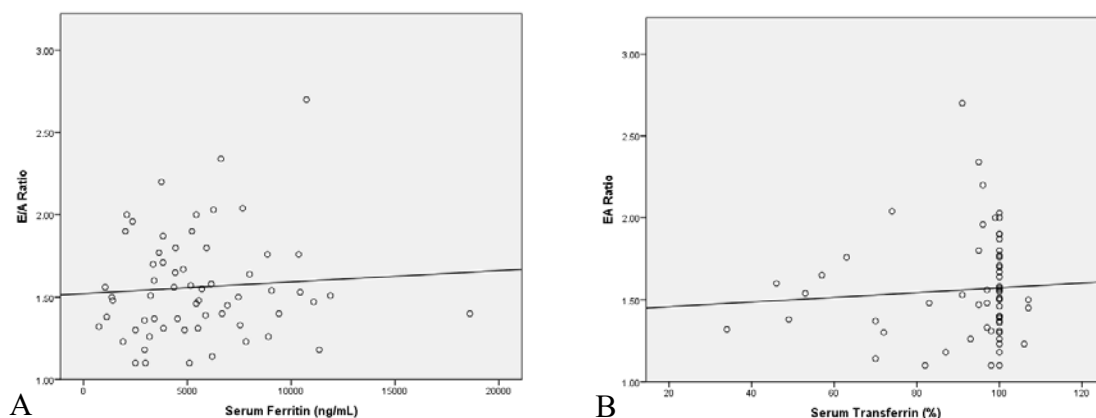


Figure 4. Correlation Between serum ferritin with diastolic function (A) and transferrin saturation with diastolic function (B)

DISCUSSION

The median of 4-months ferritin serum in this study was 5130 ng/mL. Amrita et al¹⁶ in 2020 found that the mean ferritin level in transfusion-dependent beta thalassemia in Surabaya was 5137.8 ng/mL and the median age was 23 years old, which is almost exactly identical to this study because study was done in same country. The Turkish Study found less than both study (2,072 ng/mL).¹⁷

This study showed 38.7% subjects had mean cardiac T2* <20 ms. Studies in other countries showed 30-85% subjects had mean cardiac T2* <20 ms.⁸ Patton et al showed 74% subjects had the cardiac T2* < 20 ms, while a study in Thailand showed 7.8% subjects had abnormal cardiac T2* MRI values because 12.1% of the subjects were non-transfusion dependent.¹⁸

The mean ejection fraction of this study was 67.55 % which was almost as similar as those in a study by Chinprateep, et al¹⁸ that showed the mean ejection fraction of 63.59%. Chinprateep et al.¹⁸ showed slightly lower ejection fraction values than this study (63.59% vs 67.55%) because the mean age of the subjects was older than this study (27.5 years vs 22 years).

All of the subjects in this study had impairment of diastolic function though only 38.7% subjects had abnormal cardiac MRI T2*. Chinprateep, et al¹⁸ found 89.5% subjects with mean age of 27.5 years old already had diastolic dysfunction but only 7.2% subjects had had abnormal cardiac MRI T2*. This data proved that diastolic dysfunction has happened before

abnormal cardiac MRI T2*.¹⁸ Therefore, diastolic dysfunction can be used for early detection of iron toxicity.

There was a weak correlation between serum ferritin and cardiac MRI T2* ($r=-0.272$, $p=0.032$) in this study. Study by Yuksel et al.¹⁷ found moderate negative correlation between ferritin and cardiac MRI T2* values ($r=-0.34$, $p=0.009$). The Turkish study got moderate correlation. Patton et al¹⁹ also showed a moderate negative correlation between serum ferritin and cardiac MRI T2*.

No correlation between cardiac MRI T2* and ejection fraction was found in this study. Patton, et al¹⁹ and Alpendurada, et al²⁰ showed a correlation between iron toxicity and systolic cardiac function, however mean T2* was lower (13.4 and 18.4 ms) and hemosiderosis was seen in more than 50% of study patients. Patton, et al¹⁹ found that marked decrease in cardiac function was found in subjects with cardiac MRI T2* <10 ms, indicated by median ejection fraction of 49%. According to Penell, et al^{8,21}, the ejection fraction would decline at an advanced stage of cardiac hemosiderosis. The value of MRI T2* and cardiac function has a weak correlation until it reaches its critical values when the deterioration of cardiac function occurs rapidly.¹⁹ Approximately 61,8% of subjects of this study has normal T2* value, therefore mean of EF of this study was normal. This study failed to show correlation between iron toxicity and cardiac function, particularly ejection fraction. Cardiac failure in thalassemia patients, caused

by systolic dysfunction, occurs at the late stage of the disease.

This study showed no correlation between iron toxicity and diastolic dysfunction. There were no subjects who had normal diastolic dysfunction in this study. However, several other studies showed iron toxicity and diastolic dysfunction correlation. Djer et al²² found a correlation between MRI T2 * and the E / A ratio. Aessopos et al²³ also showed a weak correlation between MRI T2 and E / A *. Diastolic dysfunction, however, occurs earlier than systolic dysfunction.¹⁴

This study showed a weak correlation between serum ferritin and left ventricular ejection fraction. The same as this study, Akpınar et al²⁰ showed a weak correlation between both. But this study didn't show correlation between serum ferritin and diastolic function because there were no subject who had normal diastolic function. Nisli et al²⁴ and Akpınar et al²⁰ found a correlation between serum ferritin and diastolic function, compared the diastolic function with healthy control subjects. This is due to the fact that from the three different forms of iron in myocytes, labile cellular iron (LCI) is the most toxic. It is only when ferritin converts to LCI that it leads to impaired contractility and congestive cardiac failure. Besides that immunoinflammatory and genetic factors also contribute such as myocarditis, the HLA genotype and apolipoprotein E genotype.¹¹

This study showed correlation between serum ferritin level and iron toxicity and correlation between serum ferritin level and systolic function, but did not show correlation between iron toxicity and cardiac function. Learning from other studies about iron toxicity and cardiac function, this correlation depends on the proportion of subjects with cardiac iron toxicity. More proportion of subjects with cardiac iron toxicity means stronger correlation to systolic dysfunction. But, correlation between iron toxicity and diastolic dysfunction would be marked if the proportion of subjects with iron toxicity was lesser because diastolic dysfunction has happened before severe iron toxicity.

Limitation of the Study

This study was a correlation study that did not account for other factors that contribute to cardiac dysfunction in Thalassemia patients beyond iron toxicity. The results from several studies can be concluded that cardiac toxicity in Thalassemia patients were influenced by other factors beyond iron toxicity. Studies that involve many factors beyond iron toxicity should be done.

CONCLUSION

Serum ferritin correlated weakly with iron toxicity and ejection fraction but did not correlate with diastolic function. There is no correlation between cardiac iron toxicity with cardiac systolic and diastolic function.

ACKNOWLEDGMENTS

We gratefully acknowledge Utami Susilowati for her contribution to statistical analysis. We also thank the participating centers, doctors, and staff who assisted in contributing patient data to this study: Adult Thalassemia Polyclinic of Cipto Mangunkusumo Hospital.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

REFERENCES

1. Rund D, Rachmilewitz E. Beta-thalassemia. *N Engl J Med.* 2005;353(11):1135-46.
2. Derchi G, Formisano F, Balocco M, et al. Clinical management of cardiovascular complications in patients with thalassaemia major: a large observational multicenter study. *Eur J Echocardiogr.* 2011;12(3):242-6.
3. Borgna-Pignatti C, Rugolotto S, De Stefano P, et al. Survival and disease complications in thalassemia major. *Ann N Y Acad Sci.* 1998;850:227-31.
4. Pepe A, Meloni A, Rossi G, et al. Cardiac and hepatic iron and ejection fraction in thalassemia major: multicentre prospective comparison of combined deferiprone and deferoxamine therapy against deferiprone or deferoxamine monotherapy. *J Cardiovasc Magn Reson.* 2013;15(1):1.
5. Borgna-Pignatti C, Cappellini M, De Stefano P, et al. Survival and complications in thalassemia. *2005;1054(1):40-7.*

6. Ladis V, Chouliaras G, Berdousi H, Kanavakis E, Kattamis C. Longitudinal study of survival and causes of death in patients with thalassemia major in Greece. *Ann N Y Acad Sci.* 2005;1054:445-50.
7. Musallam KM, Cappellini MD, Wood JC, Taher AT. Iron overload in non-transfusion-dependent thalassemia: a clinical perspective. *Blood Rev.* 2012;26 Suppl 1:S16-9.
8. Pennell DJ, Udelson JE, Arai AE, et al. Cardiovascular function and treatment in β -thalassemia major: a consensus statement from the American Heart Association. *Circulation.* 2013;128(3):281-308.
9. Eleftheriou A, Cannon L, Angastiniotis M. Thalassaemia prior and consequent to COVID-19 pandemic. The perspective of Thalassaemia International Federation (TIF). *Thalassaemia Reports.* 2020;10(1).
10. Aessopos A, Kati M, Tsironi M. Congestive heart failure and treatment in thalassemia major. *Hemoglobin.* 2008;32(1-2):63-73.
11. Aessopos A, Berdoukas V, Tsironi M, Ejuh. The heart in transfusion dependent homozygous thalassaemia today—prediction, prevention and management. 2008;80(2):93-106.
12. Gammella E, Recalcati S, Rybinska I, Buratti P, Cairo G. Iron-induced damage in cardiomyopathy: Oxidative-dependent and independent mechanisms. *Oxidative Medicine and Cellular Longevity.* 2015;2015:230182.
13. Nagueh SF, Appleton CP, Gillebert TC, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. *J Am Soc Echocardiogr.* 2009;22(2):107-33.
14. Kremastinos DT, Tsiapras DP, Kostopoulou AG, Hamodraka ES, Chaidaroglou AS, Kapsali ED, Ejuh. NT-proBNP levels and diastolic dysfunction in β -Thalassaemia major patients. 2007;9(5):531-6.
15. Moussavi F, Ghasabeh MA, Roodpeyma S, et al. Optimal method for early detection of cardiac disorders in thalassemia major patients: magnetic resonance imaging or echocardiography? *Blood Res.* 2014;49(3):182-6.
16. Amrita PNA, Bintoro SUY, Sedana MP, et al. Serum ferritin level affects T lymphocyte CD4, CD8, and CD4/CD8 ratio in transfusion-dependent beta-thalassemia. *Drug Invention Today.* 2020;13(6):887-92.
17. Yuksel IO, Koklu E, Kurtoglu E, et al. The association between serum Ferritin level, tissue doppler echocardiography, cardiac T2* MRI, and heart rate recovery in patients with beta thalassemia major. *Acta Cardiol Sin.* 2016;32(2):231-8.
18. Chinprateep B, Ratanasit N, Kaolawanich Y, et al. Prevalence of left ventricular diastolic dysfunction by cardiac magnetic resonance imaging in thalassemia major patients with normal left ventricular systolic function. *BMC Cardiovasc Disord.* 2019;19(1):245.
19. Patton N, Brown G, Leung M, et al. Observational study of iron overload as assessed by magnetic resonance imaging in an adult population of transfusion-dependent patients with β thalassaemia: significant association between low cardiac T2* < 10 ms and cardiac events. 2010;40(6):419-26.
20. Akpınar O, Acartürk E, Kanadaşı M, Ünsal Ç, Başlamışlı F. Tissue doppler imaging and NT-proBNP levels show the early impairment of ventricular function in patients with β -thalassaemia major. 2007;62(3):225-31.
21. Djer MM, Anggriawan SL, Gatot D, Amalia P, Sastroasmoro S, Widjaja P. Correlation between T2* cardiovascular magnetic resonance with left ventricular function and mass in adolescent and adult major thalassemia patients with iron overload. *Acta Med Indones.* 2013;45(4):295-301.
22. Kremastinos DT, Farmakis D. Iron overload cardiomyopathy in clinical practice. *Circulation.* 2011;124(20):2253-63.
23. Aessopos A, Giakoumis A, Fragodimitri C, et al. Correlation of echocardiography parameters with cardiac magnetic resonance imaging in transfusion-dependent thalassaemia major. 2007;78(1):58-65.
24. Nisli K, Taner Y, Naci O, et al. Electrocardiographic markers for the early detection of cardiac disease in patients with beta-thalassemia major. *J Pediatr (Rio J).* 2010;86(2):159-62.

Internal Medicine Residents' Perceptions of Point-of-Care Ultrasound in Residency Program: Highlighting the Unmet Needs

Martha Rosana¹, Oke Dimas Asmara¹, Rabbinu Rangga Pribadi^{1,2},
Kemal Fariz Kalista¹, Kuntjoro Harimurti¹

¹Department of Internal Medicine, Faculty of Medicine Universitas Indonesia, Cipto Mangunkusumo National General Hospital, Jakarta, Indonesia

²Clinical Simulation and Competency Improvement Unit/Indonesian Clinical Training and Education Centre (ICTEC), Faculty of Medicine Universitas Indonesia, Cipto Mangunkusumo National General Hospital, Jakarta, Indonesia

Corresponding Author:

Kuntjoro Harimurti, MD., PhD. Department of Internal Medicine, Faculty of Medicine Universitas Indonesia, Cipto Mangunkusumo National General Hospital, Jl. Pangeran Diponegoro No. 71, Kenari, Senen, Central Jakarta, Jakarta 10430, Indonesia. Email: kuntjoro.harimurti@gmail.com.

ABSTRAK

Latar belakang: Point-of-care ultrasound (POCUS) mulai banyak digunakan dalam bidang Ilmu Penyakit Dalam. Pelatihan Ultrasonografi telah menjadi bagian dari Program Pendidikan Dokter Spesialis (PPDS) Ilmu Penyakit Dalam. Tujuan penelitian ini adalah untuk menilai persepsi peserta PPDS mengenai kompetensi dan kebutuhan akan POCUS. **Metode:** Peneliti melakukan studi deskriptif potong-lintang dengan menggunakan data sekunder dari survei internal peserta PPDS IPD di Fakultas Kedokteran Universitas Indonesia. Survei tersebut dilakukan untuk evaluasi dan perbaikan pada PPDS. **Hasil:** 175 dari 197 (88,8%) peserta PPDS mengikuti survei. 99,4% subjek setuju POCUS merupakan ketrampilan yang harus dimiliki selama PPDS. Namun hanya 40% subjek menilai mereka mampu melaksanakan POCUS. Enam puluh dua persen peserta PPDS menyatakan durasi pelatihan USG selama PPDS belum cukup. Subjek menilai ketrampilan yang paling penting ialah USG hepatobilier, paru, jantung, ginjal dan tindakan yang dipandu USG. **Kesimpulan:** Penelitian ini menyatakan bahwa pelatihan POCUS dibutuhkan oleh peserta PPDS IPD karena mayoritas dari subjek menyatakan bahwa mereka tidak mampu melaksanakan USG. Optimalisasi pelatihan yang telah ada harus dilakukan untuk memastikan kecakapan peserta PPDS.

Kata kunci: Ilmu penyakit dalam, program pendidikan dokter spesialis, ultrasonografi.

ABSTRACT

Background: Point-of-care ultrasound (POCUS) is increasingly utilized in internal medicine field. Ultrasound training has been part of the training provided in the Internal Medicine Residency Program (IMRP). The aim of this study is to examine the residents' needs and perceptions regarding their competency and needs of POCUS. **Methods:** We conducted a cross-sectional descriptive study using secondary data from a survey among internal medicine residents at the Faculty of Medicine Universitas Indonesia, Cipto Mangunkusumo National General Hospital. The survey was conducted to evaluate and improve the curriculum in IMRP. **Results:** A total of 175 out of 197 residents (88.8%) completed the questionnaire. Almost all the residents (99.4%) agreed that POCUS is a beneficial must-have skill during residency. Nevertheless, only 40% of residents identified themselves as competent in POCUS. Sixty-two percent residents reckoned that the training received during the residency program is insufficient.

They indicated that the most useful skills are US of hepatobiliary, lungs, heart, kidneys, and US-guided procedures.

Conclusion: *This study confirms that there is a need for POCUS training for IM residents, as the majority perceived themselves as unable to perform US. It is necessary to optimize the training provided to ensure proficiency.*

Keywords: *Internal medicine, residency, ultrasonography.*

INTRODUCTION

Point-of-care ultrasound (POCUS) is increasingly used in many specialties, including internal medicine (IM). The interesting aspect of POCUS is its application in point-of-care or bedside settings. This makes POCUS a goal-directed and focused tool in answering brief yet important clinical questions regarding the patients' symptoms.¹ Recent data shows that POCUS improves diagnostic accuracy and timeliness, shortens the time to definitive therapy, and reduces the risk of blind procedures.¹ Furthermore, POCUS can be used in various clinical scenarios, not only in emergency, but also in ward and outpatient clinic settings.

For internists, POCUS is an emerging and relatively new skill in daily clinical practice. Nevertheless, the Indonesian College of Internal Medicine requires internists to be competent in abdominal ultrasounds and various emergency scenarios that would benefit from ultrasound (US) use. To achieve that competence, US training has already become part of the training provided in the Internal Medicine Residency Program (IMRP), Faculty of Medicine Universitas Indonesia and Cipto Mangunkusumo National General Hospital. However, there is limited data about the optimal content and duration of the training program. To date, in Indonesia, no data has been published the IM residents' perception of the need for and competence acquisition of US skills during residency training.

An online survey regarding residents' perception of ultrasonography training was conducted at our institution by the IMRP during February-March 2020. The primary objective of the survey was to evaluate and improve the overall curriculum in IMRP. We carried out a descriptive study presenting secondary data analysis of the survey to further investigate the residents' needs and aspiration regarding ultrasound training. In the future, the results

of this study are expected to form the basis for the development and formulation of a POCUS training program, as well as a US curriculum for IM residents at the Faculty of Medicine Universitas Indonesia, Cipto Mangunkusumo National General Hospital.

METHODS

We conducted a cross-sectional descriptive study using secondary data from a survey among IM residents at the Faculty of Medicine Universitas Indonesia, Cipto Mangunkusumo National General Hospital. The survey was organized in February-March 2020 by the IMRP at the Faculty of Medicine Universitas Indonesia. The survey was part of the evaluation and improvement program for overall curriculum in IMRP. The content of the questionnaire was developed by three researchers: an attending physician from the IM department, and two chief IM residents.

The survey was disseminated on February 18, 2020, using an online survey tool (www.docs.google.com/forms). All IM residents from the Faculty of Medicine Universitas Indonesia, Cipto Mangunkusumo National Hospital who had already begun their clinical rotations were invited to fill out the questionnaire. An invitation and regular reminder for this survey was distributed by the Internal Medicine Resident Committee (IMRC) between February 18, 2020 and March 8, 2020.

The questionnaire consisted of 18 questions concerning demographic data, the current US training and practice, and the residents' needs, perceived competence, limitations, and future expectations regarding US training during the residency program. At the beginning of the questionnaire there was also a brief illustration of what POCUS is. In the questionnaire, we divided the applications of POCUS into nine categories: (1) heart (pericardial effusion,

cardiac tamponade, ejection fraction, and tricuspid annular plane systolic excursion), (2) lung (pneumothorax, pleural effusion, lung edema, pneumonia, and lung mass), (3) vena cava (collapsibility index), (4) renal tract (hydronephrosis, urinary tract stones, acute kidney disease, and chronic kidney disease), (5) hepatobiliary (acute hepatitis, chronic liver disease, cirrhosis, splenomegaly, biliary duct dilatation, cholelithiasis, and choledocolithiasis), (6) intestinal and pancreas (acute pancreatitis, pancreatic mass, acute appendicitis, small bowel obstruction, inflammatory bowel disease), (7) deep vein thrombosis, (8) procedures (abdominal paracentesis and thoracocentesis), and (9) musculoskeletal (joint effusion). The full text of the survey can be found in Appendix. We extracted and analyzed the data in June 2020 using Microsoft Excel. The results of the survey were presented as descriptive statistics as frequencies and percentage. The study was approved by the Health Research Ethics Committee of the Faculty of Medicine Universitas Indonesia and Cipto Mangunkusumo National General Hospital (Reference no. KET-734/UN2.F1/ETIK/PPM.00.02/2020).

RESULTS

A total of 175 out of 197 residents completed this survey, yielding a response rate of 88.8%. The survey was voluntary and limited to 1 month period of dissemination. There were 22 residents who did not fill the survey. The demographics of the respondents are shown in **Table 1**.

The first part of the questionnaire consists of the questions concerning the residents' perception of the utility of POCUS. Almost all the residents (99.4%) agreed that POCUS is a must-have skill during residency training. All the residents agreed that POCUS is beneficial for improving the accuracy and timeliness of diagnosis in various conditions. They all agreed that POCUS can be used as a tool to ensure prompt management for various acute conditions, whether in the emergency room, wards, or outpatient clinics. The respondents' perception of which core applications of POCUS are useful for IM residents and internists are shown in **Table 2**.

Table 1. Demographic characteristic of respondents.

Demographics	N (%)
Age (years)	
26-30	122 (69.7)
31-35	46 (26.3)
36-40	7 (4)
Sex	
Male	88 (50.2)
Female	87 (49.8)
Residency (year)	
1	21 (12)
2	45 (25.7)
3	43 (24.6)
4	43 (24.6)
5	20 (11.4)
6	3 (1.7)
Ultrasound training	
Yes	135 (77.1)
No	40 (22.9)
Ultrasound machine available at workplace	
Yes	140 (80)
No	35 (20)
Frequency of exercising POCUS during the last 1 year	
Never	66 (37.7)
< 12 times in 1 year	77 (44)
12-24 times in 1 year	29 (16.6)
> 24 times in 1 year	3 (1.7)

Table 2. The most useful core applications indicated by respondents.

Core applications	Yes N (%)	No N (%)
Musculoskeletal	97 (55.4)	78 (44.6)
Procedures	152 (86.9)	23 (13.1)
Deep vein thrombosis	103 (58.9)	72 (41.1)
Intestinal and pancreas	109 (62.3)	66 (37.7)
Hepatobiliary	165 (94.3)	10 (5.7)
Renal tract	146 (83.4)	29 (16.6)
Vena cava	121 (69.1)	54 (31.9)
Lung	159 (90.9)	16 (1.1)
Heart	149 (85.1)	26 (14.9)

The second part of the questionnaire focused on the current use of US application and the residents' perception of their competence. Less than half of the residents (40%, n=70) identified themselves as competent in POCUS. Only 24 residents from year 4 (55.8%) and 9 residents from year 5 (45%) felt that they were competent to perform POCUS. The perceived competence of the residents for various US applications is shown in **Figure 1**. Out of the 135 respondents who had already received US training, 113

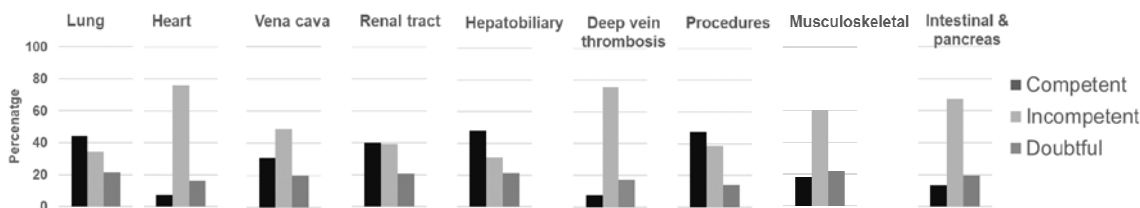


Figure 1. The residents' perceived competence in ultrasound applications.

(83.7%) felt that the training was insufficient. Forty-six percent of fourth-year residents and 40% of fifth-year residents reported that they had performed less than 12 POCUS examinations during the past year. If the residents had indeed performed ultrasounds themselves, the most common applications were hepatobiliary (42%, n=74), lung (40%, n=71), and US-guided procedures (36%, n=63).

The last part of the questionnaire was about limitations in acquiring competence and the residents' future expectations. Eighty percent of the respondents indicated the inadequacy of the US training as a major barrier. Other limitations were insufficient time to practice the skills needed, inadequate supervision from the instructors or attendings and uneven availability of the US machine at each workplace (Table 3).

All respondents agreed that POCUS skills might be needed and used in the next five years, when they are expected to be internists. All the respondents agreed that POCUS core applications must be taught during the residency program. The top five core competences they wished to acquire were heart, lung, hepatobiliary, deep vein thrombosis, and procedures (Table 4).

Table 3. Perceived barriers for POCUS use.

Limitations	Yes N (%)
Insufficient training module available	140 (80)
Insufficient practice time	110 (62.8)
Insufficient knowledge of ultrasound (POCUS)	95 (54.2)
Unavailability of ultrasound machine at workplace	95 (53.1)
Insufficient supervised practice time	82 (46.9)
Insufficient cases to practice on	25 (14.3)
No limitations	5 (2.9)

Table 4. Core applications the respondents aimed to achieve.

Core applications	Yes N (%)	No N (%)
Musculoskeletal	113 (64.6)	62 (35.4)
Procedures	134 (76.6)	41 (23.4)
Deep vein thrombosis	138 (78.9)	37 (21.1)
Intestinal and pancreas	124 (70.9)	51 (29.1)
Hepatobiliary	139 (79.4)	36 (21.6)
Renal tract	131 (74.9)	44 (25.1)
Vena cava	122 (69.7)	53 (30.3)
Lung	140 (80.0)	35 (20.0)
Heart	161 (92.0)	14 (8.0)

DISCUSSION

To the best of our knowledge, there has not been any study published in Indonesia assessing the needs and usefulness of US training for IM residents. Our study showed that all respondents believed that US skills are a must-have competence during residency training, as well as for internists. All respondents agreed that POCUS could be beneficial in improving diagnostic accuracy and promptness.

A study which was recently published in the Netherlands and carried out by Olgers, et al. also showed similar results.² These are consistent with previous studies showing a high level of interest in US training, as well as a generally low level of perceived competence among IM residents.³⁻⁶ The use of POCUS by internists has increased significantly over the past years, due to the accumulating evidence showing that POCUS may assist therapeutic procedures and improve diagnostic performance when used alongside conventional physical examination.⁷ The use of POCUS for lung and heart assessment increases accuracy when it comes to diagnosing dyspneic and shock patients.⁸ As far as the examination of the abdomen is concerned, the ultrasound has been in widely, both in trauma and

in acute medicine settings. Moreover, it is now known that the use of POCUS in certain bedside procedures decreases the likelihood of errors and complications.⁷ Based on those evidence, it is not surprising that POCUS skills are needed by IM residents in daily clinical practices.

Nevertheless, in our study, more than half of the respondents indicated that they had not achieved proficiency in US skills. It is also worth highlighting that only about half of the senior (chief) residents perceived themselves as competent US applications. They reported that the main barriers in acquiring the necessary proficiency are insufficient training modules, insufficient practice time, and a lack of knowledge regarding POCUS. These findings are in line with those of previous studies.²⁻⁵

During the Internal Medicine Residency Program at the Faculty of Medicine Universitas Indonesia, Cipto Mangunkusumo National General Hospital, the second-year residents were provided with three-day training in the US of hepatobiliary and kidney systems. After that, the residents would receive further US training during certain division rotations and would have opportunities to practice during outpatient care and on-call shifts at the emergency room and on inpatient wards. In spite of that, the residents regarded the amount of training and practice as insufficient.

At this moment, it is unclear how many hours of training an IM resident should receive to become competent in each US skills. As the US is a highly operator-dependent imaging modality, it certainly requires substantial time to study and practice. It is also up for debate whether an IM resident should acquire all the core competences of POCUS, since internal medicine consists of many subspecialties. According to the Indonesian College of Internal Medicine, abdominal US is the only US skill that an internist must achieve to the level of competence 4A, which means an internist must be proficient enough to do it without any further supervision.⁹ However, the Indonesian College of Internal Medicine also requires internists to diagnose and treat various emergency conditions, such as acute abdominal pain, shock, acute kidney injury, respiratory failure and thrombosis, as well as to carry out

procedures, such as abdominal paracentesis, thoracocentesis, and insertion of central vein catheter, which could benefit from the use of POCUS.

We believe that the results of this study reflect a real problem and limitation in our IMRP regarding ultrasound training. Although some of the US trainings are already part of the internal medicine curriculum, to date, there has not been any uniform curriculum of US training for internal medicine in Indonesia. It is necessary to have clear guidelines on the core competences and US skills an internist should acquire, in accordance with the standard competence mandated by the Indonesian College of Internal Medicine. The curriculum should include a description of core US applications and the training process needed to be achieved.^{10,11} As the residents already have a clear perspective of the importance of ultrasound skills, an optimized training program is deemed necessary. We believe that the results of this study reflect a real problem and limitation in our IMRP regarding ultrasound training.

This study has some limitations. First, this study used secondary data from a previously conducted survey. Second, the questionnaire used in the survey was not validated due to not having an established gold standard. Third, this study was conducted using a cross-sectional survey performed at a single institution.

CONCLUSION

Our study showed high interest in and a need for US training among IM residents, as US is beneficial for patient care. Despite the training they obtained during the residency program, the majority of residents still perceived themselves as insufficiently trained to perform ultrasound. More than half of the residents reckoned that the training during residency is still lacking and considered this as a major barrier. This study will hopefully serve as a starting point for the optimization of US courses for the IM residency program. Additionally, studies in more center and institutions with larger samples may be needed to develop a uniform and integrated US curriculum for the internal medicine residency program in Indonesia. We believe that US will continue to

be safe, noninvasive, and useful complement to the history and physical examination of patients for the purpose of making accurate diagnoses. Therefore, it is crucial that future internists have sufficient training to take advantage of this powerful tool.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

ACKNOWLEDGMENTS

The authors thank the Internal Medicine Residency Program Universitas Indonesia for providing the data for this study.

REFERENCES

1. Arienti V, Camaggi V. Clinical applications of bedside ultrasonography in internal and emergency medicine. *Intern Emerg Med*. 2011;6(3):195–201.
2. Olgers TJ, Ter Maaten JC. Point-of-care ultrasound curriculum for internal medicine residents: What do you desire? A national survey. *BMC Med Educ*. 2020;20(1).
3. Schnobrich DJ, Gladding S, Olson APJ, Duran-Nelson A. Point-of-care ultrasound in internal medicine: A national survey of educational leadership. *J Grad Med Educ*. 2013;5(3):498–502.
4. Kessler C, Bhandarkar S. Ultrasound training for medical students and internal medicine residents A needs assessment. *J Clin Ultrasound*. 2010;38(8):401–8.
5. Peh WM, Kang ML. A pilot survey on an understanding of point of care bedside ultrasound (POCUS) among medical doctors in internal medicine: Exposure, perceptions, interest and barriers to training. *Proceedings of Singapore Healthcare*. 2018;27(2):85–95.
6. Elhassan M, Gandhi KD, Sandhu C, Hashmi M, Bahl S. Internal medicine residents' point-of-care ultrasound skills and need assessment and the role of medical school training. *Adv Med Educ Pract*. 2019;10:379–86.
7. Ma IWY, Arishenkoff S, Wiseman J, et al. Internal medicine point-of-care ultrasound curriculum: Consensus recommendations from the Canadian Internal Medicine Ultrasound (CIMUS) Group. *J Gen Intern Med*. 2017;32(9):1052–7.
8. Smallwood N, Dachsel M. Point-of-care ultrasound (POCUS): Unnecessary gadgetry or evidence-based medicine? *Clin Med (Lond)*. 2018;18(3):219–24.
9. Kolegium Ilmu Penyakit Dalam. *Daftar Kompetensi Dokter Spesialis Penyakit Dalam dan Dokter Spesialis Penyakit Dalam Subspesialis*. Jakarta: Kolegium Ilmu Penyakit Dalam; 2018.
10. Olgers T.J, Azizi N, Blans M.J, et al. Point-of-care ultrasound (PoCUS) for the internist in acute medicine: a uniform curriculum. *Neth J Med*. 2019;77(5):168–76.
11. Torres-Macho J, Aro T, Bruckner I, et al. Point-of-care ultrasound in internal medicine: A position paper by the ultrasound working group of the European federation of internal medicine. *Eur J Intern Med*. 2020;73:67–71.

APPENDIX

POCUS SURVEY FOR INTERNAL MEDICINE RESIDENTS OF UNIVERSITAS INDONESIA – CIPTO MANGUNKUSUMO GENERAL HOSPITAL

Point-of-care ultrasound (POCUS) is increasingly used in many specialties, including internal medicine (IM). It is as goal-directed and focused tool in answering clinical questions regarding patient's symptom or sign. Recent data show that POCUS improves diagnostic accuracy and timeliness, shortens the time to definitive therapy, and reduces the risk of blind procedures in various clinical scenarios, not only in emergency settings, but also in ward and outpatient clinic settings.

POCUS (Point-of-care Ultrasound)	
Clinical settings	Core competencies
<ul style="list-style-type: none"> - Dyspnea of unknown origin - Chest pain - Patients with shock and periarrest situations - Abdominal pain - Abdominal mass - Jaundice - Renal injury - Lower limb edema 	<ul style="list-style-type: none"> - Free fluid detection (pleural, pericardial, peritoneal) - Wet or dry lung - Dilated heart ventricles - Severe ventricular systolic dysfunction - Diameter and collapsibility inferior vena cava - Palpable or suspected abdominal mass (solid vs liquid) - Splenomegaly - Gallbladder stones - Dilatation of the biliary tract - Hydronephrosis - Small bowel dilatation - Abdominal aortic aneurysm - Bladder outlet obstruction - Ultrasound assisted procedures: thoracocentesis, paracentesis, venipuncture, arterial puncture, central vein catheterization - Proximal deep vein thrombosis by compression evaluation - Catheter into the bladder

Sumber: Torres-Macho J, Aro T, Bruckner I, Cogliati C, Gilja OH, Gurghean A, et al. *Point-of-care ultrasound in internal medicine: A position paper by the ultrasound working group of the European federation of internal medicine. Eur J Intern Med.* 2019

This survey is conducted to know the perception of internal medicine residents regarding their competency and needs of POCUS, as well as regarding the resource available and the barriers. We appreciate your participation in this survey. The results will be analyzed anonymously. The survey consists of 17 questions and will take you about 5 minutes to complete.

1. What is your age?
2. What is your gender?
 - a. Male
 - b. Female
3. What year did you start residency?
4. In which semester of residency are you?
5. Do you have access to ultrasound machine?
 - a. Yes
 - b. No
6. Do you agree with this statement? POCUS needs to be performed by internal medicine residents in daily clinical practice.
 - a. Agree
 - b. Disagree
7. Do you agree with this statement? POCUS is a diagnostic modality that could contribute to faster and better diagnosis for several conditions in internal medicine.
 - a. Agree
 - b. Disagree
8. Do you agree with this statement? POCUS is beneficial for diagnosing and treating patients in various clinical scenarios in emergency room, inpatient ward, procedure room, and outpatient clinics.
 - a. Agree
 - b. Disagree
9. Which POCUS applications do you think are useful for internal medicine residents and internists? (multiple answers possible)
 - a. None
 - b. Heart (pericardial effusion, cardiac tamponade, ejection fraction, and tricuspid annular systolic excursion)
 - c. Lung (pneumothorax, pleural effusion, lung edema, pneumonia, and lung mass)
 - d. Vena cava (collapsibility index)
 - e. Renal tract (hydronephrosis, urinary tract stones, acute kidney disease, and chronic kidney disease)
 - f. Hepatobiliary (acute hepatitis, chronic liver disease, cirrhosis, splenomegaly, biliary duct dilatation, cholelithiasis, and choledocolithiasis)
 - g. Intestinal & pancreas (acute pancreatitis, pancreas mass, acute appendicitis, small bowel obstruction, inflammatory bowel disease)
 - h. Deep vein thrombosis
 - i. Procedures (abdominal paracentesis and thoracocentesis)
 - j. Musculoskeletal (joint effusion)
 - k. Others:
11. Was the ultrasound training you obtained during residency program sufficient?
 - a. Sufficient
 - b. Insufficient
 - c. Never obtain any ultrasound training
11. How many times you ever performed POCUS in the last 1 year?
 - a. Never
 - b. < 12 times in 1 year
 - c. 12-24 times in 1 year
 - d. > 24 times in 1 year
12. Which POCUS applications you have performed during residency?
 - a. Heart (pericardial effusion, cardiac tamponade, ejection fraction, and tricuspid annular systolic excursion)
 - b. Lung (pneumothorax, pleural effusion, lung edema, pneumonia, and lung mass)
 - c. Vena cava (collapsibility index)
 - d. Renal tract (hydronephrosis, urinary tract stones, acute kidney disease, and chronic kidney disease)
 - e. Hepatobiliary (acute hepatitis, chronic liver disease, cirrhosis, splenomegaly, biliary duct dilatation, cholelithiasis, and choledocolithiasis)
 - f. Intestinal and pancreas (acute pancreatitis, pancreas mass, acute appendicitis, small bowel obstruction, inflammatory bowel disease)
 - g. Deep vein thrombosis
 - h. Procedures (abdominal paracentesis and thoracocentesis)
 - i. Musculoskeletal (joint effusion)
 - j. Others:
13. Do you agree with this statement? I feel competent to perform POCUS.
 - a. Agree
 - b. Disagree

14. Do you agree with this statement? I feel competent to perform these POCUS applications without supervision. (agree, disagree, doubtful)
- Heart (pericardial effusion, cardiac tamponade, ejection fraction, and tricuspid annular systolic excursion)
 - Lung (pneumothorax, pleural effusion, lung edema, pneumonia, and lung mass)
 - Vena cava (collapsibility index)
 - Renal tract (hydronephrosis, urinary tract stones, acute kidney disease, and chronic kidney disease)
 - Hepatobiliary (acute hepatitis, chronic liver disease, cirrhosis, splenomegaly, biliary duct dilatation, cholelithiasis, and choledocolithiasis)
 - Intestinal and pancreas (acute pancreatitis, pancreas mass, acute appendicitis, small bowel obstruction, inflammatory bowel disease)
 - Deep vein thrombosis
 - Procedures (abdominal paracentesis and thoracocentesis)
 - Musculoskeletal (joint effusion)
 - Others:
15. What are the biggest limitations or barriers to obtain the competency of and perform POCUS? (multiple answers possible)
- Insufficient knowledge of ultrasound (POCUS)
 - Insufficient training module available
 - Insufficient practice time
 - Unavailability of ultrasound machine at workplace
 - Insufficient supervised practice time
 - Insufficient cases to practice on
 - No limitations
 - Others:
16. Do you agree with this statement? It is very likely that I will use POCUS within 5 years.
- Agree
 - Disagree
17. Do you agree with this statement? Internal medicine residents need to obtain POCUS training.
- Agree
 - Disagree

The Challenging Anticoagulant Therapy in Patient with COVID-19-Associated Coagulopathy

Raissa Virgy Rianda¹, Mohammad Subkhan^{2,3}, Aditya Doni Pradana⁴, Fahrur Nisa'i Fatimah⁵, Rama Azalix Rianda⁶, Muhammad P. Airlangga^{2,7}, Muhammad Miftahussurur^{8,9}

¹ Department of Child Health, Faculty of Medicine Universitas Airlangga-Dr. Soetomo Hospital, Surabaya, Indonesia.

² Siti Khodijah Hospital, Sidoarjo, Indonesia.

³ Department of Pulmonary and Respiratory Medicine, Faculty of Medicine, Universitas Muhammadiyah Surabaya, Surabaya, Indonesia.

⁴ Department of Cardiology and Vascular Medicine, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia.

⁵ Department of Cardiology and Vascular Medicine, Faculty of Medicine Universitas Airlangga, Dr. Soetomo General Hospital, Surabaya, Indonesia.

⁶ Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

⁷ Department of Cardiology and Vascular Medicine, Faculty of Medicine, Universitas Muhammadiyah Surabaya, Surabaya, Indonesia.

⁸ Gastroentero-Hepatology Division, Department of Internal Medicine, Faculty of Medicine Universitas Airlangga-Dr. Soetomo Teaching Hospital, Surabaya, Indonesia.

⁹ Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia.

Corresponding Author:

Muhammad Miftahussurur, MD., PhD. Gastroentero-Hepatology Division, Department of Internal Medicine, Faculty of Medicine-Dr. Soetomo Teaching Hospital, Universitas Airlangga, Surabaya. Jl. Mayjen Prof. Dr. Moestopo 47, Surabaya 60131, Indonesia. Email: muhammad-m@fk.unair.ac.id.

ABSTRAK

COVID-19 menjadi penyakit infeksius yang meluas di seluruh dunia di akhir tahun 2019. Saat ini Indonesia menduduki angka kematian tertinggi di Asia, sekitar 4-5 persen. Menariknya, koagulopati terkait COVID-19 yang ditandai dengan meningkatnya faktor prokoagulan termasuk fibrinogen dan D-Dimer, yang berhubungan dengan tingginya mortalitas dan prognosis yang buruk. Kami melaporkan kasus laki-laki usia 30 tahun datang ke rumah sakit dengan keluhan muntah, demam yang memburuk, dan sesak, dengan diagnosis COVID-19 terkait koagulopati. Tujuh hari setelah masuk, dia mengalami perburukan kondisi dengan penurunan saturasi oksigen yang signifikan, dan parameter koagulasi meningkat dengan tinggi dicurigai adanya emboli paru. Pasien mendapat terapi azithromycin, isoprinosine, lopinavir, dan fondaparinux dengan dosis tromboprolifaksis sejak awal masuk. Peningkatan dosis fondaparinux berperan pada saat perburukan klinis yang kemudian diikuti dengan perbaikan klinis dan penurunan kadar D-dimer. Terapi antikoagulan, terutama dengan fondaparinux, menunjukkan prognosis yang lebih baik pada pasien dengan D-Dimer yang meningkat tinggi. Fondaparinux perlu dipantau secara tepat untuk mencegah perdarahan dan efek samping. Pasien keluar dari rumah sakit dalam kondisi yang lebih baik dan tingkat D-Dimer normal. Tidak ada kejadian perdarahan atau efek samping utama yang ditemukan dalam kasus ini. Keputusan untuk meningkatkan dosis antikoagulan dapat dinilai secara individu, dengan mempertimbangkan risiko, manfaat, dan yang terpenting adalah temuan klinis.

Kata kunci: COVID-19, koagulopati, antikoagulan, trombosis.

ABSTRACT

COVID-19 became a widespread infectious disease in late 2019. Indonesia currently has the highest COVID-19 mortality rate in Asia, between 4-5 percent. Interestingly, COVID-19-associated coagulopathy characterized by an increase of several procoagulant factor levels, including fibrinogen and D-dimer, that has been associated with higher mortality and unfavorable outcomes. We report a case of a 30-year-old male admitted to the hospital with a profuse vomiting and worsening fever, cough and shortness of breath, and was diagnosed with COVID-19-associated coagulopathy. Seven days after admission, he became deteriorated with significant reduction of oxygen saturation and his coagulation parameter levels were increased with highly suspicion of pulmonary embolism. He was treated with azithromycin, isoprinosine, lopinavir, and fondaparinux with thromboprophylaxis dosage since admission. The role of increased fondaparinux dosage at the time of clinical deterioration was then followed by clinical improvement and reduced D-dimer level. Anticoagulant therapy, mainly with fondaparinux, showed a better prognosis in patients with markedly elevated D-Dimer. Fondaparinux needs to be monitored appropriately to prevent bleeding and adverse. The patient was discharged from the hospital in an improved condition and normal D-Dimer levels. There were no bleeding event nor other major side effects that had been found. The decision for increasing dose of anticoagulant may be determined on individual basis, considering risks, benefits, and also the most important is clinical findings.

Keywords: COVID-19, coagulopathy, anticoagulation, thrombosis.

INTRODUCTION

The coronavirus disease-2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), which had been declared a pandemic by the World Health Organization.¹ As of August 2020, the global death toll from COVID-19 has reached 725.330, with more than 19 million confirmed cases. Indonesia has 123.503 confirmed cases, with 5.658 deaths and 79.306 recoveries. According to WHO, East Java province had contributed the highest case in Indonesia with 24.992 confirmed cases (20.2%) followed by DKI Jakarta, and Central Java province.² Coagulation abnormalities have been found in COVID-19 infection, characterized by an increase of several procoagulant factor levels, including fibrinogen and D-dimer. COVID-19-associated coagulopathy has been associated with higher mortality and unfavorable outcomes.¹ Recent studies show that the D-dimer level frequently increases in 36% to 43% COVID-19 patients. Compared to mild cases, D-dimer in severe COVID-19 has a higher predictive value for the patient's outcome and worsening condition.^{3,4} We reported a case of COVID-19 with clinically significant coagulopathy and deterioration which was treated with fondaparinux as thromboprophylaxis during the first week of hospitalization, which

was later converted to therapeutic anticoagulant dose in spite of the current clinical guideline not recommending it solely based on clinical findings. This issue remains controversial since guidelines do not recommend the decision to increase anticoagulant dose only based on clinical findings. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or COVID-19 infection in this case was confirmed by reverse-transcriptase-polymerase-chain-reaction (RT-PCR) assay.

CASE ILLUSTRATION

A 30-year-old Javanese male was admitted to hospital because of nausea and profuse vomiting for three days and was getting worse 6 hours before admission. He reported syncope after few episodes of profuse vomiting. There was no history of eating either spicy or sour food before vomiting. The patient denied any complaints of painful swallowing, fever, cough, and shortness of breath. There was no history of previous illnesses such as diabetes mellitus or hypertension, but he was a heavy smoker. He stayed with his mother in Surabaya, East Java, family members denied any complaint and remained healthy but local transmission of COVID-19 has been recorded in his residential area. There was no known COVID-19 contact, and he has not recently traveled.

Our initial examination revealed a weak general condition, but the patient remained alert. Vital signs were stable: blood pressure, 110/70 mmHg; body temperature, 36.5°C; heart rate, 85 times per minute; respiratory rate was 20 times per minute, and oxygen saturation was 99% in room air. Cardiac examination revealed unremarkable results. Lung auscultation was bilaterally clear. Abdominal physical examination results were unremarkable. During admission, there was no sign and symptoms of dehydration, vital signs were normal, capillary refill time was normal, and skin turgor was normal. His blood laboratory results are summarized in (Table 1) but had notably high NLR of 4.4 when he first arrived to the hospital. The 12-lead electrocardiogram showed normal sinus rhythm.

The next day, the patient developed a fever and cough. The body temperature rose to 38°C and his chest x-ray showed atypical pneumonia (Figure 1). Rapid diagnostic test using IgM and IgG as screening test was non-reactive, and other lab results showed fibrinogen was 342.7 mg/dL (normal range 180-350 mg/dL), CRP, D-dimer, Procalcitonin increased respectively 2.14 mg/dL (normal range <0.3 mg/dL), 0.51 µg/mL (normal range <0.5 µg/mL) and 0.1 ng/mL (normal range <0.05 ng/mL). He had no prolonged activated partial thromboplastin time (a PTT) of 24.8 s and plasma prothrombin time (PPT) of 11.5 s. The reverse transcription polymerase chain reaction (RT-PCR) SARS-CoV-PCR nasopharyngeal swab result was confirmed positive COVID-19 (Standard M nCoV Real-Time Detection Kit, SD

Table 1. Clinical laboratory results.

Laboratory Characteristic	On admission	Day-7	Discharge	Normal limits
Haemoglobin (g/dL)	15.9	10.6	11.2	13-18
Red Blood Cell (10 ⁶ /uL)	5.44	3.75	3.89	4.5-5.5
Haematocrit (%)	44.5	32.8	34.3	40-50
White Blood Cell (10 ³ /uL)	6.29	13.75	7.37	4-11
Neutrophil %	75.8	88.0	73.0	50-70
Lymphocyte %	16.4	9.5	19.0	20-40
NLR	4.62	9.26	3.84	
Platelet count (10 ³ /uL)	216	413	529	150-400
Blood Glucose (mg/dL)	187	-	-	<200
Creatinine (mg/dL)	0.9	-	-	0.7-1.2
BUN (mg/dL)	22.0	-	-	7-20
Ureum (mg/dL)	47.1	-	-	17-43
Bilirubin Direct (mg/dL)	0.38	-	-	<0.2
Bilirubin Total (mg/dL)	1.12	-	-	0.1-1.2
Alkaline Phosphatase (U/L)	83	-	-	<50
Alanine Transferase (U/L)	161	-	-	<50
Sodium (mmol/L)	135	-	-	136-145
Potassium (mmol/L)	4.0	-	-	3.5-5.1
Chloride (mmol/L)	98	-	-	98-107
D-dimer (µg/mL)	0.51	2.52	0.39	<0.5
Fibrinogen (mg/dL)	342.7	596.4	-	180-350
Procalcitonin (ng/mL)	0.10	-	-	<0.05
CRP (mg/L)	2.14	-	-	<0.3

Table 2. Blood gas analysis obtained in day-9 hospitalization.

Parameters	Results	Normal limits
pH	7.43	7.35 - 7.45
PCO2	37	35-45 mmHg
PO2	60	90 – 100 mmHg
BE	0.1	-2 - +2 mmol/L
tCO2	25.2	mmol/L
HCO3	24.1	22-26 mmol/L
SO2	90%	≥ 95 %
FiO2	1.00	

Biosensor, Inc, Suwon, South Korea).

The patient was treated with Azithromycin orally 500 mg/day for five days, and Isoprinosine orally in doses of 500 mg every 8 hours. The patient had been well until five days of treatment. On the sixth day of hospitalization, the patient's clinical condition rapidly deteriorated. He underwent shortness of breath abruptly, and the respiratory rate became 28 times per minute, oxygen saturation declined to 83% in room air. Lung auscultation revealed rhonchi

symmetrically all over his chest and diminished vesicular sounds. At the hospital, he underwent an evaluation chest x-ray examination (**Figure 1**). Blood gas analysis revealed mild hypoxemia with pO_2 60 mmHg, pH 7.43, pCO_2 37 mmHg (**Table 2**). He was planned to be referred for mechanical ventilation, but all hospital in the adjacent area had limited capacity. Later, the patient was put on a non-rebreathing mask with 10 L/min flow (**Figure 2**). The oxygen saturation was slowly increased to 94%. The

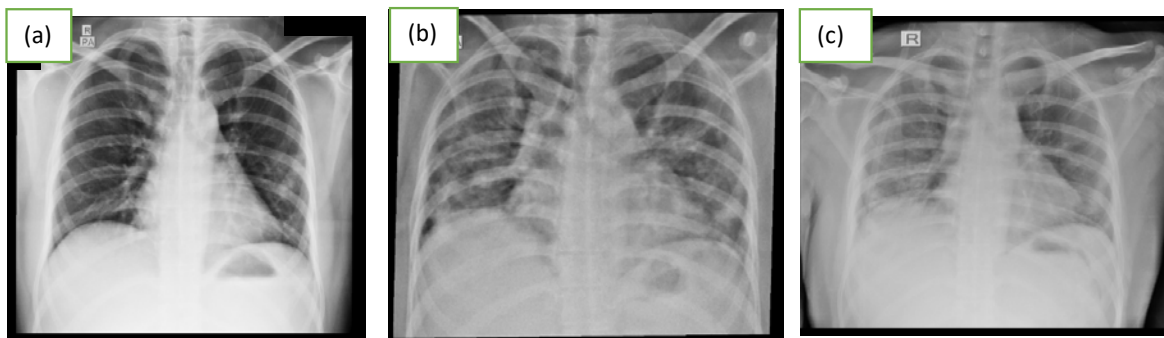


Figure 1. Chest x-ray imaging of the patient. (a) chest x-ray at the first admission to the hospital revealed atypical pneumonia (b) chest x-ray evaluation images on day 16 demonstrated infiltrates pneumonia in both lungs which was worse than the ones at the first visit (c) Chest x-ray re-evaluation image on the day 24 of hospitalization showed improvement which was no infiltrate in lungs.

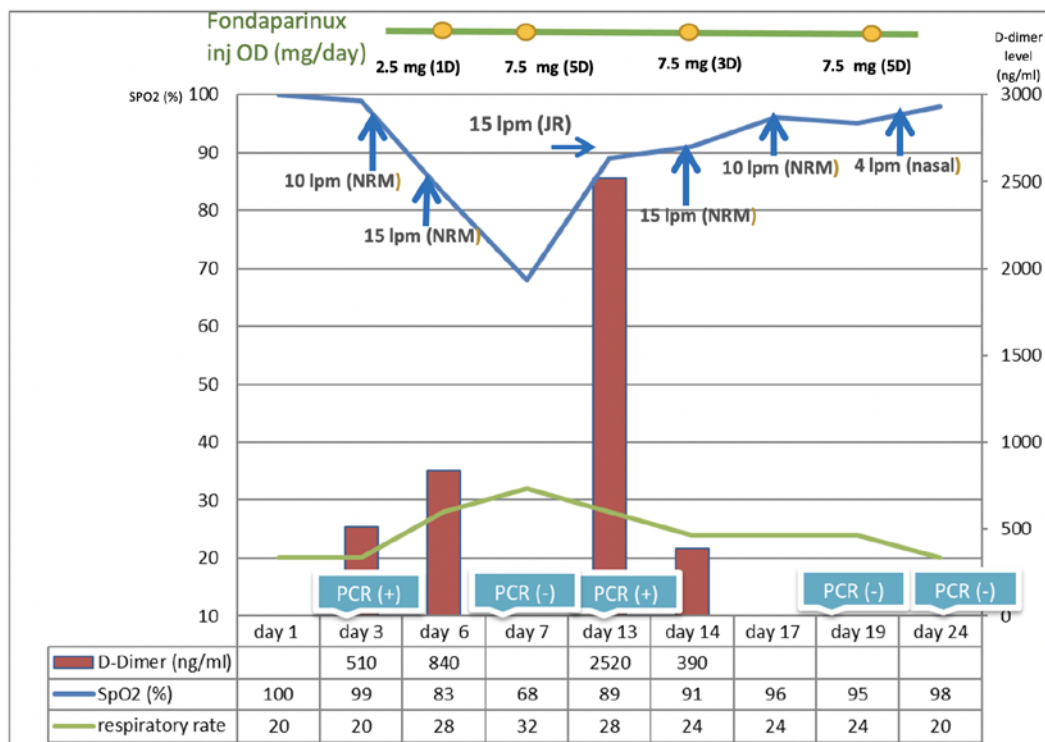


Figure 2. Summary of clinical patient's course of the present case. NRM: non-rebreathing mask. JR: Jackson Rees.

fibrinogen became 596.4 mg/dL, and the D-Dimer level was 0.84 $\mu\text{g/L}$ (normal range $<0.5 \mu\text{g/mL}$). Fondaparinux at a dose of 2.5 mg was administered subcutaneously for three days as thromboprophylaxis, and a combination of Lopinavir/Ritonavir was given orally every 12 hours. On the next day, his general condition was deteriorating, and respiratory rate was 32 times per minute, oxygen saturation declined to 64%, the patient was put on Jackson Rees with 100% oxygen at a flow rate up to 15 L/min. The oxygen saturation was increased to 91%. The dosage of anticoagulant (Fondaparinux) was increased to 7.5 mg subcutaneously for five days.

On the fourteenth day of hospitalization, the patient's condition gradually improved. Lung auscultation revealed diminished rhonchi all over his chest. The patient began to wear a non-rebreathing oxygen mask with 15 L/min flow, and oxygen saturation was 96%, respiratory rate was 24 times per minute. However, D-Dimer was still increased, 2.52 $\mu\text{g/mL}$ (normal range $<0.5 \mu\text{g/mL}$), therefore Fondaparinux injection was still given in the same dose of 7.5 mg/day for the next three days, but lopinavir/ritonavir was stopped. D-Dimer was decreased significantly to 0.39 $\mu\text{g/mL}$ (normal range $<0.5 \mu\text{g/mL}$), and the patient clinical condition markedly improved. On the nineteenth day, respiratory rate was 24 per minute with O₂ nasal 4 L/min, and Fondaparinux was given 7.5 mg/day for five days. The patient was discharged from the hospital after 24 days of hospitalization without complaint according to WHO criteria, lung auscultation revealed vesicular sounds symmetrically and the last chest x-ray was taken (**Figure 1**). There was no bleeding and no other adverse events after fondaparinux usage in patient.

DISCUSSION

Case definition of COVID-19 guidelines based on symptoms, laboratory results showed decrease in lymphocytes and white blood cells, chest radiography showing new pulmonary infiltrates, and no improvement in symptoms after 3 days of antibiotics treatment.⁵ The confirmation of diagnosis is based on the positive result of RT-PCR on a nasal swab.⁶ The most commonly

reported symptoms are fever (83%), cough (82%), shortness of breath (31%), myalgia (11%) or fatigue (9%).^{5,7} whereas forms with digestive symptoms were anorexia, nausea, diarrhea, abdominal pain or initially non-febrile may be at the forefront.⁶ In our patient, digestive symptoms were presented in first day of admission and then followed by respiratory symptoms. Confirmation of COVID-19 diagnosis is based on positive RT-PCR nasal swab result.

Coagulopathy is one of the most significant factor for poor outcome in COVID-19.^{8,9} Several putative mechanisms had been suggested, including inflammation-induced hypercoagulability, hypoxia-mediated endothelial dysfunction, and focal-to-extensive microvascular thrombosis.^{10,11} Thrombosis incidence in severe COVID-19 cases is triggered by hypoxia through the increase of blood viscosity and hypoxia-induced transcription factor-dependent signaling pathway.¹² Our case reported symptoms of COVID-19-associated coagulopathy during the first two days of hospitalization. Laboratory results revealed a marked increase of D-dimer, CRP, and Fibrinogen. Coagulopathy, which is defined as spontaneous prolongation of prothrombin time by more than 3 seconds or activated partial thromboplastin time (aPTT) by more than 5 seconds, was reported as an independent predictor of thrombotic complications. Hematologic abnormalities noted in COVID-19 coagulopathy include: decreased platelet counts, decreased fibrinogen levels, elevated PT/INR, and elevated D-dimer.¹³

D-dimer is one of the markers that thought to be beneficial for COVID-19 management. Recent studies stated that anticoagulant therapy, especially with low molecular weight heparin (LMWH) and Fondaparinux, appears to give a better outcome in severe COVID-19 patients that meet sepsis-induced coagulopathy criteria or with significantly elevated D-dimer.⁹ According to ISTH ad interim guideline, thromboprophylaxis with LMWH or fondaparinux prophylactic dosage should be given to all hospitalized COVID-19 patients with no contraindications (high bleeding risk, active bleeding, platelets $<25,000/\text{uL}$).¹⁴ Fondaparinux is more preferred than unfractionated heparin (UFH) in terms of

bleeding risk and clinical efficacy. A previous study also showed that fondaparinux had anti-inflammatory properties, bringing extra benefit for COVID infection where there is a marked increase of pro-inflammatory cytokines.¹⁵ Several cost-effectiveness analysis studies also show superior efficacy of Fondaparinux compared to other anticoagulants as thromboprophylaxis.^{16,17} Besides the satisfactory clinical result in terms of reduction in thromboembolism and mortality, Fondaparinux should not be used in patients with severe renal impairment.¹⁵ However, if COVID-19 patients are at risks of venous thromboembolism, pulmonary embolism, and renal insufficiency, UFH might act as an alternative anticoagulant.¹⁸

In our patient, the SIC score was normal which according to the dosage guideline, Fondaparinux should only used in thromboprophylaxis dosage. However, we decided to increase the fondaparinux dose to 7.5 mg/day empirically although it was not recommended by ISTH solely based on the patient's clinical condition (deteriorating pulmonary status with high suspicion of PE), D-dimer level on Day 6, and no contraindication was found in the patient. The physician used recommendations from the ESC/ERS PE guidelines, that highlight the importance of induction or step-up approaches of anticoagulation therapy in all patients suspected for pulmonary embolism (PE) with high or intermediate clinical probability without delay. We used the treatment dosage until the patient was discharged on day 24 from our hospital. We also observed clinical improvement on Day 13 after being given a treatment dosage of Fondaparinux for seven days. This increased dosage therapy was based on a recent consensus as long as no contraindications were found, and closed monitoring was performed due to bleeding risk.¹⁹ According to the IMPROVE bleeding risk score, our patients receive 1 point (low risk of major bleeding) for each component for a maximum score of 30,5. The score is stratified into low (<7 points) and high (≥ 7 points) bleeding risk.²⁰

Previous studies reported that the COVID-19 mortality rate was estimated to be 4.3% to 14.6%.^{1,21} A recent study that included 449

patients severe COVID-19, in which 99 patients were treated with heparin at prophylactic dosage, showed that anticoagulant therapy was reported to be associated with a better outcome in correlation to mortality rate (40.0% versus 64.2%, $P = 0.029$).⁹ Another study also reported that patients with increased D-dimer levels (6 times the upper limit of normal) treated heparin showcased lower mortality rates.⁴ Fibrinogen count is also recommended by ISTH guidance for disseminated intravascular coagulation (DIC) in addition to platelet count, PT, and D-dimers.¹⁴

Several case reports suggested thrombolysis agents such as tissue plasminogen activator (tPA) in pulmonary embolism cases with refractory hypoxia, where improvement of perfusion / diffuse ratio (P / F) was reported.²² DIC reported increasing mortality rate, where 71.4% of patients with DIC did not survive during infection compared to one patient (0.6%) who survived. Researchers also noted that there was a significant increase of D-dimer level and PT, and decreased fibrinogen level in non-survivors at day 10 and 14 of treatment.⁹

CONCLUSION

The increasing dose of anticoagulant from thromboprophylaxis to an empirical therapeutic dosage of anticoagulant solely based on clinical condition, D-dimer level, and no contraindication is considered safe and effective anticoagulant treatment for COVID-19-associated coagulopathy. No bleeding risk nor other major side effects have been found in this case.

ACKNOWLEDGMENTS

We thank the healthcare workers at the COVID ward at the Siti Khodijah Hospital, for their assistance in the clinical management of this case. The COVID-19 management team who is working hard day and night after wearing cumbersome personal protective equipment. We declare no conflict of interest.

REFERENCES

1. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020;395:1054–62. <https://doi.org/10.1016/S0140->

- 6736(20)30566-3.
2. WHO. Coronavirus disease situation report. World Health Organization. vol. 19. 2020.
 3. Lippi G. D-dimer is associated with severity of Coronavirus disease 2019: A pooled D-dimer is associated with severity of Coronavirus disease 2019: A pooled analysis. 2020:2019–21. <https://doi.org/10.1055/s-0040-1709650>.
 4. Fogarty H, Townsend L, Ni Cheallaigh C, et al. COVID19 coagulopathy in Caucasian patients. *Br J Haematol*. 2020. <https://doi.org/10.1111/bjh.16749>.
 5. Adhikari SP, Meng S, Wu Y, et al. Epidemiology, causes, clinical manifestation and diagnosis, prevention and control of coronavirus disease (COVID-19) during the early outbreak period: a scoping review. 2020:1–12.
 6. Mezaletk ZT, Khibri H, Ammouri W, et al. COVID-19 associated coagulopathy and thrombotic complications 2020. <https://doi.org/10.1177/1076029620948137>.
 7. Ozma MA, Maroufi P, Khodadadi E. Clinical manifestation, diagnosis, prevention and control of SARS-CoV-2 (COVID-19) during the outbreak period 2020;2.
 8. Helms J, Tacquard C, Severac F, et al. High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. *Intensive Care Med*. 2020;46:1089–98. <https://doi.org/10.1007/s00134-020-06062-x>.
 9. Tang N, Bai H, Chen X, Gong J, Li D, Sun Z. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. *J Thromb Haemost*. 2020:1094–9. <https://doi.org/10.1111/jth.14817>.
 10. McGonagle D, O'Donnell JS, Sharif K, Emery P, Bridgewood C. Immune mechanisms of pulmonary intravascular coagulopathy in COVID-19 pneumonia. *Lancet Rheumatol*. 2020;2019:1–9. [https://doi.org/10.1016/S2665-9913\(20\)30121-1](https://doi.org/10.1016/S2665-9913(20)30121-1).
 11. García LF. Immune response, inflammation, and the clinical spectrum of COVID-19. *Front Immunol*. 2020;11:4–8. <https://doi.org/10.3389/fimmu.2020.01441>.
 12. Suresh M V, Balijepalli S, Zhang B, et al. Hypoxia-inducible factor (HIF)-1 α promotes inflammation and injury following aspiration-induced lung injury in mice. *Shock*. 2019;52.
 13. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J Thromb Haemost*. 2020;18:844–7. <https://doi.org/10.1111/jth.14768>.
 14. Thachil J, Tang N, Gando S, et al. ISTH interim guidance on recognition and management of coagulopathy in COVID-19. *J Thromb Haemost*. 2020:1023–6. <https://doi.org/10.1111/jth.14810>.
 15. Turpie AGG. Selective factor Xa inhibition with fondaparinux: From concept to clinical benefit. *Eur Hear Journal*. 2008;10 (Suppl):1–7. <https://doi.org/10.1093/eurheartj/sun003>.
 16. Bjorvatn A, Kristiansen F. Fondaparinux sodium compared with enoxaparin sodium: a cost-effectiveness analysis. *Am J Cardiovasc Drugs*. 2005;5:121–30. <https://doi.org/10.2165/00129784-200505020-00006>.
 17. Turpie AGG, Bauer KA, Eriksson BI, Lassen MR. Fondaparinux vs enoxaparin for the prevention of venous thromboembolism in major orthopedic surgery: a meta-analysis of 4 randomized double-blind studies. *Arch Intern Med*. 2002;162:1833–40. <https://doi.org/10.1001/archinte.162.16.1833>.
 18. British Thoracic Society. BTS Guidance on Venous Thromboembolic Disease in patients with COVID-19. *Br Thorac Soc*. 2020;2:7.
 19. Ramacciotti E, Macedo AS, Biagioni RB, et al. Evidence-based practical guidance for the antithrombotic management in patients with Coronavirus disease (COVID-19) in 2020. *Clin Appl Thromb*. 2020;26. <https://doi.org/10.1177/1076029620936350>.
 20. AlHajri L, Gebran N. The use of low molecular weight heparin for venous thromboembolism prophylaxis in medical patients: How much are we adherent to the guidelines? *Open J Intern Med*. 2015;5:81–91. <https://doi.org/10.4236/ojim.2015.54012>.
 21. Bi X, Su Z, Yan H, et al. Prediction of severe illness due to COVID-19 based on an analysis of initial fibrinogen to albumin ratio and platelet count prediction of severe illness due to COVID-19 based on an analysis of initial fibrinogen to albumin ratio and platelet count. *Platelets*. 2020;31:674–9. <https://doi.org/10.1080/09537104.2020.1760230>.
 22. Wang J, Hajizadeh N, Moore EE, et al. Tissue plasminogen activator (tPA) treatment for COVID-19 associated acute respiratory distress syndrome (ARDS): A case series 2020:1–4. <https://doi.org/10.1111/jth.14828>.

Atypical Exanthem as Cutaneous Manifestation Related to COVID-19 at a Primary Healthcare Facility

Prasetyadi Mawardi¹, Danu Yuliarta¹, Waode Nuraida²

¹ Department of Dermatology and Venereology, Faculty of Medicine, Sebelas Maret University/Dr. Moewardi General Hospital, Surakarta, Central Java, Indonesia.

² Badak Baru Primary Health Care, Kutai Kartanegara, East Kalimantan, Indonesia.

Corresponding Author:

Prasetyadi Mawardi, MD., PhD. Department of Dermatology and Venereology, Faculty of Medicine, Sebelas Maret University/Dr. Moewardi General Hospital, Jl. Kol. Sutarto 132 Jebres Surakarta 57126, Indonesia. email: prasetyadimawardi@gmail.com; prasetyadi_m@staff.uns.ac.id.

ABSTRAK

Infeksi penyakit Coronavirus (COVID-19) menyerang struktur mukosa saluran pernapasan, terutama mukosa bronkial dan sel imun. Perubahan kulit dan manifestasi terkait infeksi COVID-19 tetap tidak dipahami dengan jelas. Manifestasi kulit terkait COVID-19 telah dilaporkan. Pasien kami menunjukkan eksantema kulit atipikal di kakinya tanpa kelainan lain yang ditemukan. Kami menggunakan azitromisin oral 500 mg, deksametason 0,5 mg, vitamin C 100 mg, dan parasetamol 500 mg, yang tersedia di Puskesmas Badak Baru. Exanthem telah membaik setelah 10 hari pengobatan.

Kata kunci: manifestasi kulit atipikal, covid-19, exanthem, perawatan kesehatan primer.

ABSTRACT

Coronavirus disease (COVID-19) infection attacks the mucosal structures of the respiratory tract, especially the bronchial mucosa and immune cells. The skin changes and manifestations related to COVID-19 infection remain not clearly understood. Cutaneous manifestations related to COVID-19 had been reported. Our patient manifested atypical cutaneous exanthem on her legs, with no other abnormalities found. We used oral azithromycin 500 mg, dexamethasone 0.5 mg, vitamin C 100 mg, and paracetamol 500 mg, which are available at Badak Baru Primary Health Care. The exanthem has improved after 10 days of treatment.

Keywords: atypical cutaneous manifestation, covid-19, exanthem, primary health care.

INTRODUCTION

The first cases of coronavirus disease (COVID-19) were found in Wuhan, China. The World Health Organization reported that the outbreak was caused by a new type of coronavirus called COVID-19 on February 11, 2020.¹ COVID-19 is a public health emergency characterized by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or acute respiratory distress syndrome (ARDS).²

COVID-19 infection attacks the mucosal structures of the respiratory tract, especially the bronchial mucosa and immune cells as evidenced by the histopathological findings in biopsy specimens from various organs such as the lungs, heart, kidneys, spleen, or bone marrow. However, SARS-CoV-2 symptoms are unpredictable; each person exhibits various symptoms that are different from others, which making difficult to establish a certain diagnosis.³ Several

dermatologists found cutaneous manifestations such as rash, urticaria, chicken pox-like eruptions, transient livedo reticularis, chilblain lesions, and erythema multiformes.^{4,5} However, cutaneous manifestations related to COVID-19 infection remain not clearly understood whether they were caused by the infection or treatment reaction.^{3,6}

CASE ILLUSTRATION

A 30-year-old female patient visited the Badak Baru Primary Health Care and presented with rashes on her lower legs since 3 days prior (**Figure 1**). A week before the rash emerged, the patient complained of a mild fever, cold, cough, sore throat, and discomfort when swallowing. The patient denied any history of drug consumption. Her vital signs were normal. The physical examination exhibited symmetrical exanthem on both lower legs. The laboratory finding showed reactive antibody COVID-19 test and positive PCR swab COVID-19 test. However, the physician did not conduct histopathological examination of the skin due to high risk virus transmission and limited facilities. The patient was diagnosed with exanthematous rash related to COVID-19. She was treated with oral azithromycin 500 mg once a day for 10 days, oral

dexamethasone 0.5 mg twice a day (four tablets in the morning and two tablets in the afternoon) for 5 days and then tapered to two tablets daily in the morning and two tablets daily in the afternoon for 5 days, oral vitamin C 100 mg 3 times a day for 10 days, and paracetamol 500 mg as needed for high fever. Moreover, patient self-isolated for 10–14 days. On the 10th day of the treatment, the evaluation exhibited improvement in cutaneous manifestation (**Figure 2**). No other abnormalities were found.



Figure 1: Lesion on lower leg: exanthem upon admission.



Figure 2: Both lower legs: atypical exanthem 10 days after admission.

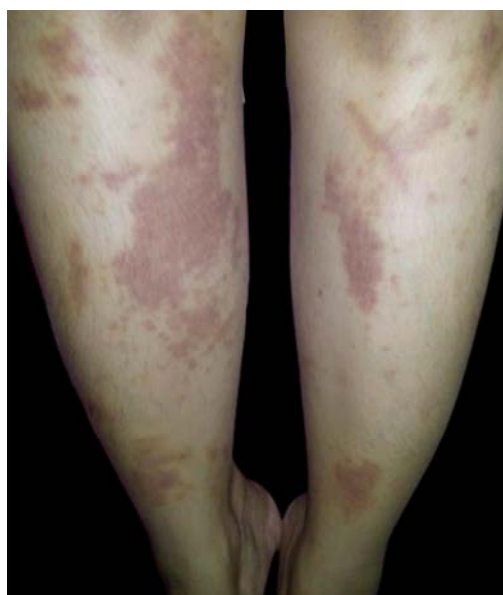


Figure 3: Lower leg atypical exanthem after 14 days.



Figure 4: Improvement after 40 days.

DISCUSSION

Cutaneous manifestation related to COVID-19 was first reported by Recalcati, who examined 148 confirmed COVID-19-positive patients who were hospitalized at Lecco Hospital, Lombardi, Italy. Of the 148 patients, it turned out that 60 patients have received previous therapy so they were excluded from observation. The most common types of skin disorder were erythematous rash (77.8%), extensive urticaria (16.7%) and varicella-like vesicles (5.5%).⁷

Our patient had atypical exanthem on both lower legs. An exanthem is defined as a manifestation of infection of the skin or a reddening reaction of an inflammatory process related to the adverse effects of systemic drug use.⁸ Casas et al. classified cutaneous manifestation with COVID-19: pseudo chilblain, urticarial eruption, vesicular eruption, and maculopapular eruption.⁹ According to Garcia, viral exanthem is classified into three types, namely, classic or typical viral, atypical, and paraviral exanthem. The six classic types of rashes are measles, rubella, scarlet fever, exanthem subitum, erythema infectiosum, and varicella.¹⁰ Viral infection is preceded by a very complicated viral infection, both at the time of binding of the virus by certain human

receptors, through different stages until new virion are released outside. Initial viral infection is stochastic, depending on human exposures to the virus; it may cause systemic infection or complete elimination of the virus by antibodies due to minimal exposure to the virus. Although the pathobiology of cutaneous manifestation related to COVID-19 is not well understood, exanthem is common in viral infections. On the basis of the cells that are likely infected, COVID-19 can be divided into three phases that correspond to different clinical stages of the disease: asymptomatic state (initial 1–2 days of infection), upper airway and conducting airway response (next few days), and then progression to ARDS with hypoxia, ground glass infiltrates. The 20% of confirmed COVID-19 patients can progress to the third stage, and approximately 80% of infected patients have asymptomatic and mild diseases.¹¹

Therapy using oral azithromycin 500 mg, dexamethasone 0.5 mg, vitamin C 100 mg, and paracetamol 500 mg for high fever and self-isolation for 10–14 days was recommended. These medications are available at Badak Baru Primary Health Care (Puskesmas). On the 10th day, the patient came to the Primary Health Care (Puskesmas) with fading rashes on both her legs (Figure 2). No other abnormalities were found. The exanthem has improved after 14 and 40 days (Figures 3 and 4). The anti-microbial azithromycin has immunomodulatory and anti-inflammatory effects.¹² Oral dexamethasone is also given as an anti-inflammatory drug. Dexamethasone inhibits the expression of inflammatory mediators by macrophages and other cells and is used in the treatment of many immune-mediated inflammatory diseases. Dexamethasone has a long-lasting action and higher anti-inflammatory and lower mineralocorticoid effects.¹³ Meanwhile, oral vitamin C is indicated to increase the potentiation of anti-inflammatory drugs.

Limitations

Limitations in this case report include the use of minimal facilities and equipment such as a camera that is not available in our public health facility. Atypical manifestations can be misdiagnosed in cases of COVID-19.

CONCLUSION

Besides respiratory problems, the SARS-CoV-2 virus manifested multi-organ symptoms with each person having different manifestations, including those on the skin. Comprehensive identification by conducting a complex history and physical examination is useful in treating SARS-CoV-2 infection. We showed the rare manifestations, which cutaneous manifestations might be related to immunity.

DECLARATION OF PATIENT CONSENT

The authors certify that they obtained all appropriate patient consent forms. In the form, the patient has given her consent for her images and other clinical information to be reported in a journal. The patient has understood that her name and initials will not be published and due efforts will be made to conceal her identity, but anonymity cannot be guaranteed.

CONFLICTS OF INTEREST

There is no conflict of interest.

REFERENCES

1. WHO. Coronavirus disease 2019 (COVID-19) situation report - 59. Accessed April 10, 2020. <https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200319-sitrep-59-covid-19.pdf>
2. Zhu N, Zhang D, Wang W, et al. A novel Coronavirus from patients with pneumonia in China, 2019. *N Engl J Med.* 2020;382(8):727-33.
3. Iancu GM, Solomon A, Birlutiu V. Viral exanthema as manifestation of SARS-CoV-2 infection: A case report. *Medicine (Baltimore).* 2020;99(35):e21810.
4. Richardson S, Hirsch JS, Narasimhan M, et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City area. *JAMA.* 2020;323(20):2052-9.
5. Gisondi P, Piaserico S, Bordin C, et al. Cutaneous manifestations of SARS-CoV-2 infection: a clinical update. *J Eur Acad Dermatol Venereol.* 2020;34(11):2499-504.
6. Yao X, Li T, He Z, et al. Histopathological study of new Coronavirus pneumonia (COVID-19) in three patients. *Chinese J Pathol.* 2020;49.
7. Recalcati S. Cutaneous manifestations in COVID-19: a first perspective. *J Eur Acad Dermatol Venereol.* 2020;34(5):e212-3.
8. Drago F, Rampini P, Rampini E, et al. Atypical exanthems: Morphology and laboratory investigations may lead to an aetiological diagnosis in about 70% of cases. *Br J Dermatol.* 2002;147(2):255-60.
9. Casas CG, Catal A, Hernández GC, et al. Classification of the cutaneous manifestations of COVID-19: a rapid prospective nationwide consensus study in Spain with 375 cases. *Br J Dermatol.* 2020;183(1):71-7.
10. Garcia JJG. Differential diagnosis of viral Exanthemas. *Open Vaccine J.* 2010;3:65-8.
11. Mason RJ. Pathogenesis of COVID-19 from a cell biology perspective. *Eur Respir J.* 2020;55(4):9-11.
12. Pradhan S, Madke B, Kabra P, et al. Anti-inflammatory and immunomodulatory effects of antibiotics and their use in dermatology. *Indian J Dermatol.* 2016;61(5):469-81.
13. Newton R. Molecular mechanism of glucocorticoid action : what is important?. *Thorax.* 2000;55:603-13.

The Importance of the Timing of Tocilizumab Administration in Moderate to Severely Ill COVID-19: Single Centered Experience Case series

Eric Daniel Tenda¹, Setiabakti Andrian², Sedjahtera Albert², Moses M. Asaf^f, Ceva W. Pitoyo¹, Siti Setiati³, Imam Subekti⁴

¹ Division of Respiriology and Critical Illness, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

² Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

³ Division of Geriatric Medicine, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - CEEBM Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

⁴ Division of Endocrine Metabolic and Diabetes, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - COVID-19 Board Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

Corresponding Author:

Eric Daniel Tenda, MD., PhD. Division of Respiriology and Critical Care, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital. Jl. Diponegoro no. 71, Jakarta 10430, Indonesia. Email: eric.tenda@ui.ac.id.

ABSTRAK

Salah satu penyebab utama kematian pada COVID-19 adalah disregulasi sistem kekebalan tubuh yang menyebabkan badai sitokin, sindrom inflamasi sistemik yang berpotensi fatal. Interleukin 6 (IL-6) adalah sitokin pro-inflamasi yang diproduksi sebagai respons terhadap infeksi dan cedera jaringan dan diyakini memainkan peran penting dalam peristiwa badai sitokin, yang ditandai oleh peningkatannya dalam proses tersebut. Dengan pertimbangan peran IL-6 sebagai sitokin pro-inflamasi dalam proses badai sitokin pada COVID-19, melihat IL-6 sebagai target terapeutik sangat mungkin. Tocilizumab adalah antibodi monoklonal yang secara kompetitif menghambat pengikatan IL-6 ke reseptornya (IL-6R). Penggunaan IL-6R blocker direkomendasikan untuk pasien COVID-19 derajat berat dalam pedoman terapi terbaru yang diterbitkan oleh Organisasi Kesehatan Dunia (WHO), tetapi waktu pemberiannya belum ditentukan. Sementara, beberapa penelitian sebelumnya tentang penggunaan tocilizumab pada pasien COVID-19 menunjukkan hasil yang beragam serta belum merujuk pada kadar IL-6 plasma tertentu untuk memutuskan waktu pemberian tocilizumab. Dalam rangkaian kasus ini, kami menyajikan tiga pasien dengan infeksi COVID-19 sedang hingga berat yang menerima tocilizumab sebagai tambahan untuk terapi perawatan standar. Seri kasus ini memperkenalkan gagasan baru bahwa penggunaan tocilizumab tepat waktu sebagaimana ditandai oleh kadar IL-6 plasma pada pasien COVID-19 sedang hingga berat berpotensi meningkatkan kondisi klinis secara keseluruhan dan meningkatkan tingkat kelangsungan hidup.

Kata kunci: *case series, COVID-19, interleukin-6, timely, tocilizumab.*

ABSTRACT

One of the main causes of death in COVID-19 is the dysregulation of the host's immune system which leads to cytokine storm, a potentially fatal systemic inflammatory syndrome. Interleukin 6 (IL-6) is a pro-inflammatory

cytokine that is produced in response to infections and tissue injuries and is believed to play a pivotal role in the event of a cytokine storm, as signified by its increase in the process. Considering the role of IL-6 as a pro-inflammatory cytokine in the process of cytokine storm in COVID-19, perceiving IL-6 as a therapeutic target could prove to be promising. Tocilizumab is a monoclonal antibody that competitively inhibits the binding of IL-6 to its receptor (IL-6R). The use of IL-6R blocker is recommended for severe COVID-19 patients in the latest therapeutic guideline published by the World Health Organization (WHO), but the timing of the administration has not been specified. While previous studies about the use of tocilizumab in COVID-19 patients have shown various results, these studies do not emphasize on plasma IL-6 levels when deciding the time of tocilizumab administration. In this case series, we present three patients with moderate to severe COVID-19 infections that receive tocilizumab as an adjunct to the standard of care therapy. This case series introduces the novel idea that the timely use of tocilizumab as signified by plasma IL-6 levels in moderate to severe COVID-19 patients could potentially improve overall clinical condition and increase survival rate.

Keywords: case series, COVID-19, interleukin-6, timely, tocilizumab.

INTRODUCTION

COVID-19 infected patients exhibit initial phase of viral replication followed by host driven immune response which sometimes leads to acute respiratory distress syndrome (ARDS) due to the dysregulation of immune system. Dysregulation of immune system during the infection may lead to a cytokine storm, as signified by increased levels of pro-inflammatory cytokines, such as IL-6.¹ IL-6 is a pro-inflammatory cytokine that is produced in response to acute environmental stress, such as infections and tissue injuries.² IL-6 also induces haemostasis and coagulation, increases vascular permeability, which could lead to pulmonary damage and ARDS.³ A publication by Coapescu et al. has suggested that an increase in IL-6 in SARS-CoV-2 infection is synonymous with increased disease severity that translates into sepsis, ARDS/mechanical ventilation, and mortality. Hence, seeing IL-6 as a therapeutic target for SARS-CoV-2 infection is highly plausible and a possibility worth studying.⁴ Tocilizumab is an IL-6 antagonist, primarily applied in autoimmune diseases such as rheumatoid arthritis. Cytokine-directed therapy has recently been proposed especially in critically ill COVID-19 patients in order to preserve organ functions amidst the inflammation storm. One recent open-label random clinical trial on IL-6 receptor (IL-6R) antagonist shows promising results in severe cases of COVID-19 in adjunction with the standard care of therapy.⁵ Studies from China reported that the use of IL-6 antagonists in COVID-19

patients resulted in the resolution of fever and hypoxemia, and subsequent improvements in serum CRP levels and CT findings.⁶ The widely used COVID-19 management guideline in Indonesia that was published in December 2020 included the usage of tocilizumab in COVID-19 once the standard regimen therapy does not show clinical improvement.⁷ It is recommended to use IL-6R blockers at the earliest signs of clinical deterioration or at the earliest indication of clinical transition into severe COVID-19, which usually happens around after the first week of infection.⁸ Moreover, in World Health Organization's (WHO) latest COVID-19 therapeutic guideline, the use of tocilizumab and sarilumab as IL-6 receptor blockers are strongly recommended in severe and critical COVID.⁹ However, the WHO guideline did not specify the recommended timing for the administration of IL-6R blockers. This absence of information is what prompted the authors to write this manuscript and is the main point of discussion of this case series. The concept that the use of IL-6R blockers in a specific period of time (as signified by plasma IL-6 levels) could potentially increase its efficacy in treating moderate to severe COVID-19 patients is the main novelty of this case series. The inclusion criteria in this case series include: patients positive for COVID-19 infection as diagnosed by SARS-CoV-2 RT-PCR, moderate to severe COVID-19 patients as classified by the COVID-19 management guideline in Indonesia, and patients that have consensually agreed to be featured in this research. In this case series, we

present the successful timely use of tocilizumab in the management of moderate to severe COVID-19 patients.

CASE ILLUSTRATIONS

Case 1

A 61-year-old male patient was admitted to hospital with an initial complaint of fever that started four days prior to hospital admission. Additional complaints included unproductive cough, shortness of breath and fatigue. The patient also had a comorbidity of controlled hypertension. COVID-19 diagnosis was confirmed based on the positive SARS-CoV-2 RT-PCR done on January 6th, 2020. Physical examination on admission showed a body temperature of 38.3°C, a heart rate of 111x/min, a respiration rate of 20x/min with bilateral rales more prominent at the base of the lungs, a peripheral oxygen saturation measurement of 94% on room air. Favipiravir was given on admission as part of the initial treatment along with the standard of care therapy. Breathing difficulty worsened on the 3rd day of hospitalization (7th day of symptoms' onset) with peripheral saturation of 88% in room air, which increased to 97% with supplemental oxygen delivered through non-rebreathing mask at 11 lpm. On the 4th day of hospitalization (8th day of symptoms' onset), remdesivir was given as a replacement for favipiravir as there was no observable clinical improvement and an intravenous antiviral was deemed necessary by the primary physician, along with the addition of intravenous dexamethasone. After 10 days of intravenous remdesivir and dexamethasone administration, the patient's condition plateaued and there was still no observable clinical improvement. Blood gas analysis on the 14th day (18th day of symptoms' onset) of hospitalization showed acute respiratory distress syndrome and blood test showed elevated inflammatory markers. Empirical antibiotics of cefepime 1 g tid and levofloxacin 750 mg qd were started on 14th day of hospitalization while waiting for sputum culture. Thorax CT-scan on the 14th day of hospitalization showed multifocal ground glass opacity with crazy paving and thickening

of the interlobular septae. IL-6 was found to be elevated to 118.70 pg/mL on the 16th day of hospitalization (20th day of symptoms' onset) and the sputum culture was found to be sterile. The patient was then admitted into intensive care unit on the 16th day of hospitalization and supplemental oxygenation was changed to high-flow nasal cannula with an FiO₂ of 80% and a flow of 50 lpm. The patient received plasma convalescent therapy of 200 mL per day on the 15th and 16th day of hospital care, and tocilizumab 400 mg qd single dose was given on the 18th day of hospitalization (22nd day of symptoms' onset). The patient was weaned for oxygen and supplemental oxygen delivery was changed back to non-rebreathing mask on the 20th day of hospitalization (24th day of symptoms' onset) and the patient was subsequently discharged from the ICU. On the 23rd day of hospitalization (27th day of symptoms' onset), SARS-CoV-2 RT-PCR was found to be negative and the patient was admitted into the general ward. A follow-up thorax CT-scan was done, and the result showed decreased ground glass opacities and the development of fibrosis of the lungs. After five days of hospitalization in the general ward, the patient was then discharged and scheduled for outpatient control.

Case 2

A 69-year-old male patient complained shortness of breath six days prior to hospital admission. Additional complaints included unproductive cough and fatigue. The patient had a medical history of valvular heart disease and a comorbidity of controlled hypertension. Physical examination on admission showed an oxygen saturation of 93% on room air with a respiration rate of 22x/min, a heart rate of 114x/min, and a temperature of 37.1°C. Chest X-ray performed on admission showed inhomogeneous opacity in the right lung. Initially, the patient was treated with remdesivir 200 mg qd, subsequently adjusted to 100 mg qd for 10 days, ceftriaxone 2 g qd, levofloxacin 750 mg qd along with the standard of care therapy. On the 3rd day of hospitalization (9th day of symptoms' onset), the patient's breathing difficulty worsened and the patient subsequently experienced septic shock,

with blood pressure dropping to 69/46 mmHg, procalcitonin was also found to be elevated to 0.86 ng/mL. Antibiotic was escalated to meropenem 2 g tid IV while norepinephrine was initiated. IL-6 value was found to be elevated to 134.4 pg/mL on the 8th of hospitalization (14th day of symptoms' onset), and tocilizumab 400 mg qd single dose was given subsequently. The patient's condition improved clinically (MAP >65 mmHg, RR 22x/min, SpO₂ 99% with supplemental oxygen delivered through nasal cannula at 3 lpm) and norepinephrine was stopped on the 10th day of hospitalization (16th day of symptoms' onset). Blood test showed that IL-6 level had decreased to 31.28 pg/mL on the 4th day after tocilizumab administration. After the administration of tocilizumab, the patient's clinical condition improved progressively. Patient was then discharged from the hospital after 30 days of hospitalization.

Case 3

A 35-year-old male patient complained shortness of breath followed by cough, high fever, and fatigue three days prior to hospital admission. Based on the SARS-CoV-2 RT-PCR result, the patient was confirmed positive for COVID-19 one day before admission, and chest radiograph showed heterogeneous opacity on the basal region of the right lung. Upon admission, the physical examination showed the patient had a breathing rate of 20x/min with peripheral oxygen saturation of 98% on room air, a heart rate of 89x/min, and a temperature of 41.0°C. The patient was given the standard of care until day five of hospitalization. On the 5th day of hospitalization (8th day of symptoms' onset), the patient's oxygen saturation decreased to 93% on room air with a respiratory rate of 24-26x/min. Laboratory examination showed that IL-6 serum level was significantly elevated to 141.9 pg/mL. The patient was then given tocilizumab 400 mg qd single dose and dexamethasone 4 mg tid. On day six of hospitalization (9th day

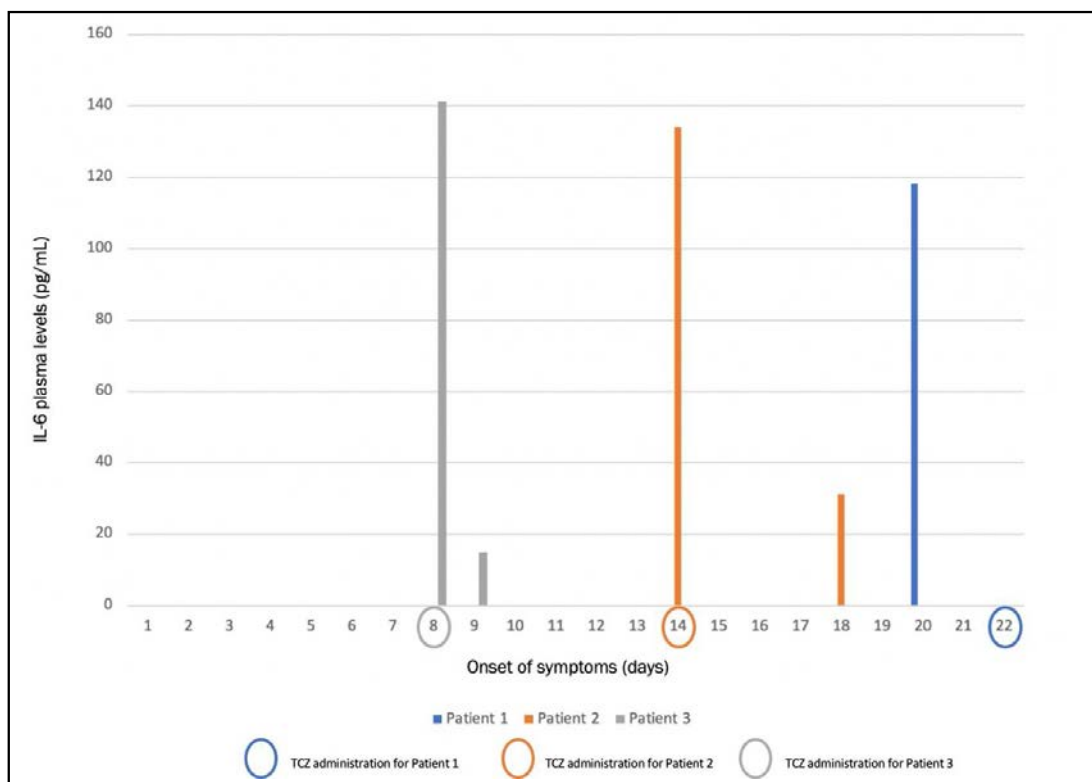


Figure 1. Bar graph showing the timeline of each patient's respective IL-6 levels and the timing of tocilizumab administration.

of symptoms' onset) and a day post tocilizumab administration, blood tests showed that IL-6 level was significantly lower at 14.97 pg/mL, with a simultaneous decrease in CRP. On day eight of hospitalization, evaluation of chest radiograph showed multiple heterogeneous opacities in both lungs. On day 11 of hospitalization, repeated SARS-CoV-2 RT-PCR came out negative, and the patient was subsequently discharged on the same day.

DISCUSSION

In this case report, we'd like to present the timely and selected use of tocilizumab in three patients with moderate-to-severely ill cases of COVID-19. All patients in this case report were given a standard therapy of remdesivir, prophylactic anticoagulant, azithromycin, dexamethasone, vitamin C, vitamin D and supportive oxygen supplementation. All patients consensually agreed to be subjects in this case report. Studies have shown that COVID-19 patients that were admitted to ICU showed higher plasma levels of inflammatory cytokines such as IL-6.^{10, 11} Recent clinical trial data, however, resulted in mixed conclusions on the efficacy of tocilizumab in COVID-19 patients. Study done by Xu et al. in China suggested that tocilizumab administration to severely ill patients with elevated IL-6 resulted in better clinical symptoms such as reduced oxygen supplementation exigency and fever, as well as reduction in inflammatory markers such as procalcitonin and C-reactive protein (CRP).¹² Interestingly, the level of IL-6 did not decrease even after clinical symptoms improved.¹² Other studies, such as one done by Stone et al., concluded that tocilizumab failed in preventing intubation or death in COVID-19 patient when compared to placebo, although the level of IL-6 in the patient recruited in their study did not show high level of IL-6, unlike the levels of IL-6 found in our cases.¹³ Some studies suggested that a cut-off value of 80 pg/mL was a good predictor for poorer prognosis in the patient,¹⁴⁻¹⁶ Tocilizumab monoclonal antibody that competitively inhibits the binding between IL-6 and its receptor (IL-6R). Inhibiting the entire receptor complex will inhibit the subsequent inflammatory cascade due to IL-6

signal transduction. Measurement of IL-6 level before and after administration of tocilizumab is a prognostic factor of disease progression as it represents the level of inflammation in patients and the subsequent outcomes of the patients.¹⁷ The timely introduction of tocilizumab as indicated by high IL-6 serum levels is crucial, as one observational study by Galván-Román et al. found that only those with high IL-6 levels (IL-6 cut-off of 30 pg/mL) with early introduction of tocilizumab showed clinical benefits in terms of PaO₂/FiO₂ ratio increase and survival rate, while patients with low IL-6 did not show improvement in their PaO₂/FiO₂ ratio nor increased survival rate after treatment with tocilizumab.¹⁸ Another possible biomarker analogue of IL-6 that could potentially be used to validate the use of tocilizumab in COVID-19 patients is CRP, as the liver-produced CRP is induced primarily by plasma IL-6.¹⁹ The preliminary results of the RECOVERY trial - a multicentre, randomised, controlled, open-label, platform trial which recruited more than 27,000 hospitalized adult patients with clinically suspected or confirmed COVID-19 - has shown that the efficacy of tocilizumab in severely ill COVID-19 patients (as shown by CRP levels of ≥ 75 mg/L) is significant and worth using.²⁰ Instead of overall condition improvement, the premature use of IL-6 antagonist could prove to be harmful as the extensive obstruction of IL-6 receptor simultaneously impedes $\alpha 1$ -antitrypsin (AAT), which in turn could cause airway inflammation and lung tissue damage.¹⁹ In our case series, all three patients' IL-6 serum levels exceeded both proposed cut-off of 30 pg/mL and 80 pg/mL by some studies, as it was also the hospital's therapeutic policy to administer tocilizumab using an IL-6 cut-off of 40 pg/mL in patients who are unresponsive to systemic corticosteroid therapy as signified by their oxygen demand and need for oxygen support. All three patients improved clinically post tocilizumab administration. However, it should be kept in mind that for the first patient, plasma convalescent was administered three days prior to tocilizumab administration and could act as a confounding factor to the patient's overall clinical improvement. COVID-19

infection might trigger inflammatory cytokines dysregulation and cause worsening clinical symptoms. IL-6 is one such pro-inflammatory cytokines that is responsible for the cytokine dysregulation. Tocilizumab as an IL-6 receptor blocker shows promising result, as shown in this case series when given at the correct time in patients with high serum levels of IL-6. Moreover, data from RECOVERY and REMAP-CAP trials have shown that the introduction of tocilizumab was beneficial, especially when given in adjunction with corticosteroid administration in patients with deterioration of clinical symptoms, usually accompanied by the increase in IL-6 and CRP levels.^{5,20} The use of tocilizumab as recommended in this manuscript is also supported by WHO's latest therapeutic guideline (6 July 2021) which strongly recommends the use of IL-6R blockers (tocilizumab and sarilumab) in severe and critical COVID-19 patients in conjunction with systemic corticosteroids.⁹ This recommendation was made based on the evidence that the use of IL-6R blockers in such patients reduces mortality (OR=0.86, 95% CI 0.79-0.95) and the need for invasive mechanical ventilation (OR=0.72, 95% CI 0.57-0.90) significantly.^{21,22} Although the timing of administration has not been specified by the WHO, using plasma levels of IL-6 and CRP as reference for administration should be contemplated based on prior findings.

CONCLUSION

Based on our case series we see that the timely use of tocilizumab could prove useful in moderate to severe COVID-19 patients. The definition of timely in our case series refers to the plasma biomarker levels, specifically IL-6 plasma levels, as the administration of tocilizumab in all three of our cases differ in days of symptom's onset, yet all three patients showed clinical improvements. We suggest that further studies should emphasize on the IL-6 reference levels and CRP levels for tocilizumab administration.

STATEMENT OF ETHICS

All patients in this case series have given their written informed consent for this article to be published.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

FUNDING SOURCES

No external funding sources were provided in preparation of the study.

AUTHOR CONTRIBUTIONS

Writers contribute equally in the preparation of the manuscript.

REFERENCES

1. Min CK, Cheon S, Ha NY, et al. Comparative and kinetic analysis of viral shedding and immunological responses in MERS patients representing a broad spectrum of disease severity. *Sci Rep.* 2016;6:25359. doi: 10.1038/srep25359. PMID: 27146253; PMCID: PMC4857172.
2. Tanaka T, Narazaki M, Kishimoto T. Immunotherapeutic implications of IL-6 blockade for cytokine storm. *Immunother.* 2016;8(8):959-70. doi: 10.2217/imt-2016-0020. PMID: 27381687.
3. Soy M, Keser G, Atagündüz P, Tabak F, Atagündüz I, Kayhan S. Cytokine storm in COVID-19: pathogenesis and overview of anti-inflammatory agents used in treatment. *Clin Rheumatol.* 2020;39(7):2085-94. doi: 10.1007/s10067-020-05190-5. Epub 2020 May 30. PMID: 32474885; PMCID: PMC7260446.
4. Copaescu A, Smibert O, Gibson A, Phillips EJ, Trubiano JA. The role of IL-6 and other mediators in the cytokine storm associated with SARS-CoV-2 infection. *J Allergy Clin Immunol.* 2020;146(3):518-34.e1. doi: 10.1016/j.jaci.2020.07.001. PMID: 32896310; PMCID: PMC7471766.
5. REMAP-CAP Investigators, Gordon AC, Mouncey PR, Al-Beidh F, et al. Interleukin-6 receptor antagonists in critically ill patients with COVID-19. *N Engl J Med.* 2021;384(16):1491-502. doi: 10.1056/NEJMoa2100433. Epub 2021 Feb 25. PMID: 33631065; PMCID: PMC7953461.
6. Xu X, Han M, Li T, et al. Effective treatment of severe COVID-19 patients with tocilizumab. *Proc Natl Acad Sci U S A.* 2020;117(20):10970-5. doi: 289 10.1073/pnas.2005615117. Epub 2020 Apr 29. PMID: 32350134; PMCID: 290 PMC7245089.
7. Erlina burhan, Agus Dwi Susanto, Sally Aman Nasution, et al. *Pedoman tatalaksana COVID-19.* 3rd ed. Jakarta: Perhimpunan Dokter Paru Indonesia (PDPI), Perhimpunan Dokter Spesialis Kardiovaskular Indonesia (PERKI), Perhimpunan Dokter Spesialis Penyakit Dalam Indonesia (PAPDI), Perhimpunan Dokter Anestesiologi dan Terapi Intensif Indonesia (PERDATIN); 2020.

8. Widysanto A, Kurniawan A, Lugito NPH, et al. Experience of using tocilizumab for treatment in Indonesian patients with severe COVID-19. *Cytokine*. 2021;138:155393. 300 doi: 10.1016/j.cyto.2020.155393. Epub 2020 Dec 14. PMID: 33333393; PMCID: 301 PMC7833085.
9. World Health Organization. Therapeutics and COVID-19: living guideline, 6 July 2021. World Health Organization; 2021.
10. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet*. 2020;395(10223):507-13. doi: 10.1016/S0140-6736(20)30211-7. 308 Epub 2020 Jan 30. PMID: 32007143; PMCID: PMC7135076.
11. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395(10223):497-506. doi: 10.1016/S0140-6736(20)30183-5. Epub 2020 Jan 24. Erratum in: *Lancet*. 2020 Jan 30; PMID: 31986264; PMCID: PMC7159299.
12. Xu X, Han M, Li T, et al. Effective treatment of severe COVID-19 patients with tocilizumab. *Proc Natl Acad Sci U S A*. 2020;117(20):10970-5. doi: 10.1073/pnas.2005615117. Epub 2020 Apr 29. PMID: 32350134; PMCID: 319 PMC7245089.
13. Stone JH, Frigault MJ, Serling-Boyd NJ, et al; BACC Bay Tocilizumab Trial Investigators. Efficacy of Tocilizumab in patients hospitalized with COVID-19. *N Engl J Med*. 2020;383(24):2333-29,44. doi: 10.1056/NEJMoa2028836. Epub 2020 Oct 21. PMID: 33085857; 330 PMCID: PMC7646626.
14. Herold T, Jurinovic V, Arnreich C, et al. Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19. *J Allergy Clin Immunol*. 2020;146(1):128-36.e4. doi: 10.1016/j.jaci.2020.05.008. Epub 2020 May 18. PMID: 33532425269; PMCID: PMC7233239.
15. Laguna-Goya R, Utrero-Rico A, Talayero P, et al. IL-6-based mortality risk model for hospitalized patients with COVID-19. *J Allergy Clin Immunol*. 2020;146(4):799-807.e9. doi: 341 10.1016/j.jaci.2020.07.009. Epub 2020 Jul 22. PMID: 32710975; PMCID: 342 PMC7375283.
16. Chen LYC, Hoiland RL, Stukas S, Wellington CL, Sekhon MS. Confronting the controversy: interleukin-6 and the COVID-19 cytokine storm syndrome. *Eur Respir J*. 2020;56(4):2003006. doi: 10.1183/13993003.03006-2020. PMID: 34632883678; PMCID: PMC7474149.
17. Quartuccio L, Sonaglia A, Pecori D, Peghin M, Fabris M, Tascini C, De Vita S. Higher levels of IL-6 early after tocilizumab distinguish survivors from nonsurvivors in COVID-19 pneumonia: A possible indication for deeper targeting 350 of IL-6. *J Med Virol*. 2020;92(11):2852-6. doi: 10.1002/jmv.26149. Epub 351 2020 Jul 22. PMID: 32515499; PMCID: PMC7301025.
18. Galván-Román JM, Rodríguez-García SC, Roy-Vallejo E, et al; REINMUN-COVID Group. IL-6 serum levels predict severity and response to tocilizumab in COVID-19: An observational study. *J Allergy Clin Immunol*. 2021;147(1):72-362 80.e8. doi: 10.1016/j.jaci.2020.09.018. Epub 2020 Sep 30. PMID: 33010257; 363 PMCID: PMC7525244.
19. McElvaney OJ, Curley GF, Rose-John S, McElvaney NG. Interleukin-6: obstacle to targeting a complex cytokine in critical illness. *Lancet Respir Med*. 2021;9(6):643-54. doi: 10.1016/S2213-2600(21)00103-X. Epub 2021 Apr 16. 367 PMID: 33872590; PMCID: PMC8051931.
20. RECOVERY Collaborative Group. Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform 370 trial. *Lancet*. 2021;397(10285):1637-45. doi: 10.1016/S0140-3716736(21)00676-0. PMID: 33933206; PMCID: PMC8084355.
21. Zeraatkar D, Cusano E, Diaz Martinez JP, et al. Tocilizumab and sarilumab alone or in combination with corticosteroids for COVID-19: a systematic review and network meta-analysis. 2021.
22. The WHO Rapid Evidence Appraisal for COVID-19 Therapies [REACT] Working Group. Association of administration of interleukin-6 antagonists with mortality and other outcomes among hospitalized patients with COVID-19: a prospective meta-analysis. *JAMA*. 2021.

COVID Arm After Moderna Booster in Healthcare Worker: A Case Report

Anshari Saifuddin¹, Sukamto Koesnoe^{1}, Nia Kurniati², Sondang Sirait³, Riesye Arisanty⁴, Evy Yuniastuti¹*

¹Division of Allergy and Clinical Immunology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

²Division of Allergy and Clinical Immunology, Department of Child Health, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

³Department of Dermatovenereology, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

⁴Department of Pathology and Anatomy, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

Corresponding Author:

Sukamto Koesnoe, MD, PhD. Division of Allergy and Clinical Immunology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital. Jl. Diponegoro no.71, Jakarta 10430, Indonesia. Email: sukamtokoesnoe@gmail.com.

ABSTRAK

Virus SARS CoV-2 telah menginfeksi lebih dari 200 juta orang di seluruh dunia dan lebih 4,4 juta orang di Indonesia. Program vaksinasi menjadi salah satu solusi yang dicanangkan oleh berbagai negara di dunia, termasuk di Indonesia, untuk mengurangi laju transmisi COVID-19. Platform vaksinasi yang diproduksi bermacam-macam, seperti inactivated, viral vector, mRNA dan protein subunit. Program vaksinasi booster dengan platform mRNA (Moderna) direncanakan oleh pemerintah Indonesia untuk melindungi tenaga kesehatan lebih lanjut dari infeksi COVID-19 khususnya varian delta. Pada tulisan ini, kami membahas mengenai salah satu efek samping yang khas didapatkan dari vaksin Moderna, yang disebut sebagai COVID arm.

Kata kunci: COVID arm, COVID-19, booster, Moderna, vaksinasi, tenaga kesehatan.

ABSTRACT

SARS CoV-2 virus has infected more than 200 million people worldwide and more than 4.4 million in Indonesia. The vaccination program has become one of the solutions launched by many countries globally, including Indonesia, to reduce the transmission rate of COVID-19. Various vaccination platforms are produced, such as inactivated, viral vector, mRNA, and protein subunit. The vaccination booster program with mRNA platform (Moderna) was launched by the Indonesian government to give better protection for health care workers, particularly from delta variant. In this case report, we discuss one of the typical side effects of Moderna vaccine, which is referred to as the COVID arm.

Keywords: COVID arm, COVID-19, booster, Moderna, vaccination, healthcare worker.

INTRODUCTION

SARS CoV-2/ Coronavirus disease 2019 (COVID-19) has been declared a pandemic by WHO since March 2020. As of August 25th 2021, more than 214 million positive cases had been reported worldwide, with more than 4.4 million deaths.¹ There are more than 4 million confirmed cases in Indonesia, with 128 thousand deaths.² Many countries compete to develop vaccines in order to reduce the SARS-CoV-2 transmission rate. Indonesia launched the COVID-19 vaccination program to approximately 180 million populations in February 2021. Healthcare workers and first responders are among the first to get vaccinated, which reached approximately 1.7 million people.³ The vaccine schedule was two doses of inactivated vaccine (CoronaVac) 2 weeks apart. In July 2020, the emergence of the second wave, the majority caused by the new delta variant, raises concern, especially for healthcare workers. The mortality rate of doctors and other healthcare workers significantly increased on the second wave. It raised the idea to give a booster vaccination with an mRNA vaccine platform (Moderna).⁴ Side effects that arise after Moderna vaccination are varied, similar to other vaccine platforms. Mild side effects are common, either local or systemic. However, from many studies, Moderna has more severe local side effects than other platforms, referred to as the COVID arm. We will report a COVID arm case manifested early after receiving the booster.

CASE ILLUSTRATION

A 56 years old doctor complained that her left arm had become red, swollen, and tender post-vaccination with Moderna. She received two doses of CoronaVac vaccine four months before without any side effects. The Moderna shot was given in the evening. During the night, she suffered from pain at the site of the injection. The pain disturbed her when she laid on the left side and needed one paracetamol tablet to reduce the pain. The following morning, her temperature increased to 40.2°C. She took another tablet of paracetamol, and a few hours later, the temperature dropped to 38.9°C. She took paracetamol every four hours for the next

48 hours, with temperatures ranging between 38.6 – 39.2°C. Hence, making the patient stay home and miss work. She started to feel her left arm becoming more redd and swollen. On the 3rd day, the fever broke, but she still felt lethargic. RT-PCR SARS-CoV-2 on the nasopharyngeal swab was negative. The upper arm started to swell, redness, and warm to the touch. Her range of motion was reduced due to pain (VAS 3-4). Within the next two days, the swelling extended to the upper part of the lower arm, and she used surface cooling to reduce the symptoms. On the 6th-day post-vaccination, the swelling, redness, and pain were decreased, although the lower part of the upper arm and around the elbow still showed inflammation signs. The next day she used compression stocking to reduce the symptoms further. The symptoms disappeared after the 8th-day post-vaccination. She has a history of breast cancer in 2017 and underwent radical mastectomy on the right mammae. She took tamoxifen 20 mg OD routinely. She has an atopic history in the form of eczema and allergic rhinitis. She also had a thalassemia trait which had not been evaluated for a long time.

A skin biopsy was done to get a proper diagnosis. Histological appearance in the epidermis was mild focal spongiosis with a scattered vacuolar alteration. Histological appearance in the dermis was mild inflammatory infiltrate perivascular on superficial plexus, periadnexal, deep plexus, and subcutaneous fat, consisting of lymphocytes and some histiocytes with few intravascular neutrophils. Eosinophils are not present. No sign of vascular wall damage. The biopsy result is consistent with delayed-type hypersensitivity that can be found in vaccine reactions.

DISCUSSION

COVID arm is one of the side effects that rarely occurs after the administration of the Moderna vaccine. Symptoms usually are swelling, redness, pain, warmth, and pruritus at the location of vaccine injection. COVID arm is used to explain the delayed-type hypersensitivity reaction that occurs approximately one week after using COVID-19 mRNA type vaccine.

In one study of COVID mRNA type vaccine,

persons who received the second dose had a higher incidence of erythema than when receiving the first dose. Participants that used Pfizer-Biontech got erythema at the injection site as much as 4.6% after the first dose and 6.5% after the second dose within seven days. Participants who used Moderna reported moderate to severe degree of erythema: 3.1% after the first dose and 11.9% after the second dose within seven days.⁶

In the third phase clinical trial of Moderna report, the most common local side effect post-vaccination was pain at the injection site. Delayed type reactions at the injection site (with onset in the 8th day or later) were experienced by 244 subjects (0.8%) after the first dose and 68 subjects (0.2%) after the second dose from the total of 30,420 subjects. The reactions were marked by erythema, induration, and pain that usually improved spontaneously within 4-5 days.⁷

In this case, the patient had signs of COVID arm in the form of an erythema, swollen, and pain at the injection site appeared three days post-vaccination. The symptoms of swelling, pain, and erythema began to improve spontaneously on the 4th day after the onset. She only took paracetamol for a few days post-vaccination because of the fever that arose. She did not take any antibiotics. The description of the disease course and the symptom was matched with the COVID arm. Skin biopsy result confirmed COVID arm diagnosis. As reported in previous studies, generally COVID arm appeared approximately one week after the administration of Moderna vaccine.^{5,7} In this case, the onset of symptoms was relatively fast compared to COVID arm cases in general. Whether it was related to the patient's history of receiving two doses of inactivated vaccine still needs to be investigated.

With current evidence, it is still unknown how many people are experiencing the delayed-type hypersensitivity due to COVID arm after the first dose and whether they have the same reaction or are even more severe after the second dose.⁷ In one study, patients who had symptoms after the second dose experienced symptoms with faster onset than the first dose (1-3 days). Six patients did not experience recurrence of severe reaction as they have experienced at the first

dose. In comparison, three patients experienced the same reaction as the first dose, and three patients experienced a lighter reaction than the first dose.⁸

A delayed-type hypersensitivity reaction mediated by T cell occurred due to a reaction to the vaccine component. The reaction will be self-resolving, which will not extend systemically and will not be a contraindication for the next vaccination in the future.^{9,10} Reactions reported are against substances such as thimerosal, neomycin, aluminum, but there is no composition from those substances contained in the Moderna or Pfizer vaccine.^{11,12} Pfizer and Moderna have polyethylene glycol (PEG2000). Both vaccines also have salt, sugar, acid, and acid stabilizer.¹³ PEG2000 was the only ingredient in these vaccines that previously known to cause delayed-type hypersensitivity reaction. Further studies are needed to prove that PEG2000 plays a role in the occurrence of the COVID arm.¹⁴

Local skin reactions after vaccination, especially if accompanied by systemic symptoms, are often incorrectly interpreted as cellulitis. Differences between the COVID arm with cellulitis are following. First, pruritus that is commonly found in the COVID arm usually occurs within a week after vaccination. Second, the symptoms improved spontaneously within 4-5 days and responded well using topical steroids or oral antihistamines.⁵

Halperin, et al.¹⁵ define the diagnosis criteria to differentiate between cellulitis and local reaction post-vaccination. Criteria for cellulitis is a reaction in the injection site with at least 3 of these symptoms: local pain, erythema, induration/swelling, and warmth. The median time from onset of cellulitis is approximately five days post-vaccination compared to delayed-type hypersensitivity reaction that occurs in 7 days or more post-vaccination. In addition to that, the role of bacteria in cellulitis, such as *Staphylococcus aureus* or *Group A Beta Hemolytic Streptococcus* could be found from the injection site. The antibiotic response is one of distinction. If the patient has cellulitis and is not given antibiotics, it could cause the formation of an abscess. While in local reaction post-vaccination, the symptoms will improve

spontaneously.¹⁶

The growing population that got COVID-19 vaccines, particularly mRNA platforms, will increase COVID arm cases that consulted to primary care doctors. Primary care doctors

should be able to distinguish between the delayed-type hypersensitivity reaction post-vaccination with other differential diagnoses, including cellulitis. In the United States, misdiagnosed cellulitis causing 50,000–130,000



Figure 1. Clinical picture from patient's left arm, 4 days after vaccination.



Figure 2. Clinical picture from patient's left arm, 5 days after vaccination.

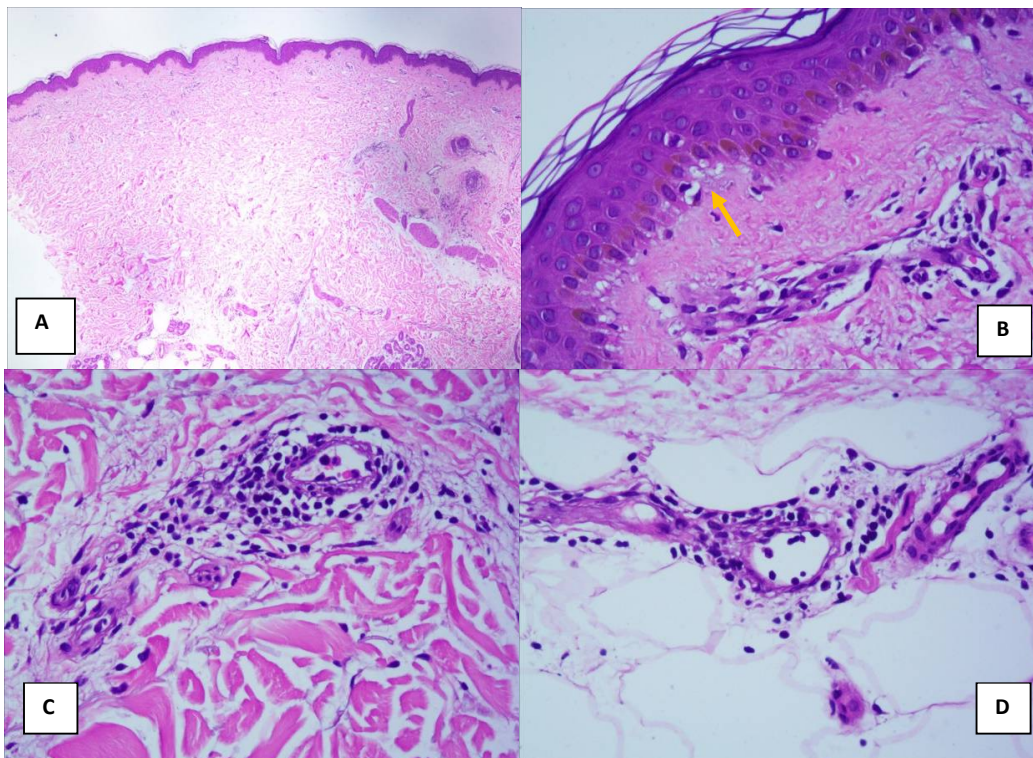


Figure 3. A). Skin biopsy result (100x magnification). B). Vacuolar degeneration in epidermis (400x magnification). C). Mid dermis (400x magnification). D). Subcutaneous (400x magnification).

individuals hospitalized and 195-515 million dollars spent every year¹⁷ In addition, the use of antibiotics in misdiagnosed cellulitis cases could cause increased risk to antibiotic resistance and related to 9,000 nosocomial infection cases every year in the United States.¹⁶

CONCLUSION

COVID arm is one of the adverse events that can occur after Moderna vaccination. This side effect is self-resolving. Clinicians should be able to differentiate the COVID arm from other differential diagnoses, such as cellulitis. It is essential to give appropriate and effective treatment to the patients.

REFERENCES

1. Worldometers. Coronavirus cases in world. [cited 2021 August 26]. Available from <https://www.worldometers.info/coronavirus/>
2. Kementerian Komunikasi dan Informatika. Sebaran kasus COVID-19 di Indonesia. [cited 2021 August 26]. Available from <https://covid19.go.id/>
3. Kementerian Kesehatan RI. Materi Penanganan COVID-19. Kemenkes. Desember 2020.
4. Tim mitigasi Ikatan Dokter Indonesia. Data kematian dokter di Indonesia akibat COVID-19. IDI. 2021
5. Lingren AL, Austin AH, Welsh K. COVID Arm: Delayed Hypersensitivity Reactions to SARS-CoV-2 Vaccines Misdiagnosed as Cellulitis. *J Primary Care Comm Health*. 2021;12:1-5
6. Allergic Reactions. Centers for Disease Control and Prevention. [cited 2021 August 26]. Available from <https://www.cdc.gov/coronavirus/2019-ncov/vaccines/safety/allergic-reaction.html>
7. Baen LR, Sahly E, Essink B, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl J Med*. 2021;384:403-16.
8. Blumenthal KG, Freeman EF, Saff RR, et al. Delayed large local reactions to mRNA-1273 vaccine against SARS-CoV-2. *N Engl J Med*. 2021;384:1273-7.
9. Kelso JM, Greenhawt MJ, Li JT, et al. Adverse reactions to vaccines practice parameter 2012 update. *J Allergy Clin Immunol*. 2012;130:25-43.
10. McNeil MM, DeStefano F. Vaccine-associated hypersensitivity. *J Allergy Clin Immunol*. 2018;141:463-72.
11. U.S. Food and Drug Administration. Fact Sheet for Recipients and Caregivers. Emergency Use Authorization (EUA) of the Pfizer-BioNTech COVID-19 Vaccine to Prevent Coronavirus Disease 2019 (COVID-19) in Individuals 16 Years of Age and Older [fact sheet]. [cited 2021 August 26]. Available from <https://www.fda.gov/media/144414/download#page=2>
12. U.S. Food and Drug Administration. Fact Sheet for Recipients and Caregivers. Emergency Use Authorization (EUA) of the Moderna COVID-19 Vaccine to Prevent Coronavirus Disease 2019 (COVID-19) in Individuals 18 Years of Age and Older [fact sheet]. [cited 2021 August 26]. Available from <https://www.fda.gov/media/144638/download#page=2>
13. Allergic Reactions. Centers for Disease Control and Prevention. [cited 2021 August 26]. Available from <https://www.cdc.gov/coronavirus/2019-ncov/vaccines/safety/allergic-reaction.html>
14. Fisher AA. Immediate and delayed allergic contact reactions to polyethylene glycol. *Contact Dermatitis*. 1978;4:135-8.
15. Halperin S, Kohl KS, Gidudu J, et al. Cellulitis at injection site: case definition and guidelines for collection, analysis, and presentation of immunization safety data. *Vaccine*. 2007;25:5803-20.
16. Davies HD, McGeer A, Schwartz B, et al. Invasive group A streptococcal infections in Ontario, Canada. Ontario Group A Streptococcal Study Group. *N Engl J Med*. 1996;335(8):547-54.
17. Weng QY, Raff AB, Cohen JM, et al. Costs and consequences associated with misdiagnosed lower extremity cellulitis. *JAMA Dermatol*. 2017;153(2):141-6.

Risk factor, diagnosis, and current treatment of *H. pylori* Infection in Indonesia: A Literature Review

Rizani P. Iman¹, Tiroy Junita¹, Rinaldo I. Rachman¹, Ari F. Syam²

¹ Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia.

² Division of Gastroenterology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

Corresponding author:

Prof. Ari Fahrial Syam, MD., PhD. Division of Gastroenterology, Dept. of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital. Jl. Diponegoro no. 71, Jakarta 10430, Indonesia. Email: ari_syam@hotmail.com.

ABSTRAK

Infeksi Helicobacter pylori (H. pylori) di Indonesia merupakan suatu masalah yang telah berkembang, meskipun prevalensi di Indonesia dilaporkan lebih rendah dibanding negara ASEAN dan Asia lainnya. Dominasi jenis bakteri H. pylori dengan virulensi genotipe yang lebih lemah di Indonesia diduga menjadi penyebab rendahnya prevalensi infeksi tersebut serta kanker gaster sebagai komplikasinya. Walaupun pemeriksaan endoskopi masih menjadi alat utama untuk mengevaluasi status mukosa gaster pada pasien yang terinfeksi H. pylori, infeksi H. pylori sendiri dapat secara sederhana didiagnosis menggunakan strategi test-and-treat, terutama pada daerah dengan sumber daya terbatas. Beberapa temuan menunjukkan angka resistensi terhadap berbagai antibiotik yang tinggi di banyak daerah dan etnik Indonesia, di mana hal tersebut mengindikasikan bahwa pengobatan menggunakan triple therapy regimen tidak dapat diaplikasikan terhadap semua populasi secara keseluruhan. Pengobatan infeksi H. pylori sebaiknya didasarkan terhadap pola resistensi pada area tersebut, dan regimen menggunakan antibiotik jenis baru seperti furazolidone, rifabutin, dan sitafloxacin dapat dipertimbangkan sebagai terapi potensial untuk mengeradikasi infeksi tersebut. Untuk menentukan pendekatan yang paling adekuat, studi multisenter menggunakan sampel yang lebih besar penting untuk dilakukan.

Kata kunci: faktor risiko, diagnosis, penatalaksanaan terkini, infeksi, *Helicobacter pylori*.

ABSTRACT

Helicobacter pylori (H. pylori) infection has become an emerging problem in Indonesia despite its relatively low prevalence as opposed to other Southeast Asian and Asian countries. Strains containing less virulent genotypes predominantly found in Indonesia is suggested to be the rationale for why the disease prevalence, as well as its gastric cancer complication, remain inferior in respect of neighboring counterparts. Although endoscopic evaluation is still necessary to determine the gastric mucosal status of those infected with H. pylori, the infection itself can be easily diagnosed with test-and-treat strategy especially in areas with limited resources. Several findings revealed high rates of antibiotic resistance varying among Indonesian regions and ethnicities, suggesting that triple therapy regimen may not be suitable for all populations. Whereas treatment should be based on the pattern of resistance in respective regions, novel regimens involving furazolidone, rifabutin, and sitafloxacin are proposed as potential drugs of choice to eradicate H. pylori infection. In order to determine the adequate approach for H. pylori infection in Indonesia, further multicenter studies involving larger sample size should be conducted.

Keywords: risk factor, diagnosis, current treatment, infection, *Helicobacter pylori*.

INTRODUCTION

Indonesia is one of the most populous countries in the world. Indonesia is unique in that its land mass is formed of an archipelago consisting of more than 1,000 ethnicities and even more sub-ethnic groups. *Helicobacter pylori* (*H. pylori*) infection has been an emerging problem in Indonesia with a prevalence of 22.1% reported by Syam et al.¹ Globally, 4.4 billion people are infected with *H. pylori*.² The prevalence of *H. pylori* infection in Indonesia is relatively low compared with that of other countries in Southeast Asia and Asia. In developing countries, the prevalence of *H. pylori* infection ranges from 85% to 90%; however, this figure is significantly lower in developed countries, ranging between 30% and 50%.^{3,4} For instance, the United Arab Emirates has an overall prevalence of 41%.⁵ In Southern Asia, Pakistan and India have the highest *H. pylori* infection prevalence—81% and 63.5%, respectively. In line with the low prevalence of *H. pylori* infection, Indonesia has only the fifth highest incidence of gastric cancer among Southeast Asian countries, reported as 3,014 cases per year according to GLOBOCAN 2018.⁶ In contrast to neighboring countries, Vietnam ranks first, with 17,527 new cases of gastric cancer per year.⁶ China leads Asia in term of gastric cancer incidence, with 456,124 cases per year. Southeast Asia in general has a gastric cancer incidence of 8.2 per 100,000 and 4.1 per 100,000 per year for men and women, respectively. These relatively high numbers are only less than a third of those in East Asia (the highest in the world).⁷ These data spark the question: “*Is the low prevalence of gastric cancer in Indonesia due to the low prevalence of H. pylori infection?*”.

ETIOPATHOGENESIS AND RISK FACTORS

The low prevalence of *H. pylori* infection remains a point of curiosity. Initially, the prevalence of *H. pylori* in Indonesia was expected to be as high as in other developing Asian countries, as sanitation infrastructures are not yet well constructed. Several studies have assessed the risk factors of *H. pylori* infection in Indonesia. A study by Goto et al. showed that the risk factors significantly associated with *H.*

pylori infection are infrequent hand washing before meals (OR 4.10; 95% CI: 1.15-14.6), cucumber intake more than once a week (OR 6.61; 95% CI: 1.87-23.3), chicken intake more than once a week (OR 1.40; 95% CI: 0.43-4.56), and former and current alcohol drinking (OR 61.9; 95% CI: 1.67-2300.8).⁸ On the other hand, drinking soy milk more than once a week decreased the risk with marginal significance (OR 0.10; 95% CI: 0.01-0.97). The Buginese and Batak ethnicities were shown to have a higher risk of *H. pylori* infection than the Javanese, as Buginese and Batakese eat using their fingers more frequently.⁸ Drinking tap water instead of mineral water is also a risk factor for *H. pylori* infection.⁹ The same risk factors are also seen in the United Arab Emirates.⁵

With regard to ethnicity, a study conducted by Syam et al. assessing the prevalence of *H. pylori* on the five largest islands of Indonesia revealed much interesting data. They found differences in the prevalence of *H. pylori* infection between ethnicities, hence establishing ethnicity as an independent risk factor for *H. pylori* infection. The Papuan, Batak, and Buginese ethnicities had a higher risk of developing *H. pylori* infection than the Javanese, Dayak, and Chinese ethnicities.¹⁰ Ethnicity is also a risk factor for *H. pylori* infection in the United Arab Emirates ($p < 0.001$). Khoder et al. showed that people of African descent have a significantly higher prevalence than those of Asian or Arab ethnicity. Among Asians, Indians have the highest prevalence of *H. pylori* infection, followed by the people of Pakistan and the Philippines.⁵

There is an ongoing debate over whether or not psychological stress is a risk factor for *H. pylori* infection. The idea is that psychological stress provokes gastric acid secretion, increasing the level of aggressive factors and inhibiting protective factor of the gastric mucosa. These conditions facilitate infection of the stomach by *H. pylori*. A study by Darwin et al. in Padang showed that psychological stress appears to have no correlation with the expression of IL-6 in the gastric mucous of patients with functional dyspepsia. However, there is evidence of increasing activity of *H. pylori* in patients with increased psychological stress.¹¹

H. pylori can manifest as a broad spectrum of clinical symptoms and diseases ranging from peptic ulcer to gastric cancer and from bloating to dyspepsia. A cross-sectional study in Medan conducted by Siregar et al. involving 80 patients with gastritis showed that serum TNF- α and VEGF levels were significantly increased in the *H. pylori*-infected group, but there were no significant differences in serum levels of IL-8 between *H. pylori*-positive and negative groups. High levels of TNF- α were associated with a severe degree of chronic inflammation, high levels of IL-8 were associated with severe degree of chronic inflammation and neutrophil infiltration, and high levels of VEGF were associated with a severe degree of premalignant gastric lesion.¹²

One of the clinical manifestations of *H. pylori* infection is Gastroesophageal Reflux Disease (GERD). It was suggested that there is a parallel association of increasing GERD incidence with decreasing *H. pylori* infection prevalence in Asia. Miftahussurur et al. conducted a study involving 104 dyspeptic patients evaluated by endoscopy in Indonesia. This study found a high prevalence of GERD in areas with a low *H. pylori* prevalence, although the association could not be measured due to an insufficient number of cases.¹²

Peptic ulcer disease is still a significant health burden, despite decreasing rates of *H. pylori* infection due to eradication therapy. In Indonesia the prevalence of *H. pylori* infection in peptic ulcer disease is 90%-100%. Peptic ulcer disease itself can be differentiated into duodenal and gastric ulcer disease. *H. pylori* plays a role in those two ulcer locations. The location of *H. pylori* infection determines whether gastric acid secretion will be up- or downregulated, based on the inflammatory zone. Untreated *H. pylori* infection may lead to gastric cancer, as *H. pylori* is classified as a Class 1 carcinogen in humans. However, the risk of malignancy depends on the virulence factor of *H. pylori*.¹³

An interesting point concerning the prevalence of gastric cancer in Indonesia is that it is relatively low compared with other Asian countries. A multicenter study conducted by Miftahussurur et al. in Java, Papua, Sulawesi, Borneo, and Sumatera showed that virulence

factors of genotypes presenting in *H. pylori* strains were suggested to contribute to the low rate of gastric cancer in Indonesia. Their study revealed the genetic profile of *H. pylori* in Indonesia. Of the strains examined, 97.7% possessed the *cagA* gene, 60.5% of which were East-Asian-type *cagA*, while 20.9% were Western-type-*cagA*. In all, 18.6% were of the novel ABB type, most of which were obtained from Papuan subjects. Patients infected with East-Asian-type-*cagA* strains possessing a 6-bp deletion showed significantly lower inflammation and atrophy scores in the corpus area than those with strains expressing the Western-type *cagA*. These findings suggest that the low incidence of gastric cancer in Indonesia may also be due to less virulent genotypes in the predominant strains.¹⁰ Variations in *H. pylori* strains affect the prevalence of gastric cancer within a country. *hspE*Asia isolates among Chinese people appear linked to a higher incidence than *hpAsia2* or *hpEurope* strains, found among Indians and Malays.⁷

H. pylori vacA and *cagA* genes are associated with higher virulence, whereas Vascular Endothelial Growth Factor (VEGF) is one important marker for neo-angiogenesis. Siregar et al. conducted a study in Medan and concluded that an increased level of VEGF is correlated with *H. pylori* infection and its virulence status. There was a significant rise in serum levels of VEGF in *H. pylori*-infected patients compared with those without infection. Moreover, *cagA*-positive strains of *H. pylori* also showed significantly higher VEGF levels than *cagA*-negative strains.¹⁴

DIAGNOSIS OF *H. PYLORI* INFECTION

Several studies were performed in Indonesia to identify other diagnostic tools and biomarkers and to compare them with the urea breath test (UBT) and endoscopic evaluation, for the diagnosis of *H. pylori* infection in the Indonesian population.

Budyono et al.¹⁵ conducted a cross-sectional study from November 2016 to March 2017 to analyze the association between the severity of dyspepsia, measured with a modified Glasgow Dyspepsia Severity Score (GDSS) questionnaire,

and *H. pylori* infection, measured with 14C UBT Examination. There was a significant difference in GDSS scores between *H. pylori*-negative and positive patients. However, clinical assessment failed to show a significant difference in severity between the two groups (GDSS of 3.06 in positive and GDSS of 1.67 negative, out of 10). Therefore, it is difficult to recommend modified GDSS as a substitute for the current examination method, as it has a sensitivity of 41.6% and specificity of 85.5%, taking UBT as the gold standard. *H. pylori* eradication should be considered in patients with a modified GDSS score of 4 and above if there is no better examination available.

Nurdin et al.¹⁶ conducted a study to investigate *H. pylori* detection in gastric biopsy. They compared immunohistochemistry and Giemsa staining for the detection of *H. pylori* in active chronic gastritis. The Giemsa sensitivity test performed upon immunohistochemistry showed a sensitivity value of 65% and a specificity value of 100%. Immunohistochemistry staining in active chronic gastritis was more sensitive than Giemsa, particularly in detecting coccoid-shaped bacteria.

As for the use of serology in *H. pylori* infection, Miftahussurur et al. found that an ELISA test for *H. pylori* infection had sensitivity of 66.7% and specificity of 97.2%, compared with immunohistochemistry as the gold standard. This showed the low accuracy of the ELISA kit when used in an Indonesia population.¹⁷

Other studies attempted to generate markers that could be used to evaluate gastric mucosal status in patients with *H. pylori* infection. Miftahussurur et al. studied the use of serum pepsinogen (PGs) as a non-invasive method to determine gastric mucosal status, in order to favor areas in Indonesia in which gastrointestinal endoscopy is unavailable. PG levels were determined using the PG ELISA kit. The samples were then classified according to the ABC method as follows: *H. pylori*-negative/PG-negative (group A), *H. pylori*-positive/PG-negative (group B), *H. pylori*-positive/PG-positive (group C), and *H. pylori*-negative/PG-positive (group D). *H. pylori* (+) patients had significantly higher PG II levels and lower

PG I/PG II ratios than *H. pylori* (-) patients. The prevalence of chronic and atrophic gastritis was also significantly higher in *H. pylori* (+) patients. PG levels can be useful to determine chronic gastritis but have modest sensitivity for atrophic gastritis in Indonesia.¹⁷

A cross-sectional study conducted by Dairi et al. aimed to determine serum malondialdehyde (MDA) levels in patients with *H. pylori* (+) gastritis. *H. pylori* infection produces reactive oxygen species (ROS), whereas ROS itself are an important factor for the development of gastritis. *H. pylori* infection also causes recruitment of neutrophils and macrophages, which in turn increase free radicals. This oxidative stress causes tissue damage that can be measured by MDA, the product of lipid peroxidase. The mean MDA level in *H. pylori* (+) patients was 1.58, whereas in *H. pylori* (-) it was 1.19, with $p = 0.013$ for the difference. There is still no normal value of MDA that can be used as standard, as it can be affected by age, enzyme activities, antioxidant supplements, diseases, and environmental factors.¹⁸

From a study using a survey of 26 experts from nine Southeast Asian countries (Cambodia, Indonesia, Laos, Malaysia, Myanmar, the Philippines, Singapore, Thailand, and Vietnam), the rapid urease test (RUT) was the most common method used to diagnose *H. pylori* infection, followed by UBT. Most Southeast Asian countries used a test-and-treat strategy for patients with dyspepsia without alarming symptoms, except Laos and the Philippines. In these cases, stool antigen and urea breath tests were commonly used. Among health centers and regions in the same countries, there were significantly different proportions of patients with findings of endoscopic gastritis who underwent RUT and histological evaluation.¹⁹

COMPLICATIONS OF *H. PYLORI*/INFECTION

Colonization with *H. pylori* is known to increase the risk of certain diseases, such as duodenal ulcer, gastric ulcer, non-cardiac gastric carcinoma, B-cell lymphoma, and possibly thrombocytopenic purpura. A case-control study was conducted by Darnindro et al. between June 2014 and August 2014 in Jakarta with 69

cases and 71 controls using medical record and histopathology evaluation. *H. pylori* infection can cause atrophy and metaplasia of the gastric mucosa. According to histopathological findings, active chronic gastritis was found in 62.3% of *H. pylori* (+) patients, compared with only 12.7% of *H. pylori* (-) patients (OR = 11.31; 95% CI: 4.86-26.7). Mild and moderate atrophy was higher among *H. pylori* (+) patients, though the difference was not statistically significant ($p = 0.09$). The frequency of gastric mucosal metaplasia was found to be higher in *H. pylori* (+) patients (10.1% vs 1.4%; $p = 0.03$).²⁰

Increased metaplasia in the *H. pylori* (+) population was also observed by Tenggara et al. Of 1127 patients who underwent endoscopy between 2001 and 2011 in North Jakarta, metaplasia was found in 3.31% of *H. pylori* (-) patients and 9.23% of *H. pylori* (+) patients. Only 7 patients had gastric malignancies, and all of them were *H. pylori* (-). They concluded that there was an association between metaplasia and *H. pylori* infection. However, there was no association between gastric malignancy and *H. pylori* infection.²¹

According to Quach et al., the prevalence of gastric cancer is the highest in Vietnam (17/100.000) and the lowest in Indonesia (1/100.000). In Indonesia, ethnicities including Chinese, Batakese, and Minahasanese were considered as high-risk factors for the development of gastric cancer. In most Southeast Asian countries, a familial history of gastric cancer and precancerous gastric lesions were considered risk factors for gastric cancer development.²⁰

It was suggested that *H. pylori* infection decreases the risk of gastroesophageal reflux disease and its complications, including Barrett's esophagus, adenocarcinoma of the esophagus, and gastric cardia. A study conducted by Miftahussurur et al. in Surabaya involved 104 patients with dyspeptic syndrome in Indonesia between October 2015 and November 2015 who underwent endoscopic evaluation. GERD was diagnosed by endoscopic evaluation, and patients were asked to complete GERD-Q questionnaires. The presence of *H. pylori* strains was identified on the basis of histology, culture,

and immunohistochemistry. Of all patients, 53.8% were confirmed to have GERD. Only two patients (1.9%) were diagnosed as *H. pylori* positive, although both were also diagnosed with GERD. In conclusion, this study found a high prevalence of GERD in areas with a low *H. pylori* prevalence, although the association could not be measured due to an insufficient number of cases.¹²

CURRENT TREATMENT MODALITIES FOR *H. PYLORI* INFECTION

The currently available guidelines for dyspepsia with *H. pylori* infection management in Indonesia by Syam et al. (2017) recommend a minimum 7-day course of either triple or quadruple therapy for the first-line regimen, depending on the rate of resistance to clarithromycin in the affected area.²² The combination of a proton pump inhibitor (PPI), such as rabeprazole, lansoprazole, omeprazole, pantoprazole, or esomeprazole, to reduce gastric acid production and antibiotics effective against *H. pylori* is reported to be beneficial to eradicate the infection.

Triple therapy with PPI, amoxicillin, and clarithromycin/metronidazole remains the treatment of choice in areas with a rate of resistance of less than 20% to clarithromycin.²¹ Meanwhile, those residing in areas with high resistance to clarithromycin are suggested to undergo a culture and resistance test with samples isolated from an endoscopic specimen, or a molecular test directly through gastric biopsy, prior to treatment initiation. A quadruple therapy comprising bismuth subsalicylate, PPI, metronidazole, and tetracycline is recommended in such areas. Under circumstances where bismuth is not available, the same regimen can still be used without including bismuth.

When the first-line clarithromycin-based regimen fails, bismuth quadruple or triple therapy with PPI, levofloxacin, and amoxicillin are favorable as the second-line treatment, whereas in areas with high resistance to clarithromycin, the PPI-levofloxacin-amoxicillin regimen may be the first line of treatment.²²

A confirmation test needs to be conducted within at least 4 weeks after each treatment

course.²² A UBT or *H. pylori* stool antigen monoclonal test can be beneficial to identify any treatment failure.

ANTIBIOTIC RESISTANCE IN *H. PYLORI* INFECTION

The high prevalence of clarithromycin-, levofloxacin-, and metronidazole-resistant *H. pylori* remains a burden of many Asia-Pacific countries, as reported in a meta-analysis by Kuo et al.²² Among many other reasons, such as genetic polymorphisms and patient compliance, antibiotic resistance has been proposed as a major factor contributing to the low eradication rate of *H. pylori* infection after standard therapy.²⁴ Therefore, in *H. pylori* Management in ASEAN: The Bangkok Consensus Report, Mahachai et al. recommend that the first-line regimen should be customized for each region in accordance with its antibiotic resistance pattern.²⁵

Miftahussurur et al.²⁶ conducted a prospective study to evaluate resistance rates from August 2012 to November 2015 involving 849 adult dyspeptic patients who underwent endoscopy examinations in 11 cities spreading across the five largest islands of Indonesia. Among them, 77 strains of *H. Pylori* were isolated to undergo sensitivity assays for metronidazole, clarithromycin, levofloxacin, amoxicillin, and tetracycline. There were high rates of antibiotic resistance to metronidazole and levofloxacin (46.8% and 31.2%, respectively), whereas a lower prevalence of resistance to clarithromycin (9.1%), amoxicillin (5.2%), and tetracycline (2.5%) were observed. The patterns of resistance were also found to vary significantly among several ethnicities and regions. The highest rate of resistance to levofloxacin was found in Java (50%) and Sumatera (44.4%), while Sumatera also showed a significantly higher rate of metronidazole resistance (88.9%) than other islands. Of all ethnic groups, Ambonese were associated with significantly higher rates of metronidazole and tetracycline resistance compared with others. Moreover, high levels of clarithromycin resistance were also observed in Ambonese, Chinese, and Balinese. Only strains isolated from Dayak individuals were found to be sensitive to all antibiotics. The study

suggested that standard triple therapy based on metronidazole and levofloxacin may not be effective at eradicating *H. pylori* infection in Indonesia.

To investigate this matter further, Miftahussurur et al.²⁶ initiated a follow-up study where a total of 105 strains isolated from 1,039 dyspeptic subjects from August 2012 to February 2016 were analyzed in order to investigate possible alternative regimens for *H. pylori* infection in Indonesian regions with high rates of metronidazole and levofloxacin resistance. Sensitivity to five alternative antibiotics comprising rifaximin, rifabutin, furazolidone, garenoxacin, and sitafloxacin were assessed in this study. It was revealed that all strains were sensitive to rifabutin, furazolidone, and sitafloxacin. A mild rate of resistance to garenoxacin was found, whereas those treated with rifaximin showed a much higher rate (6.7% and 38.9% respectively). The resistance rates to both drugs were found to be the highest in Java. Garenoxacin resistance was found neither in Bali, Kalimantan, and Timor nor in those belonging to Ambonese, Balinese, Dayak, Javanese, Minahasanese, and Timor ethnic groups. In contrast, rifaximin resistance was found to be evenly distributed among all ethnicities. The study suggested that alternative therapies with furazolidone, rifabutin, and sitafloxacin might be considered as the treatment of choice to eradicate *H. pylori* in Indonesian regions with metronidazole and levofloxacin, as well as clarithromycin resistance.

In an attempt to identify the virulence factors that might contribute to the high prevalence of antibiotic-resistant *H. pylori* strains in Indonesia, genetic sequencing assays were conducted in both studies. An association between *H. pylori* resistance to several antibiotics and their genotypes were then discovered.^{26,27} Tetracycline-resistant *H. pylori* was shown to be significantly associated with CagA-positive strains, whereas those with genetic expression of VacA were associated with rifaximin resistance.

CONCLUSION

The prevalence of *H. pylori* infection in Indonesia is relatively low in comparison with

other Southeast Asian and Asian countries, as is the incidence of gastric cancer. We suggest that this phenomenon is due to East-Asian-type-cagA strains as a predominant strain in Indonesia that possesses a 6-bp deletion, resulting in less virulent disease. In areas with limited resources, the test-and-treat strategy is preferred to diagnose dyspeptic patients without alarming symptoms. To date in Indonesia, there is still no noninvasive examination proven to be significantly valuable for evaluating gastric mucosal status in *H. pylori* infected patients. Triple therapy remains the mainstay of treatment in areas of low resistance rates to clarithromycin, while a PPI-levofloxacin-amoxicillin regimen should be used in high-resistance areas. However, the Indonesian population also has high resistance rate to levofloxacin, especially in the regions of Java and Sumatera. Therefore, in these cases, furazolidone, rifabutin, and sitafloxacin might be considered as drugs of choice to eradicate *H. pylori*. A multicenter study involving a large number of samples should be conducted to determine the optimal *H. pylori* treatment regimen in Indonesia.

REFERENCES:

1. Syam AF, Miftahussurur M, Makmun D, et al. Risk factors and prevalence of *Helicobacter pylori* in five largest islands of Indonesia: a preliminary study. PLoS ONE;10(11):e0140186.
2. Hooi JKY, Lai WY, Ng WK, et al. Global prevalence of *Helicobacter pylori* infection: Systematic review and meta-analysis. Gastroenterology. 2017;153:420–9.
3. World Gastroenterology. World Gastroenterology Organisation Global Guideline: *Helicobacter pylori* in developing countries. J Clin Gastroenterol. 2011; 45:383–8.
4. Buruoa C, Axon A. Epidemiology of *Helicobacter pylori* infection. *Helicobacter*. 2017;22 (1).
5. Khoder G, Muhammad JS, Mahmoud I, Soliman SSM, Buruoa C. Prevalence of *Helicobacter pylori* and its associated factors among healthy asymptomatic residents in the United Arab Emirates. Pathogens. 2019;8(44);1-14.
6. Globocan Indonesia: Estimated age-standardized incidence and mortality rates: both sexes. [Internet] 2012 [cited 2016 Jan 20]. Available from: www. http://globocan.iarc.fr/Pages/fact_sheets_population.aspx
7. Gheelim, Lim KG, Palayan K. A review of gastric cancer research in Malaysia. Asian Pacific J Cancer Prevent. 2019;20(1):5.
8. Goto Y, Syam AF, Darnindro N, Puspita Hapsari FC. Risk factors for and prevalence of *Helicobacter Pylori* infection among healthy inhabitants in Northern Jakarta, Indonesia. Asian Pac J Cancer Prev. 2016;17(9):4469-75.
9. Darnindro N, Syam AF, Fauzi A, Rumende CM. Seroprevalence and socio-demographic factors of *Helicobacter pylori* infection in patients with dyspepsia in Kalibaru primary health care north Jakarta. Acta Med Indones. 2015;47(4):297–303.
10. Miftahussurur M, Syam AF, Makmun D, et al. *Helicobacter pylori* virulence genes in the five largest islands of Indonesia. Gut pathogens. 2015;7(1):26.
11. Darwin E, Murni AW, Nurdin AE. The Eff Darwin E, Murni AW, Nurdin AE. The effect of psychological stress on mucosal IL-6 and *Helicobacter pylori* activity in functional dyspepsia. Acta Medica Indonesiana. 2017 Apr;49(2):99-104.
12. Miftahussurur M, Doohan D, Nusi IA, et al. Gastroesophageal reflux disease in an area with low *Helicobacter pylori* infection prevalence. PloS one. 2018;13(11):e0205644.
13. Koncoro H, Wibawa IDN. Peptic ulcer disease different pathogenesis of duodenal and gastric ulcer. Indones J Gastroenterol Hepatol Digest Endosc. 2015;16(3);179-82.
14. Siregar GA, Halim S, Sitepu RR. Serum TNF- α , Il-8, VEGF levels in *Helicobacter pylori* infection and their association with degree of gastritis. Acta Medica Indonesiana. 2015;47(2).
15. Budyono C, Widita H, Herardi R, Syam AF. Association between severity of dyspepsia and urea breath test results in patients with positive *Helicobacter pylori* serology. Indones J Gastroenterol Hepatol Digest Endosc. 2016;19(2);79-82.
16. Nurdin W, Krisnuhoni E, Kusmardi. Comparison of *Helicobacter pylori* detection using immunohistochemistry and giemsa and its association with morphological changes in active chronic gastritis. Indones J Gastroenterol Hepatol Digest Endosc. 2016;17(1);21-7.
17. Miftahussurur M, Nusi IA, Akil F, et al, Adi P. Gastric mucosal status in populations with a low prevalence of *Helicobacter pylori* in Indonesia. PloS one. 2017;12(5):e0176203.
18. Dairi L, Siregar A, Sungkar T. The comparison of serum malondialdehyde level between *H. pylori positive* and *H. pylori negative gastritis* patients. Indones J Gastroenterol Hepatol Digest Endosc. 2018;19(1);3-6.
19. Quach DT, Vilaichone R, Vu KV, Yamaoka Y, Sugano K, Mahachai V. *Helicobacter pylori* infection and related gastrointestinal diseases in Southeast Asian Countries: an expert opinion. Asian Pac J Cancer Prev. 2018;19 (12):3565-9.
20. Darnindro N, Syam AF, Handjari DR, Makmun D. Gastric mucosa atrophy and metaplasia in patient with

- Helicobacter pylori* infection. Indones J Gastroenterol Hepatol Digest Endosc. 2015;16(1):13-6.
21. Tenggara R, Irawan VR. The association between metaplasia and gastric malignancy with *Helicobacter pylori* infection. Indones J Gastroenterol Hepatol Digest Endosc. 2017;18(2):94-7.
 22. Syam AF, Simadibrata M, Makmun D, Abdullah M, Fauzi A, Renaldi K, Maulahela H, Utari AP. National consensus on management of dyspepsia and *Helicobacter pylori* infection. Acta Medica Indonesiana. 2017;49(3):279.
 23. Kuo YT, Liou JM, El-Omar EM, et al. Primary antibiotic resistance in *Helicobacter pylori* in the Asia-Pacific region: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol. 2017;2(10):707-15.
 24. Vilaichone RK, Quach DT, Yamaoka Y, Sugano K, Mahachai V. Prevalence and pattern of antibiotic resistant strains of *Helicobacter pylori* infection in ASEAN. Asian Pacific J Cancer Prevent. 2018;19(5):1411.
 25. Mahachai V, Vilaichone RK, Pittayanon R, et al. *Helicobacter pylori* management in ASEAN: the Bangkok consensus report. J Gastroenterol Hepatol. 2018;33(1):37-56.
 26. Miftahussurur M, Syam AF, Nusi IA, et al. Surveillance of *Helicobacter pylori* antibiotic susceptibility in Indonesia: different resistance types among regions and with novel genetic mutations. PloS one. 2016;11(12):e0166199.
 27. Miftahussurur M, Waskito LA, Syam AF, et al. Alternative eradication regimens for *Helicobacter pylori* infection in Indonesian regions with high metronidazole and levofloxacin resistance. Infection and Drug Resistance. 2019;12:345-58.

Lung Transplantation as a way to Escape Pneumonia in Patients with COVID-19: Lessons from ARDS and Influenza

Reyhaneh Shams Khozani¹, Dorsa Shekouh², Sayyed Amirreza Hosseini³, Mehrnush Saghab Torbati⁴, Mahsa Noorian⁵, Reyhaneh Rabiei⁶, Qazal Mohseni³, Hossein Rostami Rad³, Shakiba Shaterzadeh Bojd⁷, Parastoo Hosseini⁸, Arina Ansari⁹, Kimia Godazandeh¹⁰, Niloofar Deravi^{7*}

¹ Department of Medical Biotechnology, Venom and Biotherapeutics Molecules Lab, Pasteur Institute of Iran, Tehran, Iran.

² Student Research Committee, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.

³ Student Research Committee, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.

⁴ Student Research Committee, School of Medicine, Zahedan Azad University of Medical Sciences, Zahedan, Iran.

⁵ Student Research Committee, School of Medicine, Hamadan University of Medical Science, Hamadan, Iran.

⁶ Student Research Committee, School of Medicine, Arak University of Medical Sciences, Arak, Iran.

⁷ Student Research Committee, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

⁸ Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

⁹ Student Research Committee, School of Medicine, North Khorasan University of Medical Sciences, Bojnurd, Iran.

¹⁰ UCL Medical School, UK.

Corresponding Author:

Niloofar Deravi, MD. SBUMS, Arabi Ave, Daneshjoo Blvd, Velenjak, Tehran 19839-63113, Iran. E-mail: niloofarderavi@sbmu.ac.ir

ABSTRAK

Pada masa kini, Coronavirus, yang disebut sebagai Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), virus yang mengancam jiwa dengan tingkat kematian yang tinggi (4,2%) dan belum ada pengobatan absolut, pada akhirnya dapat mengakibatkan sindrom gangguan pernafasan akut (ARDS). ARDS adalah salah satu komplikasi fatal, ditandai dengan infiltrasi paru dan hipoksemia berat. Kondisi ini dapat berkembang dari peradangan paru primer yang disebabkan oleh berbagai virus, terutama virus influenza, beberapa patogen manusia yang paling umum. Karena itu, banyak penelitian mengeksplorasi beberapa pendekatan untuk pengobatan ARDS. Transplantasi paru telah diklaim sebagai obat yang efisien untuk ARDS dan Influenza parah, yang juga dapat untuk mengobati komplikasi paru kritis pada SARS-CoV-2. Sepengetahuan kami, baru pertama kali studi untuk meninjau semua data yang tersedia tentang kemampuan transplantasi paru pada pengobatan pasien kritis dengan ARDS, influenza, dan SARS-CoV-2.

Kata kunci: SARS-CoV-2, COVID-19, ARDS, influenza, lung transplantation.

ABSTRACT

In this era, the novel Coronavirus, referred to as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), a life-threatening virus with a high mortality rate (4.2%) and with no absolute treatment as of yet, may ultimately result in acute respiratory distress syndrome (ARDS). ARDS is one of the fatal complications, highlighted by pulmonary infiltration and severe hypoxemia. This condition can be developed from primary lung inflammation caused by various viruses, particularly influenza viruses, some of the most common human pathogens. Due to this

issue, many studies explored several approaches for ARDS treatment. Lung transplantation has been claimed as an efficient cure for severe ARDS and Influenza, which can also be offered for treating critical lung complications of SARS-CoV-2. Thereupon, to the best of our knowledge for the first time, we aimed to review all available data about capability of lung transplantation for the treatment of critically ill patients with ARDS, Influenza, and SARS-CoV-2.

Keywords: SARS-CoV-2, COVID-19, ARDS, influenza, lung transplantation.

INTRODUCTION

The latest danger against global health is the current outbreak of the respiratory disease that was given the name Coronavirus Disease 2019 (COVID-19). The first cases of Covid-19 were reported in December 2019. This coronavirus was structurally related to the virus responsible for Severe Acute Respiratory Syndrome (SARS).^{1,2} An experimental antiviral medication, remdesivir, received Emergency Use Authorization by the US Food and Drug Administration in 2020 for patients afflicted with severe COVID-19.³ In addition, chloroquine and hydroxychloroquine were considered, as for decades they served as useful medications in prevention and treatment of chronic inflammatory diseases, including rheumatoid arthritis (RA).⁴ Despite their use in the treatment of viral respiratory infections, no strongly established evidence was found for the effectiveness of chloroquine/hydroxychloroquine medication on SARS or Middle East Respiratory Syndrome (MERS).⁵ In the lineup for new, more efficacious medical approaches, Lung transplantation (LT) is receiving more attention. LT is preferred for patients with end-stage lung related diseases or severe respiratory insufficiency. Also, bilateral lung recipients showed a higher survival rate.^{6,7} LT, as the only therapy for end-stage ARDS, provided the final choice for these patients to avoid death. Therefore, patients with irreversible pulmonary fibrosis, like post-COVID-19 ARDS, can be analyzed carefully for the possibility of a LT. In the Lancet section for Respiratory Medicine, Lang et al.⁸ provided a comprehensive report of a 44-year-old patient with COVID-19-associated ARDS, who underwent a pre-treatment transplant assessment process and was successfully treated with LT. In addition, for patients with persistent lung failure following COVID-19, in whom many weeks of supportive measures in the

intensive care unit (ICU) has not shown any significant improvement, LT was offered as a choice of treatment. Although this treatment is lifesaving, the true impact of LT in the acute cases of COVID-19 may have little consideration.⁹ According to the data and hypothesis that LT can be a beneficial treatment of ARDS and Influenza, its potential for curing end-stage COVID-19 patients should be assessed further. In this review, to the best of our knowledge for the first time we aimed to gather available data about the efficacy of LT in patients with respiratory diseases ARDS, Influenza, and COVID-19 to achieve a better perspective of this treatment in these patients.

CONSIDERATIONS IN A COVID-19 PATIENT EVALUATION FOR LT ELIGIBILITY

LT has been suggested as the savior treatment for selected COVID-19 patients present with persistent pulmonary disease despite several weeks or months of hospitalization in ICU.¹⁰ There are ten considerations that should be taken into account during a COVID-19 patient evaluation for LT candidacy. First, patients must not be older than 65, as cases of older patients showed poorer outcomes. Second, candidates for surgery must have single-organ dysfunction only. Third, adequate time must be considered for recovery.¹¹ Fourth, there must be radiological documentation of refractory lung disease. Fifth, the patient must be informed and able to discuss about transplantation. They should understand the impact of surgery on their quality of life. Sixth, patients must receive adequate physical health care while on the waiting list, because of better outcomes in such cases.^{12,13} Seventh, patients should be eligible for standard transplantation criteria such as sufficient body-mass index and absence of other notable comorbidities, including severe heart disease.¹⁴ Eighth, the patient's recent SARS-

CoV-2 Polymerase Chain Reaction (PCR) result should be negative. Based on observations made so far, the post-surgery fatality rate is remarkably higher for patients tested positive for infectious diseases, even in asymptomatic cases.¹⁵ Ninth, the surgical center should be qualified in performing high-risk surgeries, as this type of surgery on ARDS patients with Extracorporeal Membrane Oxygenation (ECMO) support is one of the highest-risk and most complicated procedures.¹⁶ Tenth, a wide donor pool should be accessible to qualified surgical centers to maximize survival chance of patients on the waiting-list. This will retain fair and impartial organ donor allocation and provide life-saving organ transplantation for cases that have the greatest chance of survival.¹⁷ Therefore, with ECMO support and fulfilling the aforementioned criteria, LT can be helpful in selected cases with severe, serious respiratory failure secondary to ARDS and COVID-19.

LT FOR COVID-19-ASSOCIATED ARDS IN PCR-POSITIVE PATIENTS

Although a large proportion of patients with COVID-19 develop asymptomatic or mild disease, about 10% transfer to ICU as a result of ARDS.^{18,19} Mortality rates of up to 60% have been reported for these patients.^{20,21} Lang et al.⁸ reported the first case of LT for a confirmed SARS-CoV-2 patient, a 44-year-old female patient with symptoms of cough and fever and nothing remarkable in her past medical history, except mild psoriatic arthritis which was treated previously, and a diagnosis of idiopathic CD4 lymphocytopenia with no clinical significance. According to the comprehensive examination and interdisciplinary discussions, the patient's lungs had no potential for recovery and based on following considerations, transplantation was approved: (1) negative viral culture and high real-time polymerase chain reaction (RT-PCR) Ct values, (2) more than one month after the onset of SARS-CoV-2 infection, (3) no available alternative procedure, (4) a young patient with a single-organ failure, (5) a pre-septic condition originated from the patient's lungs, and (6) no other obvious barriers for long-term recovery. A sequential bilateral LT was carried out, the

patient was transferred to the ICU in a stable condition, and was prone in order to improve the gas exchange and to decrease the pressure on the lower lobes of the lungs. Standard triple immunosuppression therapy was performed, and due to the fact that the patient was highly pre-sensitized, six additional treatment patterns of immune-absorption were done and antithymocyte globulin was provided. Regardless of the success of this procedure it is necessary to mention that most COVID-19 patients admitted to the ICU are older than the maximum age limit for transplantation or have other comorbidities that might not allow physicians to consider LT. This case report suggests that LT should be included in the therapeutic methods for COVID-19 positive patients with COVID-19-related ARDS. However, the applied criteria for patient selection and timing of LT still need to be validated in further studies.

PRE- AND POST-OPERATIVE MENTAL ASSESSMENT AND INTERVENTION FOR LT IN ELDERLY COVID-19 PATIENTS

Lyu et al.²² reported the postoperative mental status of two elderly COVID-19 patients in China who had no history of psychiatric disorders prior to the COVID-19 infection. The first patient, a 66-year-old woman without any history of chronic disease, underwent a bilateral LT and her status was observed for 28 days thereafter. She had developed symptoms such as restlessness and confusion 27 days before surgery which lasted for 7 days. After achieving full consciousness 8 days after surgery, only psychological supportive treatments were administered to enhance her mood and sedatives such as zolpidem (10 mg/day) and olanzapine (5 mg/day) were prescribed to treat her insomnia. The mental status of the second patient, a 70-year-old man with a 5-year history of hypertension and a 10-year history of diabetes, was observed for 21 days. Consciousness was fully recovered 6 days after the LT surgery and similar to the first patient, sedatives were prescribed to treat episodes of insomnia. By the time the observation period was over, no sign of any mental disturbance was declared. Muscle rehabilitation training started in both patients 10 days after operation. In regard

of these data, providing intensive supportive care may avoid post operation acute brain dysfunction in COVID-19 patients.

COVID-19 AND IMMUNE RESPONSES BEFORE AND AFTER LT

A few COVID-19 patients were diagnosed with more severe conditions, including acute lung injury (ALI) and ARDS. As previously mentioned, LT may be a beneficial method to protect patients from COVID-19-related ARDS.^{8,10} However, to attain safer outcomes, perioperative immune reactions of patients should be evaluated. The recent report by Yan et al.²⁰ surveyed the immune system reactions and the remnants of SARS-CoV-2 nucleic acids in two clinical cases before and after LT, including the very first case of LT for COVID-19 globally. They described two severely ill COVID-19 patients (group 1: a 58-year-old male with COVID-19-related ALI and ARDS) and (group 2: a 73-year-old male with COVID-19-associated multiple organ failure and ARDS). Both cases were admitted for LT, in order to be preserved from end-stage complications of COVID-19, in particular, ARDS. Whole blood lymphocytes, immunocytes (T, B, and NK cells), blood cytokines, and Ag-definite IgMs and IgGs of hospitalized COVID-19 patients were assessed. The number of lymphocytes in these cases remained below the standard amount before and after LT ($<1.1 \times 10^9/L$). The levels of blood immunocyte subclass CD3+ CD45+ T were under 60% and a decrease of CD8+ T cells was observed ($P < 0.01$). Furthermore, together with viral replication, the IL-6 and IL-10 ranges in plasma overstepped the maximum limit of normal values. Total concentrations of cytokine IL-10, IL-4, IL-6, IFN- γ , and TNF- α in a severe period of disease were considerably higher compared to the convalescence period. These findings display weak humoral and cellular immune reactions in patients infected with COVID-19. In pathological analyses, the results of the second patient illustrated vague mature CD3+ cells in tissues and large-scale fibrosis and mucosal necrosis in the bronchioles, and the number of IgA+ cells in epithelial cells declined in both

lung lobes. Residual COVID-19 in the lungs could be the main cause for the positive test result and mild symptoms in recovered SARS-CoV-2 patients, despite antiviral therapy. After the LT operation in severely ill COVID-19 patients (without antiviral treatments), there were no signs of COVID-19 infection in the transplanted lungs and no indication of residual COVID-19 nucleic acids observed on CT scans. Also, the humoral immune response was found to be negative. Moreover, according to Tabary et al.²³, COVID-19 is extremely devastating to the immune system, leading to the decrease of splenic T and B cell population as a result of necrosis and apoptosis. This may explain the incompetence of the immune system to protect the lungs from SARS-COV-2.

LT FOR ELDERLY PATIENTS WITH SEVERE COVID-19 PNEUMONIA

According to some studies, SARS-CoV-2 might result in irreversible loss of respiratory function in end-stage COVID-19 elderly patients.²⁴⁻²⁶ The International Society for Heart and Lung Transplantation (ISHLT) mentioned that LT is considered to be an efficient treatment for end-stage chronic lung disease.²⁷⁻²⁹ However, it is not recommended for patients with positive viral RNA tests since the ubiquitous virus may damage the transplanted lungs.^{8,30} Thus, the viral RNA test should be negative at least twice before surgery and the specimen tested should be extracted via sputum and bronchoalveolar lavage (BAL). Han et al.³¹ speculated that some factors such as blood transfusion or long-term medication, with high-doses of immunomodulators such as Thymalfasin, may be associated with early acute rejection. However, up to now, there hasn't been enough evidence to prove that irreversible lung injury may progress in SARS-CoV-2 lung infection patients.

Two patients with end-stage COVID-19 pneumonia who underwent LT were studied.³¹ The first case was a 66-year-old woman diagnosed with COVID-19, who received medication as well as convalescent plasma therapy, but showed no improvement in her declining respiratory condition. Afterwards, the patient was tested for

SARS-CoV-2, and due to negative result (despite her “white lung” radiographs) she was enlisted for LT. There were no detectable signs of pleural effusion after surgery, but the oxygenation index started to decrease. There were some complications pointing towards acute rejection, but after one dose of steroid pulse therapy, the patient’s oxygen saturation rapidly improved and the chest x-ray became clearer.

The other case was a 70-year-old man diagnosed with COVID-19, with progressive decline in his condition, despite receiving medication. His lungs in his most recent chest x-ray were described as a blurry X-ray. Furthermore, after testing negative on the SARS-CoV-2 nucleic acid tests, LT was performed. Outcomes were virtually the same as the first patient: demonstrating clear chest x-rays with no evidence of pleural effusion and residual COVID-19 in the lungs.³¹ Based on this study, LT could be an efficient intervention for end-stage COVID-19 patients when using medication, internal medicine guidelines, and mechanical ventilation (MV), where ECMO cannot ameliorate the lungs’ deteriorating condition.

LT FOR PROGRESSIVE COVID-19 RELATED PULMONARY FIBROSIS

LT could be a potential life-saving procedure for patients with currently non-resolving COVID-19.^{32,33} However, concerns limiting transplantation include recurrence of COVID-19 (or superinfecting pathogens associated with viral pneumonia), infection of the allograft, technical challenges imposed via virus-mediated injury to the patient’s lung, and possible risk of allograft infection with ventilator-associated pathogens causing pneumonia. Severe COVID-19 related damage to pulmonary vessels and pleura could create practical barriers to transplantation, and severe deconditioning due to long-term MV together with sedation and blockade of neuromuscular function. There remains a lack of adequate evidence about the possibility of lung recovery after severe COVID-19 pneumonia, and whether it results in better long-term outcomes than LT.³² In this regard, Bharat et al.³² reported the first results of two successful

LT surgeries in patients diagnosed with non-resolving SARSCoV-2 associated ARDS at the United States. Comparing the histology of explanted native lungs compared to the lungs of patients who expired from SARS-CoV-2 demonstrated some evidence of severe fibrosis in the former. The results of Single molecule Fluorescence In-Situ Hybridization (smFISH) for detecting COVID-19 RNA strands did not detect recurrent COVID-19 infection in the allograft. Therefore, LT may be the only choice for saving these patients.³² Their study reported a case of a female in her 20s diagnosed with SARSCoV-2 showing the symptoms of severe and treatment-resistant hypoxemia despite different interventions (such as endotracheal tube placement and MV), with the level of oxygen in her blood progressively decreasing. Thus, she was listed for LT and later underwent the bilateral LT surgery. Post-operative care included the patient’s current medication regime in addition to immunosuppressants. As well as her neurocognitive state, her muscular strength and endurance improved quickly following the transplantation. Two months after surgery, the patient was discharged home with oxygen saturation values of over 98% and was able to perform daily activities on her own.³²

Another case of a man in his 60s diagnosed with SARSCoV-2, who also suffered from comorbid conditions, including recurrent *Pseudomonas aeruginosa* pneumonia and hemothorax, was reported. Despite attempting different treatment approaches, no improvement in lung compliance and oxygen saturation was seen, therefore he was listed for LT. Despite the similarity between his first two independent intraoperative assessments, unexpected complexity was reported because of colliquative necrosis following COVID-19 related *Pseudomonas aeruginosa* pneumonia. Thirty days after transplantation surgery, the patient respired on room air with oxygen saturations above 97%, and his status, including consciousness and muscular strength, improved over time, under the care of rehabilitation services.³²

Moreover, according to the findings of this study, the lungs of both patients infected with SARS-CoV-2 were edematous and their

weight had significantly increased. Explanted lungs of both patients showed presence of pleural thickness and adherence, along with large bacterial cavities associated with necrosis. These bacteria were detected through PCR assay in BAL samples of the postoperative cultures. In addition, regions of diffuse alveolar hemorrhage, acute bronchopneumonia due to secondary bacterial infection, and uncommon micro thrombi were observed in the lungs of both cases. Lack of matrix organization was seen in the biopsies of patients who expired from severe SARS-CoV-2 pneumonia as well as in the sections of lung explants from patients who underwent transplantation.³²

LESSONS FROM LT TREATMENT FOR ARDS PATIENTS

According to American-European Consensus Conference (AECC), ARDS is the acute onset of decreasing oxygen levels in the blood, with bilateral intrusions on chest X-ray, without any evidence of left atrial hypertension.³⁴ Complications are seen as a result of sepsis, trauma, and pneumonia, although some patients may also have extra-pulmonary complications.³⁰ Coronaviruses that are highly pathogenic to humans such as SARS-COV, MERS-COV, and SARS-COV-2 have different symptoms, but one of the most important side effects is ALI or ARDS, which can even lead to death.³⁵ In patients with COVID-19, a phenomenon called cytokine storm is observed, which is also associated with lung damage, in which inflammatory cytokines are secreted in large quantities.³⁶ The exact mechanism of ARDS in COVID-19 patients is not known, but the abundant secretion of cytokines that induce inflammation such as IL-6, IL-1, and TNF- α may play an important role.³⁷⁻⁴⁰

Due to the critical condition of patients with ARDS, multiple studies were conducted on the effectiveness of various therapeutic interventions, including LT. Chang et al.⁴¹, in a retrospective study, identified 305 patients with ARDS, including 14 patients with an average age of 39 (SD 11) years and 8 females with no underlying disease. However, there were only 3 patients with underlying diseases, all of whom were utilizing MV. Most of these patients

were candidates for LT, the first major cause of ARDS in these patients being accidental inhalation of humidifier disinfectant and the second being pneumonia. The results of this study demonstrated that although LT may be able to lengthen the survival time of ARDS patients, it is in fact the final therapeutic option. More importantly, physicians should pay close attention to the reversibility of patients' lung function.

In another study conducted by Brichon et al.⁴² a 32-year-old woman with acute myeloid leukemia was first treated with chemotherapy and autologous bone marrow transplantation, and three months later received ruminant interleukin 2 (IL-2). Since the patient developed ARDS 4 days after receiving ruminant IL-2, MV was required. Although many efforts were made to treat the patient, her condition worsened, and LT was performed as a final resort. After 11 months of follow-up, it was found that the patient was in good condition. Therefore, in this study, it was suggested that LT can be a suitable method in some patients with ARDS. According to the promising results obtained from these studies, the use of LT for the treatment of patients with ARDS can be concluded, and it was anticipated that LT may be useful in the treatment of patients with COVID-19 who have ARDS.

LESSONS FROM LT TREATMENT FOR INFLUENZA PATIENTS

Invasive pulmonary aspergillosis (IPA) is an uncommon disease presented in pediatric cases with impaired immune systems. However, it may also develop secondary to severe influenza A pneumonia in adults who were previously healthy. While fatality and morbidity rate reported among children is high, patients are commonly given antifungal medication for IPA prior to radiologic and clinical resolution. In addition, ECMO, also known as extracorporeal life support (ECLS), is gradually developing as a promising treatment especially in patients with intense respiratory failure. Bates et al.⁴³ reported a critical care unit admission for a 15-year-old case with respiratory distress induced by influenza A subtype H3. Due to his rapidly deteriorating condition, MV was required for

him. Furthermore, the growth of *Aspergillus fumigatus* led to increased serum galactomannan, and despite the administration of antifungal medications, bilateral intensive pulmonary necrosis developed. Afterwards, he received veno-venous ECMO (VV-ECMO) support. Since multiple organ failure markedly lowered his chance of recovery, he was eligible for lung transplant. Thereby, the patient underwent successful bilateral LT operation after his medical support changed to a right ventricular assist device (RVAD) and oxygenator. 40 days later, he was discharged in a stable condition.

Influenza A virus subtype H1N1 provokes a broad range of clinical syndromes, including self-limited illness and worst of all, ARDS.⁴⁴ In fact, Influenza A is capable of rapid progression to ARDS and pulmonary fibrosis. Pulmonary fibrosis secondary to Influenza A Pneumonia may cause lung dysfunction, however it rarely warrants LT.^{45,46} Nonetheless, an established invaluable treatment for ARDS called ECMO is a bridging option for pulmonary transplantation, although its long-term usage is a highly controversial topic. Generally, influenza A is a communicable disease which presents with the same respiratory and gastrointestinal symptoms as COVID-19. To name a few: cough, fever, vomiting and diarrhea (particularly in pediatric patients). Manifestations vary from mild to severe, meaning some patients might show no symptoms while some might struggle with an irreversible stage of disease.⁴⁷

Also, Dr. Lisa Maragakis pointed out more similarities in addition to what has just been cited. Both infectious viruses can be easily transmitted during their incubation period, however, this period is much longer in COVID-19. Moreover, pneumonia may develop secondary to these infections. Medications commonly administered are antiviral drugs, not antibiotics. If both diseases progress into more severe stages, hospitalization and assisted ventilation may be required.⁴⁸ In the following, few cases infected with different subtypes of influenza virus who eventually recovered due to LT will be reviewed. Qi Wang et al. reported a successfully performed bilateral LT in a 45-year-old man with H1N1-induced ARDS and progressive fibrosis who had

received ECMO for the last 45 days before the operation.⁴⁹ His hospitalization and treatment processes are described below:

With no underlying lung disease, his only symptoms were cough and fever. As H1N1 pneumonia was diagnosed, he received oseltamivir. Evidence of bilateral pulmonary infiltration confirmed ARDS indicating the need for ventilation and ECMO. Also, tracheostomy along with continuous antibiotic therapy was used. Despite experiencing subsequent septic episodes, the H1N1 test result was negative, thus several antibiotics were administered. Even with immediate treatment measures for pulmonary fibrosis, the lungs were continuing to deteriorate. Therefore, he was included in the list of urgent recipients of LT and after about one week, a suitable 16-year-old donor free of infection was available. Ultimately, bilateral LT was operated successfully. On the first day after the operation, there was no longer a need for ECMO, on the 4th-day the ventilator was withdrawn, and on the 15th-day non-invasive ventilation via tracheostomy tube was stopped. Despite postoperative complications, including empyema, right ventricular (RV) failure, acute dysfunction of the liver and kidney, he recovered and was discharged 65 days later. After seven months, his condition was well and he could live independently, but he had signs of slightly reduced lung capacity plus elevated blood creatinine due to anti-rejection drugs.²

INFLUENZA 1

A severe respiratory illness associated with H1N1 virus, was identified in March 2009 in Mexico.⁵⁰ Rapid viral testing and PCR, with broad range of sensitivity, were used to detect this virus. Al Aklabi et al.² reported a case of a 50-year-old man who suffered from chronic obstructive pulmonary disease and end stage lung disease due to α 1-antitrypsin deficiency. He was hospitalized and listed for bilateral LT, but 48 hours after the injection of latent H1N1 virus vaccine, he displayed non-specific symptoms such as malaise and myalgia. A nasopharyngeal swab was taken for PCR testing, then following the positive test result and ISHLT recommendations^{51,52}, oseltamivir was started.

After transplantation, two samples of BAL from the transplanted lung happened to be positive for H1N1 virus, resulting in antiviral and anti-rejection therapy prescription based on regular protocols which previously resulted in promising outcomes. Finally, he was discharged home in a good condition 21 days after the operation. Thus, it can be concluded that LT can be successful in pandemic periods if careful clinical evaluation and laboratory screenings are performed on both donors and recipients.

INFLUENZA 2

Patients with suppressed immune system are at high risk for developing severe influenza A (H1N1) disease. D. P. Mason et al.⁵³ reported a case of 65-year-old man who suffered from end-stage interstitial lung disease and was admitted for unilateral transplantation. Before his operation, the recipient complained of a mild sore throat with no fever, malaise, and rhinorrhea, and had a normal chest x-ray. RT-PCR for influenza was positive 17 days after original nasopharyngeal swab; however postoperative BAL cultures were negative for both allograft and native lungs. 4 hours after transplantation he became agitated due to a body temperature of 40.0°C thus Oseltamivir (Tamiflu) was started empirically. During 48 hours, he developed worsening hypoxemia and increasing consolidation of the allograft. He received Peramivir after consideration of the high risk of renal insufficiency, disseminated intravascular coagulation and interrupted limb ischemia. Seventy-two hours after starting peramivir, he began to recover dramatically and chest x-rays gradually cleared. Despite these positive outcomes, the overall prognosis was assessed as poor and he died 22 days after the operation. This case report shows the important lasting threat of influenza H1N1 for patients who receive thoracic organ transplants, especially unilateral LT because of the airborne nature of the pathogen. Therefore, recommendations of ISHLT should be taken seriously: avoidance of contact with symptomatic individuals, vaccination of all H1N1 influenza patients listed for transplantation, and recipients who show even subtle signs and symptoms of upper respiratory

infection or unusual infectious symptoms should be swabbed for H1N1, scheduling surgery only when results are available.⁵¹

Fever, shortness of breath, cough, expectoration, as well as laboratory tests, such as Lymphopenia and an increased level of C-reactive protein are similar both influenza and COVID-19.⁵⁴ So it can be hypothesized that due to success in LT in influenza, the use of LT in COVID-19 cases may be useful too, but caution should be exercised when selecting patients, ensuring that they meet 10-item criteria (previously mentioned), including a negative RT-PCR test before the operating in both the donor and recipient, due to high risk of severe infection.

CONCLUSION

Despite, a large number of efforts to find treatments for critically ill patients with COVID-19 in the medical world, there is no effective treatment for end-stage SARS-CoV-2 infected patients. Moreover, conventional antiviral therapies may not be beneficial and some patients experience the end-stage acute respiratory syndrome. LT provided the potential effectiveness for the treatment of these patients and rescued them from death. Since SARS-CoV-2 has similarities to the flu and ARDS, and a number of articles have recently begun to examine the positive effect of LT on the treatment of COVID-19. Therefore, this review aimed to collect all the data on this subject, gaining experiences from ARDS and the flu for LT. Satisfactory results after LT in improving the general condition have arisen the researcher's efforts to find a better method for the treatment of acute respiratory syndrome COVID-19. This review shows that LT could be used when medical treatments consisting of ventilation and ECOM could not modify the lung action., and could be a promising choice.

CONFLICTS OF INTEREST

There is no conflict of interest on this article.

REFERENCES

1. Fauci AS, Lane HC, Redfield RR. COVID-19—navigating the uncharted. *Mass Medical Soc.* 2020.
2. Al Aklabi MM, Weinkauff JG, Humar A, Ghorpade

- N. Successful bilateral lung transplantation in a patient with end-stage lung disease and positive novel influenza virus (H1N1). *J Heart Lung Transplant*. 2010;29(8):898-9.
3. McCreary EK, Angus DC. Efficacy of Remdesivir in COVID-19. *JAMA*. 2020;324(11):1041-2.
 4. Ben-Zvi I, Kivity S, Langevitz P, Shoenfeld Y. Hydroxychloroquine: from malaria to autoimmunity. *Clin Rev Allergy Immunol*. 2012;42(2):145-53.
 5. Sanders JM, Monogue ML, Jodlowski TZ, Cutrell JB. Pharmacologic treatments for Coronavirus disease 2019 (COVID-19): a review. *JAMA*. 2020;323(18):1824-36.
 6. Meyers BF, Lynch J, Trulock EP, Guthrie TJ, Cooper JD, Patterson GA. Lung transplantation: a decade of experience. *Annals of Surgery*. 1999;230(3):362.
 7. Uhlin M, Mattsson J, Maeurer M. Update on viral infections in lung transplantation. *Curr Opin Pulm Med*. 2012;18(3):264-70.
 8. Lang C, Jaksch P, Hoda MA, et al. Lung transplantation for COVID-19-associated acute respiratory distress syndrome in a PCR-positive patient. *Lancet Respir Med*. 2020;8(10):1057-60.
 9. Cypel M, Keshavjee S. When to consider lung transplantation for COVID-19. *Lancet Respir Med*. 2020;8(10):944-6.
 10. Chen JY, Qiao K, Liu F, et al. Lung transplantation as therapeutic option in acute respiratory distress syndrome for coronavirus disease 2019-related pulmonary fibrosis. *Chin Med J (Engl)*. 2020;133(12):1390-6.
 11. Luyt C-E, Combes A, Becquemin M-H, et al. Long-term outcomes of pandemic 2009 influenza A (H1N1)-associated severe ARDS. *Chest*. 2012;142(3):583-92.
 12. Benazzo A, Schwarz S, Frommlet F, et al. Twenty-year experience with extracorporeal life support as bridge to lung transplantation. *J Thoracic Cardiovasc Surg*. 2019;157(6):2515-25. e10.
 13. Hoetzenecker K, Donahoe L, Yeung JC, et al. Extracorporeal life support as a bridge to lung transplantation—experience of a high-volume transplant center. *J Thoracic Cardiovasc Surg*. 2018;155(3):1316-28. e1.
 14. Weill D, Benden C, Corris PA, et al. A consensus document for the selection of lung transplant candidates: 2014—an update from the Pulmonary Transplantation Council of the International Society for Heart and Lung Transplantation. *J Heart Lung Transplant*. 2015;34(1):1-15.
 15. Doglietto F, Vezzoli M, Gheza F, et al. Factors associated with surgical mortality and complications among patients with and without Coronavirus disease 2019 (COVID-19) in Italy. *JAMA Surgery*. 2020.
 16. Bittner HB, Lehmann S, Rastan A, et al. Outcome of extracorporeal membrane oxygenation as a bridge to lung transplantation and graft recovery. *Annals Thoracic Surg*. 2012;94(3):942-50.
 17. Spagnolo P, Balestro E, Aliberti S, et al. Pulmonary fibrosis secondary to COVID-19: a call to arms? *Lancet Respir Med*. 2020.
 18. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395(10223):497-506.
 19. Remuzzi A, Remuzzi G. COVID-19 and Italy: what next? *Lancet*. 2020.
 20. Arentz M, Yim E, Klaff L, et al. Characteristics and outcomes of 21 critically ill patients with COVID-19 in Washington State. *JAMA*. 2020;323(16):1612-4.
 21. Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med*. 2020.
 22. Lyu H, Han W, Wang D, Liang T, Hu S. Perioperative mental evaluation and intervention for lung transplantation in elderly patients with COVID-19. *Psychiatry Clin Neurosci*. 2020;74(10):568-9.
 23. Tabary M, Khanmohammadi S, Araghi F, Dadkhahfar S, Tavangar SM. Pathologic features of COVID-19: A concise review. *Pathology-Research and Practice*. 2020:153097.
 24. Guan WJ, Ni ZY, Hu Y, et al. Clinical characteristics of Coronavirus disease 2019 in China. *N Engl J Med*. 2020;382(18):1708-20.
 25. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in different types of clinical specimens. *JAMA*. 2020;323(18):1843-4.
 26. Wu C, Chen X, Cai Y, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with Coronavirus disease 2019 pneumonia in Wuhan, China. *JAMA Intern Med*. 2020;180(7):934-43.
 27. Dhochak N, Singhal T, Kabra SK, Lodha R. Pathophysiology of COVID-19: Why children fare better than adults? *Indian J Pediatr*. 2020;87(7):537-46.
 28. Mehra MR, Canter CE, Hannan MM, et al. The 2016 International Society for Heart Lung Transplantation listing criteria for heart transplantation: A 10-year update. *J Heart Lung Transplant*. 2016;35(1):1-23.
 29. Yusef RD, Edwards LB, Kucheryavaya AY, et al. The registry of the International Society for Heart and Lung Transplantation: Thirty-second Official Adult Lung and Heart-Lung Transplantation Report--2015; Focus Theme: Early Graft Failure. *J Heart Lung Transplant*. 2015;34(10):1264-77.
 30. de Wit E, van Doremalen N, Falzarano D, Munster VJ. SARS and MERS: recent insights into emerging coronaviruses. *Nat Rev Microbiol*. 2016;14(8):523-34.
 31. Han W, Zhu M, Chen J, et al. Lung transplantation for elderly patients with end-stage COVID-19 pneumonia. *Ann Surg*. 2020;272(1):e33-e4.
 32. Bharat A, Querrey M, Markov NS, et al. Lung transplantation for pulmonary fibrosis secondary to severe COVID-19. *medRxiv*. 2020.
 33. van der Mark SC, Hoek RAS, Hellemons ME. Developments in lung transplantation over the past decade. *Eur Respir Rev*. 2020;29(157).

34. Ranieri VM, Rubenfeld GD, Thompson BT, et al. Acute respiratory distress syndrome: the Berlin definition. *JAMA*. 2012;307(23):2526-33.
35. Quan C, Li C, Ma H, Li Y, Zhang H. Immunopathogenesis of Coronavirus-induced acute respiratory distress syndrome (ARDS): Potential infection-associated hemophagocytic lymphohistiocytosis. *Clin Microbiol Rev*. 2020;34(1).
36. Ragab D, Salah Eldin H, Taeimah M, Khattab R, Salem R. The COVID-19 cytokine storm; What we know so far. *Front Immunol*. 2020;11:1446.
37. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395(10223):497-506.
38. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet*. 2020;395(10223):507-13.
39. Lai CC, Shih TP, Ko WC, Tang HJ, Hsueh PR. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges. *Int J Antimicrob Agents*. 2020;55(3):105924.
40. Cron RQ, Behrens EM. Cytokine storm syndrome. 2019.
41. Chang Y, Lee SO, Shim TS, et al. Lung Transplantation as a therapeutic option in acute respiratory distress syndrome. *Transplantation*. 2018;102(5):829-37.
42. Bricton PY, Barnoud D, Pison C, Perez I, Guignier M. Double lung transplantation for adult respiratory distress syndrome after recombinant interleukin 2. *Chest*. 1993;104(2):609-10.
43. Bates AR, Hanot J, Carroll A, Muhielidn A, Anton N, Lequier L. A pediatric case of prolonged extracorporeal support and successful lung transplantation following invasive pulmonary Aspergillosis and Influenza infection. D51 PEDIATRIC CASES II: American Thoracic Society; 2016. p. A7249-A.
44. Influenza WCotWCoCAoP. Clinical aspects of pandemic 2009 influenza A (H1N1) virus infection. *New Engl J Med*. 2010;362(18):1708-19.
45. Shieh W-J, Blau DM, Denison AM, et al. 2009 pandemic influenza A (H1N1): pathology and pathogenesis of 100 fatal cases in the United States. *The American journal of pathology*. 2010;177(1):166-75.
46. Weill D, Benden C, Corris PA, et al. A consensus document for the selection of lung transplant candidates: 2014—an update from the Pulmonary Transplantation Council of the International Society for Heart and Lung Transplantation. Elsevier; 2015.
47. Organization WH. Coronavirus disease (COVID-19): Similarities and differences with influenza 2020.
48. Lisa Lockerd Maragakis MD, M.P.H. Coronavirus Disease 2019 vs.the Flu 2020 [Available from: <http://www.who.int/westernp>].
49. Wang Q, Pan S, Zhang S, Shen G, Huang M, Wu M. Lung transplantation in pulmonary fibrosis secondary to Influenza A pneumonia. *Annals Thoracic Surg*. 2019;108(4):e233-e5.
50. Perez-Padilla R dIR-ZD, Ponce de Leon S, et al. Pneumonia and respiratory failure from swine-origin influenza A (H1N1) in Mexico. *N Engl J Med*. 2009;361:680-9.
51. Danziger-Isakov LA, Husain S, Mooney ML, Hannan MM. The novel 2009 H1N1 influenza virus pandemic: unique considerations for programs in cardiothoracic transplantation. *J Heart Lung Transplant*. 2009;28(12):1341-7.
52. Hajjar LA, Schout D, Galas FR, et al. Guidelines on management of human infection with the novel virus influenza A (H1N1)--a report from the Hospital das Clínicas of the University of São Paulo. *Clinics (Sao Paulo)*. 2009;64(10):1015-24.
53. Mason DP, Murthy SC, Yun JJ, et al. Lung transplantation in a recipient with novel 2009 H1N1 influenza: lessons learned. *Thorac Cardiovasc Surg*. 2011;59(2):126-7.
54. Yin Z, Kang Z, Yang D, Ding S, Luo H, Xiao E. A comparison of clinical and chest CT findings in patients with Influenza A (H1N1) virus infection and Coronavirus disease (COVID-19). *AJR Am J Roentgenol*. 2020;215(5):1065-71.

Black Fungus Complicated with COVID-19 in a Man with Underlying Non-Hodgkin's Lymphoma

Erni Juwita Nelwan^{1,2}, Rahajeng N. Tunjungputri², Retno S. Wardani³, Retno Wahyuningsih⁴

¹Division of Tropical and Infectious Disease. Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

²Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

³Department of Ear, Nose and Throat, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

⁴Department of Parasitology, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

Corresponding Authors:

Erni Juwita Nelwan, MD., PhD. Division of Tropical and Infectious Disease. Department of Internal Medicine Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital. Jl. Diponegoro no. 71, Jakarta 10430, Indonesia. Email: e.nelwan@gmail.com.

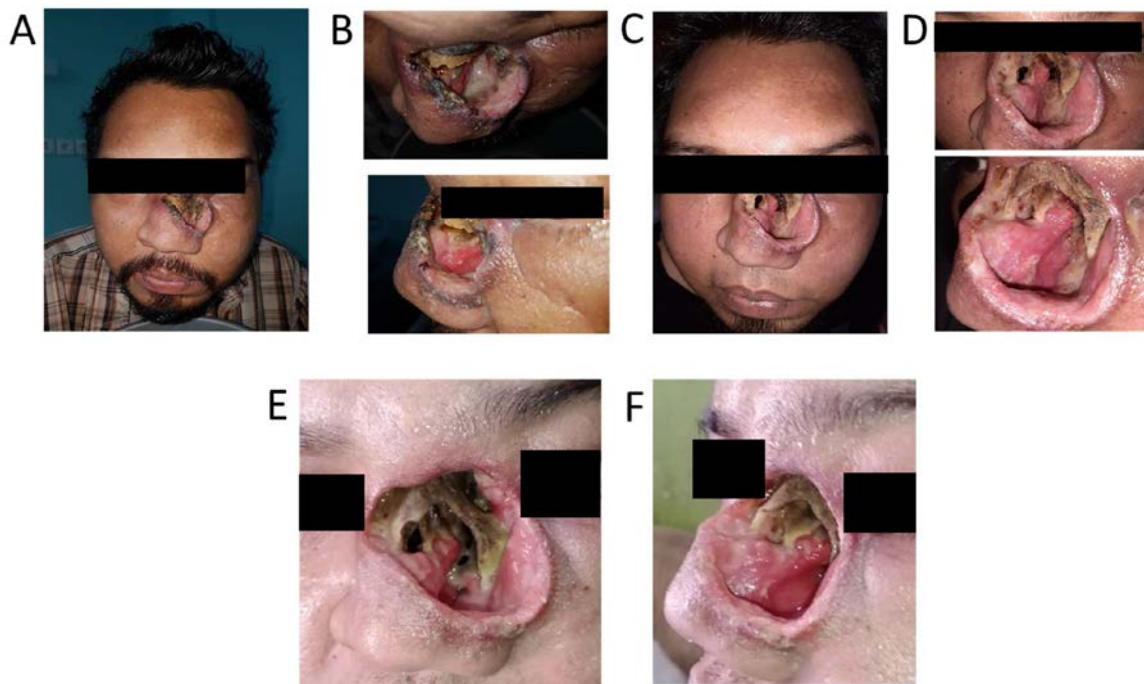


Figure 1. (A,B) The patient upon first presentation at the outpatient clinic in July 2020. (C-F) The patient after the first debridement in September 2020 during protracted hospitalization period due to his contracting COVID-19.

COVID-19 is a disease reported to suppress cellular immunity.¹ This may lead to the development of opportunistic infections,

among others black fungus, or mucormycosis. On the other hand, pre-existing defect in immunity may render patients susceptible to both

mucormycosis and COVID-19. Mucormycosis is a relatively rare fungal infection with rapid progression unless diagnosed promptly and treated adequately. Urgent surgical and medical intervention is lifesaving.² The manifestation of mucormycosis largely depends on the presence of exposure to the pathogen and the existing risk factor of the host. As black fungus is locally invasive, the majority of cases will involve tissue damage with local destruction and contiguous spread to nearby structure. We present a case of black fungus complicated with COVID-19 in a man with underlying non-Hodgkin's lymphoma.

A 34 year-old man initially visited the ENT outpatient clinic in June 2020 due to the chief complaint of pain on the left side base of the nose, and swelling of the left eye. The patient developed an open wound on the base of the nose which grew larger. He complained of congestion and pain on the left side of the nose, and greenish discharge from the nose every day, with irregular pattern of fever. The CT scan revealed soft tissue lesion on the anterior nasal cavity especially on the right side, which eroded the nasal bone, with the suspicion of mass or chronic infection; pansinusitis with erosion of the left ethmoid and maxillaris sinus, left nasal bone erosion with fistulation and ulceration to the left soft tissue nasal region and medial to the left orbita; as well as thickening of the cutaneous and subcutaneous tissue of the nasomaxillar region to the left inferior palpebra. Nasoendoscopy revealed a fistula on the left dorsal nasal cavity and suspicion of black fungus infection. Surgical and nasoendoscopic debridement was performed. As the endoscopic appearance suggest mucormycosis, and microscopic examination revealed the presence of spores and hyphae. Upon culture, dematiaceae (black fungus) colony growth was found. The patient was discharged after two-month of hospitalization for wound care and diagnosis.

He was planned to receive voriconazole or amphotericin B, and therefore he was re-admitted in September 2020. However, during this hospital admission the patient was found to be PCR positive for SARS-COV-2 due to contact tracing from another patient in the same ward, and he was promptly moved to the COVID-19

isolation ward for further management. He did not develop new COVID-19 symptoms other than fever and myalgia. Although the antifungal treatment was resumed in this period and initial debridement and repeat biopsy sample was taken, he did not receive timely nor extensive surgical debridement due to the logistical challenges related to his admission in the COVID-19 isolation ward. The patient was discharged after 21 days in late October 2021 and negative PCR SARS-COV-2 result and planned for a follow up outpatient visit. The pathology anatomy evaluation of the septal tissue obtained from nasal lesion the type NK/T-cell lymphoma although result was only available after the patient was discharged.

During the COVID-19 pandemic, co-infections of any viral, bacterial or fungal pathogen with SARS-COV-2 is very likely. In unfortunate instances such as the case presented in this report, hospital-acquired COVID-19 although did not cause additional morbidity or severe symptoms, to some extent led to suboptimal management of the fungal infection such as delaying completion of the treatment regimen or in performing extensive surgical debridement required in the management of invasive mucormycosis. Additionally, the patient was only later diagnosed with non-Hodgkin's lymphoma after repeat biopsy was taken, revealing a host risk factor for invasive fungal disease and risk of deterioration in the patient. Suboptimal or delayed diagnosis and treatment of non-COVID-19 diseases have been reported worldwide even with effective resource allocation.^{3, 4} It is important to therefore to identify cases in which delay in the management of non-COVID-19 disease could potentially be life-threatening or leading to disease progression and prioritize delivery of care in these patients.

REFERENCES

1. Liu J, Yang X, Wang H, et al. Analysis of the long-term impact on cellular immunity in COVID-19-recovered individuals reveals a profound NKT cell impairment. 2021;12(2):e00085-21.
2. Cornely OA, Alastruey-Izquierdo A, Arenz D, et al. Global guideline for the diagnosis and management of mucormycosis: an initiative of the European

- Confederation of Medical Mycology in cooperation with the Mycoses Study Group Education and Research Consortium. 2019;19(12):e405-e21.
3. Li Z, Jiang Y, Yu Y, Kang QJRM, Policy H. Effect of covid-19 pandemic on diagnosis and treatment delays in urological disease: Single-institution experience. 2021;14:895.
 4. Patt D, Gordan L, Diaz M, et al. Impact of COVID-19 on cancer care: how the pandemic is delaying cancer diagnosis and treatment for American seniors. 2020;4:1059-71.

The Role of Online Psychotherapy in COVID-19: An Evidence Based Clinical Review

Hamzah Shatri^{1,4}, Oryza Gryagus Prabu², Erpryta Nurdia Tetrasiwi², Edward Faisal¹, Rudi Putranto¹, Raden Irawati Ismail³

¹ Division of Psychosomatic and Palliative, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia, Cipto Mangunkusumo National Hospital Jakarta, Indonesia

² Resident of Internal Medicine, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia, Cipto Mangunkusumo National Hospital, Jakarta, Indonesia.

³ Department of Psychiatry, Faculty of Medicine, Universitas Indonesia dr. Cipto Mangunkusumo National Referral Hospital, Jakarta, Indonesia.

⁴ Clinical Epidemiology Unit, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia-RSUPN Cipto Mangunkusumo, Jakarta, Indonesia.

Corresponding Author:

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

ABSTRAK

Latar belakang: COVID-19 merupakan penyakit infeksi yang mudah menyebar dan memiliki manifestasi klinis penyakit ringan, sedang, atau berat. Pasien COVID-19 harus diisolasi selama perawatan untuk mengurangi penularan. Hal ini dapat menyebabkan kecemasan dan depresi, yang selanjutnya memperburuk penyakit pasien. Pemberian psikoterapi suportif dapat membantu memberikan rasa aman, nyaman dan tenang bagi pasien. Pemilihan metode dalam pemberian psikoterapi suportif dapat dilakukan secara telekonsultasi atau berbasis internet. Kajian klinis ini bertujuan untuk mengetahui pengaruh telekonsultasi atau telepsikoterapi berbasis internet terhadap pasien COVID-19. **Metode:** Pencarian literatur dilakukan dengan menggunakan database penelitian, seperti PubMed, Cochrane, EBSCO/CINAHL dan ProQuest. Artikel yang diidentifikasi disaring menggunakan kriteria kelayakan. Ada 2 penelitian (Zhou dkk. dan Wei dkk.) yang dianalisis secara kritis menggunakan Newcastle Ottawa Scale. **Hasil:** Kedua penelitian tersebut menunjukkan bahwa penatalaksanaan psikoterapi melalui telekonsultasi maupun berbasis internet pada pasien COVID-19 dapat membantu meringankan gejala psikologis pasien. Zhou dkk. mempelajari 63 suspek COVID-19 dengan 23,8% (n = 15) memiliki skor Hospital Anxiety Depression Scale (HADS) 8 atau lebih. Terdapat penurunan yang signifikan pada skor HADS-Anxiety (HADS-A) (p <0,01) dan skor HADS-Depresi (HADS-D) (p <0,01) pada 15 pasien, dan dari skor HADS keseluruhan (p <0,01). Wei dkk. Menunjukkan skor 17-HAMD dan HAMA pada kelompok intervensi psikologis online juga menunjukkan penurunan yang signifikan dalam gejala depresi dan kecemasan dibandingkan dengan kontrol. **Kesimpulan:** Psikoterapi melalui telekonsultasi daring atau berbasis internet pada pasien COVID-19 dapat membantu meringankan gejala kecemasan dan depresi serta efektif dalam menangani komplikasi psikologis pada pasien COVID-19.

Kata kunci: ansietas, depresi, COVID-19, telekonsultasi, telepsikoterapi.

ABSTRACT

Background: COVID-19 is an infectious disease that is easily widespread and has clinical manifestations as mild, moderate, or severe disease. COVID-19 patients are required to be isolated during treatment to reduce transmission. This can cause anxiety and depression, which in turn worsens the patient's illness. Providing supportive psychotherapy can help provide a feeling of safety, comfort and calm for patients. The choice of method in providing supportive psychotherapy can be done online/teleconsultation or internet-based. This clinical review aims to determine the effect of online teleconsultation or internet-based psychotherapy on COVID-19 patients. **Methods:** A systematic search was performed using online databases, such as PubMed, Cochrane, EBSCO/CINAHL and ProQuest. The identified articles were screened using eligibility criteria. There were 2 studies (Zhou et al, and Wei et al) which were analyzed critically using the Newcastle Ottawa Scale. **Results:** Both studies showed that management of psychotherapy through teleconsultation or internet-based on COVID-19 patients can help relieve the patient's psychological symptoms. Zhou et al studied 63 suspected COVID-19 with 23.8% ($n = 15$) having a Hospital Anxiety Depression Scale (HADS) score of 8 or more. There was a significant decrease in HADS-Anxiety (HADS-A) scores ($p < 0.01$) and HADS-Depression (HADS-D) scores ($p < 0.01$) in 15 patients, and from the overall HADS scores ($p < 0.01$). Wei et al. Showed 17-HAMD and HAMA scores in the online psychological intervention group also showed a significant reduction in symptoms of depression and anxiety compared to controls. **Conclusion:** Psychotherapy through online teleconsultation or internet-based on COVID-19 patients can help relieve symptoms of anxiety and depression and teleconsultation and also effective in dealing with psychological complications in patients with COVID-19.

Keywords: anxiety, depression, COVID-19, teleconsultation, telepsychoteraphy.

INTRODUCTION

SARS-CoV2 or Severe Acute Respiratory Syndrome Coronavirus-2 was at the end of 2019 causing a pneumonia epidemic in the city of Wuhan, the capital of Hubei province spreading throughout China. After infecting and causing death to thousands of people in China, the virus spread to European countries, the United States, Asia, including Indonesia.¹ The World Health Organization (WHO) declared COVID-19 a global pandemic of SARS-CoV2, in the month of March 2020. As of December 29, 2020, COVID-19 had infected 81 million people, with a death rate of 2.18%. In Indonesia, the first cases were reported in March 2020, until the end of December 2020 there were 719,219 cases with 21,452 fatalities (2.98%).² Symptoms of COVID-19 include fever, chills, cough, sore throat, myalgia, nausea and vomiting, and diarrhea. However, there are some severe symptoms which can lead to respiratory failure, heart failure, and even death, especially in patients with comorbidities.³

Apart from the physical abnormalities that occur, COVID-19 can have serious effects on the psychological health of patients. The

psychological outcomes observed in the COVID-19 pandemic can occur in individuals, communities, nationally and internationally. In individuals, it creates fear around getting infected, which may lead to debilitating disease resulting in helplessness and even death. Pandemics have serious effects on psychological health that can lead to psychosomatic crises conditions. In addition, patients who are in isolation or quarantine can also experience anxiety, anger, stress, irritability, insomnia that develop into fatigue and depression. The pandemic may also give rise to other symptoms of psychological trauma such as post-traumatic stress reactions, hyperactivity and attention disorders, and mood disorders.⁴

As COVID-19 is spread through direct transmission and the key in breaking the chain of infection is to keep distance and use protective equipment, this may cause problems with dispensing person-to-person care such as psychotherapy. One alternative is to use online technology to allow psychotherapy for patients with confirmed COVID-19 who have psychological problems.⁵ This paper will conduct a clinical review of the role of online

psychotherapy (telepsychotherapy) in patients with COVID-19.

METHODS

Protocol

This review follows established evidence-based clinical review guidelines to ensure clarity and transparency. The topic should be currently relevant and of common clinical interest. The literature search is conducted, using the following databases: PubMed, Cochrane, EBSCO/CINAHL, and ProQuest and also included are additional hand-picked sources of information related to the study.^{6,7}

Eligibility Criteria

The inclusion criteria for this clinical review includes experimental studies, longitudinal, and descriptive cohorts of teleconsultative management of COVID-19 patients. The exclusion criteria are studies of COVID-19 patients without teleconsultation management or teleconsultation in non-COVID-19 context.

Search Strategy

A systematic literature search was carried out on 25 December 2020, using the following databases: PubMed, Cochrane, EBSCO/CINAHL, and ProQuest; using the keywords attached to **Table 1**. The selection of literature is limited to English or Indonesian literature.

Data Collection

From each study, study citations, basic characteristics of the included subjects, the interventions performed, and the study results were collected. Characteristics of each study are also collected, such as: study design, study location, and patient characteristics (age, sex,

sample size, other comorbidities).

Quality Assessment and Data Synthesis

Two independent reviewers conducted independent quality assessments of the collected studies (ENT and OGP). Inclusion studies were critically reviewed using the Newcastle Ottawa Quality Assessment Scale (NOS). If there are differences, the NOS value will be discussed between the two reviewers until they reach a conclusion. A high-quality study is defined as one that meets a minimum NOS score of 7.

Data was synthesized based on 2 distinct, high-quality studies with consistent findings. The collected data were analysed by considering the variable analysis method used, number of studies, study results, odds/hazard ratios, and confidence intervals.

RESULTS

The literature search was carried out using the keywords attached to **Table 1** which resulted in 190 studies. Of the 190 studies identified, 90 articles were obtained after duplication screening. After filtering through the title and abstract, four articles were obtained. After screening with eligibility criteria, there were two articles that met the inclusion and exclusion criteria, namely Zhou, et al.⁸ and Wei, et al.⁹ for a qualitative clinical review. Two other studies were excluded because PICO was not as determined by the authors. The flow of our study selection is showed in **Figure 1**.

All studies were level 2 for evidence-based research according to Oxford 2011 and could be classified as high quality based on each NOS score. The results of the critical analysis are attached in **Table 2**. A summary of the basic

Table 1. Search queries.

Database	Queries	Hits
PubMed	"psychotherapy" AND ("teleconsultation" OR "telemedicine" OR "E-therapy" OR "internet-based") AND ("COVID-19" OR "SARS-CoV-2" OR "Severe Acute Respiratory Syndrome Corona Virus 2")	52
ProQuest	"psychotherapy" AND ("teleconsultation" OR "telemedicine" OR "E-therapy" OR "internet-based") AND ("COVID-19" OR "SARS-CoV-2" OR "Severe Acute Respiratory Syndrome Corona Virus 2")	138
Cochrane	("COVID-19" OR "2019nCoV" OR "coronavirus" OR "SARS-CoV2") AND "psychotherapy" AND "online"	15
EBSCO/CINAHL	AB "COVID-19" AND AB ("psychotherapy" or "therapy" or "counseling") AND AB "online"	267

characteristics of each study is attached in **Table 3**.

Research from Zhou et al.⁸ is a quasi-experimental study with pre-post design. This study aimed to determine the effectiveness and to evaluate the effectiveness of the intervention on the psychological condition of patients suspected with COVID-19. Patients included in this study satisfied the following criteria: 1) aged at least 18 years, 2) suspected cases of COVID-19, 3) can use a smartphone with the WeChat application and 4) have no previous mental health problems. This study excluded patients with 1) cognitive

impairment which resulted in inability to complete informed consent or questionnaires, 2) psychosis, dementia, or communication difficulties.

Zhou et al.⁸ provided psychological care and assessed the results through pretest-posttest design using Hospital Anxiety and Depression Scale (HADS). HADS has 2 parts, namely HADS-A to assess anxiety, and HADS-D to assess depression, with a score above 8 indicating significant depression or anxiety. Patients with HADS 8 and above will undergo two individual

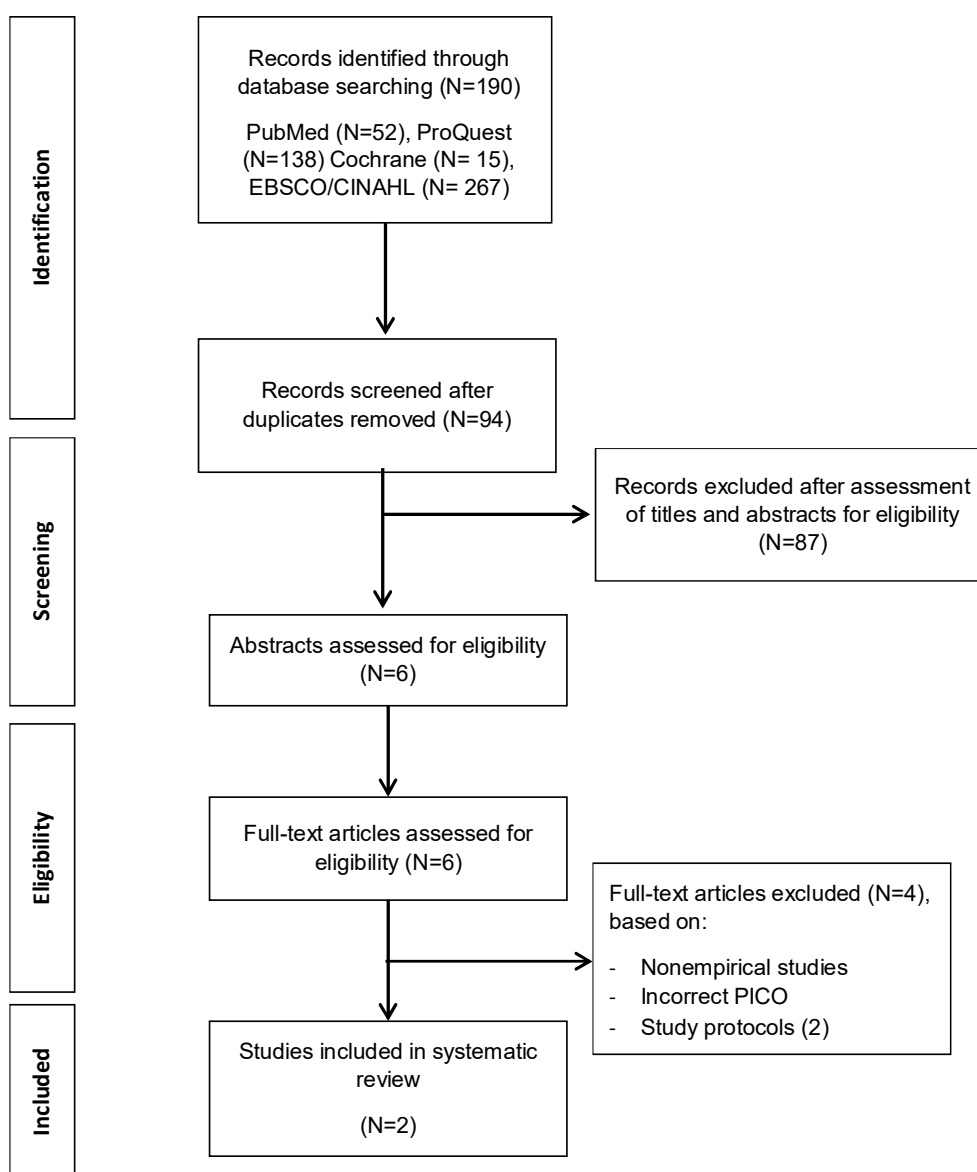


Figure 1. The Process Diagrammatically Study Selection.

Table 2. Quality of the studies based on Newcastle Ottawa Scale.

Study	Selection	Comparability	Outcome	Total
Zhou et al. ⁸	***	*	**	6
Wei et al. ⁹	***	**	***	8

Table 3. Descriptions of the studies found.

Authors	Published	Study Location	Age (years)	Total sample Total	Total sample M/F	Instrument	Results
Zhou et al. ⁸	Februari/2020	China	Median 33.9	63	30/33	HADS	Individual counseling intervention with WeChat could significantly reduce the HADS score ($p < 0.01$) in suspected COVID-19 patients with anxiety and depression.
Wei et al. ⁹	Februari/2020	China	Median 40.8	26	16/10	PHQ-9 and GAD-7; 17-HAMD and HAMA for assessing the degree of anxiety and depression	Intervention with 4 components: breath relaxation exercises, mindfulness, refuge, and butterfly hug method via smartphone. The results showed a significant decrease in the 17-HAMD value in the intervention group in the first week ($t = -2.381$; $p = 0.026$) and second ($t = -3.089$; $p = 0.005$), as well as a decrease in the value of HAMA at the end of the first week ($t = -2,263$; $p = 0.033$) and second week ($t = -3.746$; $p = 0.001$)

consultations via WeChat for 10 minutes each. A total of 63 patients with suspected COVID-19, with 23.8% ($n = 15$) having a HADS score of 8 or higher. There was a significant decrease in the HADS-A ($p < 0.01$) and HADS-D scores ($p < 0.01$) in 15 patients, and from the overall HADS score ($p < 0.01$).⁸

Research by Wei et al.⁹ conducted a prospective study in Hangzhou, China. All patients with confirmed COVID-19 in the isolation treatment room were screened using the Patient Health Questionnaire-9 (PHQ-9) and Generalized Anxiety Disorder-7 (GAD-7) for psychological disorders. Twenty-six COVID-19 patients were included in the study. After recruitment, the 26 patients were randomized into 2 groups, namely the intervention group which received intervention via the internet or was only supportive (control group). The 17-item Hamilton Depression Scale (17-HAMD) and Hamilton Anxiety Scale (HAMA) scoring were used to assess the severity of depression and anxiety

symptoms and assess the efficacy of weekly internet interventions. The intervention given consisted of 4 components, namely breathing relaxation exercises, mindfulness, "refuge" skills, and the butterfly hug method. Instructions are recorded and uploaded online. Subjects in the intervention group listened to the audio via cell phones and followed instructions for the intervention exercise every day for 2 weeks.⁹

Based on this study, there was a significant decrease in the 17-HAMD score in the patients in the intervention group compared to the control group both in the first week ($t = -2.381$; $p = 0.026$) and the second week ($t = -3.089$; $p = 0.005$). The HAMA score also showed a statistically significant decrease in the intervention group compared to the control at the end of the first week ($t = -2,263$; $p = 0.033$) and week two ($t = -3,746$; $p = 0.001$). There was no difference in age, sex, severity of disease, PHQ-9 score, GAD-7, 17-HAMD, or HAMA at baseline.⁹

DISCUSSION

COVID-19 is an infectious disease with severe impact worldwide. Its rapid transmission and high mortality can cause psychological distress.¹⁰ As the COVID-19 pandemic progresses, the use of teleconsultation or online psychotherapy has a place in the management of the psychological complications of the disease in patients, medical staff and the general population undergoing quarantine.¹¹

The results of Zhou et al.⁹ study showed that most COVID-19 suspects experienced symptoms of anxiety and depression during their quarantine at the hospital. Individual counselling using WeChat was found to significantly reduce anxiety and depression in these patients.⁶ The findings in this study suggest that the use of teleconsultation is effective in managing psychological complications in patients with COVID-19. Similar to Zhou et al.⁸ study, Wei et al.⁹ study conducted interventions using the internet and it was found that the 2-week intervention could significantly reduce the degree of depression and anxiety based on the 17-HAMD and HAMA scores. This shows that interventions via the internet or online have a role in improving the psychological outcome of patients with COVID-19.

In terms of analysis, the study of Zhou et al.⁸ included a simple analysis, without describing the characteristics of the study population in detail in order to objectively quantify the stressors that play a role in causing anxiety and depression in patients such as severity of illness or presence of comorbidities. However, with a sample size of 63 people, this study has been able to demonstrate the impact of teleconsultation, as evidenced by a significant reduction in measured anxiety and depression. The study of Zhou et al.⁸ also did not use a control group because it used a quasi-experimental study design.

In contrast to Zhou et al.⁸ study, Wei et al.⁹ study divided the subjects into two groups, namely the group that was given intervention via the internet and the control group who received only supportive therapy. However, the total amount of subjects who participated in this study were only a small number of 26 subjects, with each group consisting of 13

subjects. However, statistically there was a significant outcome after treatment, so it can be concluded that this study had also demonstrated the ability of internet consultation in reducing symptoms of depression and anxiety in patients with COVID-19, assessed in the first and second week. There was no significant difference between the basic characteristics of the two groups. Based on these results, interventions that integrate the internet have potential in creating rapid improvement in mood disorders and can be utilized in psychological management of patients with COVID-19. However in this study, as patients only exhibit mild to moderate symptoms of depression and anxiety, it was considered appropriate to dispense therapy through online-based platforms. Patients with more severe symptoms were not included in the analysis.⁹

The popularity of internet services creates an alternative for health workers to provide services including mental health services. In a meta-analysis, it was found that online-based psychotherapy was as effective as face-to-face behavioural therapy interventions in improving symptoms of depression, anxiety, and other psychological symptoms.¹² In addition, the use of online interventions has several advantages over face-to-face therapy, namely ease of access, time and location flexibility, increased privacy and autonomy, and also more cost efficient.¹³ Said factors are relevant to COVID-19 patients who are required to carry out quarantine, preventing further transmission of SARS-CoV2. Online interventions also reduce the risk of infection being transmitted to health workers.⁹

The psychological impact of the COVID-19 pandemic must be recognized along with the physical symptoms for all those affected. Telehealth, or more specifically tele-consulted health services, is a practical and feasible approach in supporting patients, family members and health care providers during this pandemic.¹⁴ As of this writing, there have been 713,000 cases of COVID-19 in Indonesia, with 21,237 deaths. Psychological symptoms associated with COVID-19 have been observed at the population level including anxiety about availability of food and other supplies as well as attendance at community events. Students, workers and tourists

who are prohibited from accessing academic institutions, workplaces, homes, respectively, are expected to experience psychological symptoms due to stress and reduced autonomy and worries about income, employment, security, and so on.¹⁵

In the absence of a medical cure for COVID-19, the global public health response is to isolate or create distance from those who are infected or at risk, reducing social contact to slow the spread of the virus, whilst maintaining good hygiene through hand washing practices to further reduce the risk of transmission. Although major isolation interventions may work in curbing transmission rates, they also lead to reduced access to their respective social support systems, leading to loneliness, and worsening of anxiety and depressive symptoms.¹⁶ If left untreated, these psychological symptoms may have long-term health effects on patients and require additional treatment adding to the burden of disease management costs. Clinical and nonclinical staff are also at risk of psychological distress because they are expected to work longer hours with a higher risk of exposure to the virus. This may also lead to stress, anxiety, fatigue, depressive symptoms, and the need for sick or stressful leave, which will negatively impact the health system's capacity to provide services during a crisis.¹⁷

Treatment protocols for people with COVID-19 must meet the physiological and psychological needs of patients and healthcare providers. Providing psychological care and support can reduce the burden of comorbid mental health conditions and ensure the well-being of those affected. Our challenge is to provide mental health services in the context of patient isolation, highlighting the role of telehealth (via video conferencing, email, telephone or smartphone applications). Providing mental health support, especially via telehealth, is likely to help patients maintain psychological well-being and better cope with acute and post-acute health requirements.

The examples and evidence supporting tele-mental health effectiveness are mixed, particularly in the context of depression, anxiety, and PTSD. Videoconferencing, online forums, smartphone applications, SMS, and e-mail have

proven to be useful communication methods for the delivery of mental health services.¹⁸⁻²² Several studies have also demonstrated the implementation of teleconsultation during the COVID-19 pandemic.²³⁻²⁷ This clinical review shows that there is evidence to apply teleconsulting with various media to reduce the psychological impact of COVID-19.

A limitation in this clinical review is the exclusion of articles written in languages other than English and Indonesian. This study also cannot proceed to quantitative analysis because of the use of heterogeneous sample and variable.

CONCLUSION

This evidence-based clinical review reports studies on the effectiveness of teleconsultation and psychotherapy in patients with COVID-19. As a preventive and curative effort, teleconsultation or online intervention can be applied more broadly and in the long term.

REFERENCES

1. Pascarella G, Strumia A, Piliago C, et al. COVID-19 diagnosis and management: a comprehensive review. *J Intern Med.* 2020;288(2):192-206.
2. Coronavirus. Available online on: <https://www.worldometers.info/coronavirus/>. Accessed 29th Desember 2020.
3. Salari N, Hosseini-Far A, Jalali R, et al. Prevalence of stress, anxiety, depression among the general population during the COVID-19 pandemic: a systematic review and meta-analysis. *Glob Health.* 2020;16:57.
4. Xiang YT, Yang Y, Li W, et al. Timely mental health care for the 2019 novel coronavirus outbreak is urgently needed. *Lancet Psychiatry.* 2020;7(3):228-9.
5. Wright JH, Caudill R. Remote treatment delivery in response to the COVID-19 pandemic. *Psychother Psychosom.* 2020. DOI: 10.1159/000507376.
6. Siwek J, Gourlay ML, David C, Slawson DC, Shaughnessy AF. How to write an evidence-based clinical review article. *Am Fam Physician.* 2002; 65(2):251-8.
7. Holloway S, Peart J. Evidence-based reviews: principles and methodological considerations. *Wounds UK.* 2018;14 (5):26-32.
8. Zhou L, Xie R-h, Yang X, et al. Feasibility and preliminary results of effectiveness of social media-based intervention on the psychological well-being of suspected COVID-19 cases during quarantine. *Can J Psychiatry.* 2020:0706743720932041.
9. Wei N, Huang B, Lu S, et al. Efficacy of internet-based

- integrated intervention on depression and anxiety symptoms in patients with COVID-19. *Biomed Biotechnol.* 2020;21(5):400-4.
10. Lakhani R, Agrawal A, Sharma M. Prevalence of depression, anxiety, and stress during COVID-19 pandemic. *J Neurosci Rural Pract.* 2020;11(4):519.
 11. Zarghami A, Farjam M, Fakhraei B, Hashemzadeh K, Yazdanpanah MH. A report of the telepsychiatric evaluation of SARS-CoV-2 patients. *Telemed J e-Health.* 2020;26(12):1461-5.
 12. Andersson G, Cuijpers P, Carlbring P, et al. Guided internet-based vs. face-to-face cognitive behavior therapy for psychiatric and somatic disorders: a systematic review and meta-analysis. *World Psychiatry.* 2014;13(3):288-95.
 13. Schröder J, Berger T, Westermann S, et al. Internet interventions for depression: new developments. *Dialogues Clin Neurosci.* 2016;18(2):203-12.
 14. Smith A, Thomas E, Snoswell C. Telehealth for global emergencies: Implications for coronavirus disease 2019 (COVID-19)[e-pub ahead of print]. *J Telemed Telecare.* <https://doi.org/10.1177/1357633X20916567>. Accessed May. 2020;12.
 15. Zhou X, Snoswell CL, Harding LE, et al. The role of telehealth in reducing the mental health burden from COVID-19. *Telemed e-Health.* 2020;26(4):377-9.
 16. Smith EM. Ethnic minorities: Life stress, social support, and mental health issues. *Counsel Psychol.* 1985;13(4):537-79.
 17. Benfante A, Di Tella M, Romeo A, Castelli L. Traumatic stress in healthcare workers during COVID-19 pandemic: a review of the immediate impact. *Front Psychol.* 2020;11:2816.
 18. Turgoose D, Ashwick R, Murphy D. Systematic review of lessons learned from delivering tele-therapy to veterans with post-traumatic stress disorder. *J Telemed Telecare.* 2018;24(9):575-85.
 19. Backhaus A, Agha Z, Maglione ML, et al. Videoconferencing psychotherapy: a systematic review. *Psychol Services.* 2012;9(2):111.
 20. Kauer SD, Mangan C, Sancil L. Do online mental health services improve help-seeking for young people? A systematic review. *J Med Int Res.* 2014;16(3):e66.
 21. Kerst A, Zielasek J, Gaebel W. Smartphone applications for depression: a systematic literature review and a survey of health care professionals' attitudes towards their use in clinical practice. *Eur Arch Psych Clin Neurosci.* 2020;270(2):139-52.
 22. Tomiainen-Holm M, Pankakoski M, Lehto T, et al. The effectiveness of email-based exercises in promoting psychological wellbeing and healthy lifestyle: a two-year follow-up study. *BMC Psychol.* 2016;4(1):21.
 23. Peralta EA, Taveras M. Effectiveness of teleconsultation use in access to mental health services during the coronavirus disease 2019 pandemic in the Dominican Republic. *Indian journal of psychiatry.* 2020;62(Suppl 3):S492.
 24. Pajević I, Hasanović M, Avdibegović E, Džubur-Kulenović A, Burgić-Radmanović M, Babić D, Mehić-Basara N, Zivlak-Radulović N, Račetović G. Organization of mental healthcare in Bosnia and Herzegovina during coronavirus disease 2019 pandemic. *Indian J Psychiatry.* 2020;62(Suppl 3): S479.
 25. Naik SS, Rahul P, Harihara S, et al. Telephonic follow-up during COVID-19 to maintain continuity of care for persons with psychiatric disorders. *Asian J Psychiatry.* 2021;57:102564.
 26. Looi JC, Allison S, Bastiampillai T, Pring W, Reay R, Kisely SR. Increased Australian outpatient private practice psychiatric care during the COVID-19 pandemic: usage of new MBS-telehealth item and face-to-face psychiatrist office-based services in Quarter 3, 2020. *Australasian Psychiatry.* 2021;29(2):194-9.
 27. Diwan MN, Awan HA, Aamir A, De Filippis R, Ullah I. Telepsychiatry in low-and middle-income countries during COVID-19: Pandemic, barriers, and road model. *J Nervous Mental Dis.* 2021;209(2):144.

Anemia in Inflammatory Bowel Disease: A Neglected Issue in Comprehensive Inflammatory Bowel Disease Management

Randy Adiwinata¹, Andrea Livina¹, Bradley Jimmy Waleleng², Harlinda Haroen³, Linda Rotty³, Fandy Gosal², Luciana Rotty², Cecilia Hendratta³, Pearla Lasut³, Jeanne Winarta², Andrew Waleleng², Marcellus Simadibrata⁴

¹ Department of Internal Medicine, Faculty of Medicine, Universitas Sam Ratulangi/Prof. dr. R. D. Kandou Hospital, Manado, Indonesia

² Division of Gastroenterology-Hepatology, Department of Internal Medicine, Faculty of Medicine, Universitas Sam Ratulangi/Prof. dr. R. D. Kandou Hospital, Manado, Indonesia

³ Division of Hematology and Medical Oncology, Department of Internal Medicine, Faculty of Medicine, Universitas Sam Ratulangi/Prof. dr. R. D. Kandou Hospital, Manado, Indonesia

⁴ Division of Gastroenterology, Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia

Corresponding author:

Bradley Jimmy Waleleng, MD. Division of Gastroenterology-Hepatology, Department of Internal Medicine, Faculty of Medicine Universitas Sam Ratulangi - Prof. dr. R. D. Kandou Hospital, Manado 95115, Indonesia. Email: walelengbradley@gmail.com

ABSTRAK

Anemia merupakan komplikasi ekstraintestinal tersering dari inflammatory bowel disease (IBD) dan berkorelasi signifikan dengan berbagai keluaran penyakit yang lebih buruk seperti angka rawat inap, angka relaps, kebutuhan tindakan pembedahan yang lebih tinggi, dan kualitas hidup yang lebih rendah. Prevalensi anemia pada pasien IBD dilaporkan bervariasi, akan tetapi diperkirakan berkisar antara 8,8% hingga 74%. Meskipun tingginya prevalensi tersebut, hingga saat ini masih terdapat kesenjangan antara protokol skrining dan pengobatan anemia pada praktik klinis sehari-hari. Sehingga dapat disimpulkan bahwa anemia pada IBD merupakan suatu komplikasi IBD yang bersifat signifikan dan perlu ditatalaksana secara komprehensif namun seringkali dikesampingkan. Anemia pada IBD dapat disebabkan oleh berbagai faktor, dengan anemia defisiensi besi sebagai etiologi tersering. Manajemen komprehensif anemia pada IBD mencakup skrining aktif, evaluasi dari etiologi, tatalaksana holistik, dan monitoring. Optimalisasi terapi IBD juga merupakan hal penting karena akan berkontribusi pada perbaikan kondisi anemia. Pendekatan dan kolaborasi multidisiplin diperlukan untuk memberikan pelayanan pasien IBD dengan lebih baik.

Kata kunci: *Anemia, inflammatory bowel disease, kolitis ulseratif, penyakit crohn.*

ABSTRACT

Anemia is the most common extraintestinal inflammatory bowel disease (IBD) manifestations and is significantly correlated with several adverse impacts such as higher hospitalization rate, relapse rate, surgical intervention requirement, and low quality of life. The prevalence of anemia in IBD patients is greatly varied between reports, with an estimated prevalence of 8.8% to 74%. However, studies showed there were still gaps in the screening protocol and anemia treatment in daily practice. Anemia in IBD tends to be an overlooked complication of significance and must be adequately addressed. Anemia in IBD may be caused by interplay of

several factors, with iron deficiency anemia being the most common etiology. Comprehensive management of anemia in IBD should consist of active screening, evaluation of the etiology, holistic treatment, and follow-up monitoring. Optimization of IBD therapy should be emphasized because it also may improve the anemic condition. A multidisciplinary approach and collaboration are needed to ensure better IBD care.

Keywords: *Anemia, inflammatory bowel disease, ulcerative colitis, Crohn disease.*

INTRODUCTION

Inflammatory bowel disease (IBD), which consists of ulcerative colitis (UC) and Crohn's Disease (CD), is characterized by chronic inflammation of the gastrointestinal tract. Pathogenesis of IBD is multifactorial and is generally thought of as an interaction of genetic, environmental, host immunological factors, and intestinal microbiota.¹ Prolonged inflammation in IBD patients may cause several intestinal and extraintestinal complications, with anemia being the most common extraintestinal complication. Anemia in IBD patients can be caused by complex interactions of all factors such as iron deficiency, persistent inflammation, blood loss due to gastrointestinal bleeding during IBD flare, folate and vitamin B12 deficiency, and the side effects of drugs. Therefore, a complete evaluation and stepwise diagnostic approach of anemia in IBD should be implemented, as many factors may interplay as the cause of anemia.² Many studies showed the significant impact of anemia on decreased quality of life of IBD patients. Anemia is also associated with higher rates of hospitalization and prolonged hospital length of stay.³ While practitioners generally recognize the significant adverse impact of anemia in IBD; a study showed there were still gaps in the screening protocol and anemia treatment in daily practice.^{3,4} Therefore, anemia in IBD tends to be an overlooked yet significant complication which needs to be adequately addressed.³

THE BURDEN OF ANEMIA IN IBD PATIENTS

The prevalence of anemia in IBD patients is greatly varied between reports. Anemia is recognized as the most common extraintestinal manifestation of IBD.⁵ The prevalence was estimated to be between 8.8 to as high as 74%; it greatly depends on subgroup IBD patients being studied. A study in a Brazil outpatient

clinic consisting of 100 UC patients and 100 CD patients reported the prevalence of anemia of 21%. They found no significant difference between the anemia prevalence of UC and CD patients (18% vs. 24%). Iron deficiency anemia (IDA) being the most common etiology of anemia (6% in UC and 10% in CD), followed by anemia of chronic disease (ACD), which represented 6% of both groups.⁴ A large five-year cohort study in the United States involving 1821 IBD patients (1077 CD, 744 UC) showed that the prevalence of anemia was 50.1% (CD: 53.3% vs. UC 44.7%). Several risk factors were analyzed, which showed that disease severity was closely related to anemia.⁶ Meta-analysis by Filmann et al.⁷ in 2014 revealed that the prevalence of anemia in IBD cases in Europe to be 27% in the CD group and 21% in the UC group. Further analysis showed that anemia was associated with the usage of IBD-specific medications and disease activity status. Patients who were treated with IBD-specific drugs (odds ratio 1.54) and patients with active disease (odds ratio 2.72) were more likely to have anemia. This meta-analysis also showed that UC patients were less likely to experience anemia than CD patients (odds ratio 0.77). Assessment of iron status among anemic patients revealed that more than 50% were iron deficient.

A large multicenter study (ECCO-EPICOM study) evaluating 1871 newly diagnosed patients, including 686 CD patients, 1021 UC patients, and 164 unclassified IBD did follow up monitoring for one year. The prevalence of anemia at diagnosis was higher among CD patients than UC patients (44% vs. 31%) in Western European countries. During one-year follow-up, they found that 49% of CD and 39% of UC patients experienced at least one episode of anemia. Further analysis regarding the type of anemia found IDA approximately 12-17%, ACD

10-27%, and mixed causes in 23-31%.⁸

A study done in an Asian country, namely India, showed that anemia was found in more than half of UC patients even in clinical remission, with IDA being the most common finding.⁹ Another long-term cohort study of IBD patients in Turkey showed that anemia was detected in 548 (58.2%) patients, from the total of 941 IBD patients. They also found that anemia was more prevalent among female and CD patients. Age of the IBD disease was highly correlated with anemia, with an incidence rate calculated as 103.45 per 1,000 patient-years. This finding showed that anemia incidence in IBD might present upon presentation and correlated with the IBD disease course.¹⁰

IMPACT OF ANEMIA IN IBD PATIENTS

Many studies showed that anemia significantly affects patients' quality of life and may be associated with a more severe IBD course. Anemia may affect the productivity of patients due to fatigue, affecting emotional and also cognitive functioning. Anemia in IBD is also implicated with more healthcare resources utilization and higher medical cost burden.¹¹ A study showed that anemia therapy might lead to quality of life (QOL) improvement in IBD patients.¹²

A five-year-longitudinal analysis performed by Koutroubakis et al. involving 410 IBD patients (245 CD, 165 UC) showed that the prevalence of anemia was 37.1%, and IBD patients with anemia required more health care, had lower QOL, and was associated with more severe disease activity. Further analysis also showed that persistent or recurrent anemia for three or more years was significantly associated with more frequent hospitalizations, health care related visits, and surgeries for IBD.⁶ Therefore, more aggressive management of anemia in IBD patients should be emphasized. Analysis of 15,761 patients IBD by Michailidou et al. found that more than half were having anemia, and anemia was strongly correlated with increased risk for an emergency operation and sepsis. Anemia was also served as a predictor for severe morbidity and increased length of stay.¹³

PATHOPHYSIOLOGY

Anemia in IBD may result from multiple factors, with iron deficiency becoming the most common cause.⁴ Interplay of multiple factors may occur, such as iron deficiency, vitamin deficiency, chronic inflammation, diet, micronutrient malabsorption, surgical history, disease activity, medications being used, IBD location and duration, and gastrointestinal bleeding (either visible or occult bleeding). Many journals stated that anemia in IBD is a model of combination between iron deficiency and chronic inflammation.²

Iron Deficiency Anemia in IBD

Iron deficiency anemia may be caused by absolute or functional iron deficiency. Absolute iron deficiency is defined as severely reduced or absence of iron stores in the body. Functional iron deficiency state is defined as adequate body iron stores but insufficient iron availability for incorporation into erythroid precursors; these findings are commonly caused by elevated hepcidin levels.¹⁴ Dietary iron is absorbed mainly in the duodenum and proximal jejunum, with approximately 2 mg of iron absorbed daily. Approximately 1-2 mg iron daily is mainly lost from skin desquamation, shedding of intestinal epithelial cells, and blood loss. Absorbed iron will be bound to transferrin, an iron transport protein, transported in circulation and delivered to bone marrow as primary resources for erythropoiesis. Old red blood cells will be phagocytized by macrophages and recycled for new red blood cells. Excess iron will be bound to a storage protein called apoferritin, forming ferritin.^{15,16} Hepcidin also plays significant roles in systemic iron regulation. High hepcidin levels may inhibit intestinal iron absorption, macrophage iron recycling and inhibit erythropoiesis.¹⁷ The iron deficiency state in IBD patients may be caused by chronic gastrointestinal bleeding or blood loss during IBD flare episodes, therefore contributing to negative iron balance. Inflammation lesion located at duodenum or proximal jejunum caused by CD may contribute to further iron absorption impairment. Also notably, strict dietary restrictions may also contribute to the development of iron deficiency anemia due

to low intake. Iron metabolism may also be impaired in chronic inflammation state due to the IBD condition. Elevated proinflammatory cytokines may impair the transportation of iron for erythropoiesis from iron stores to bone marrow, which is reflected in adequate ferritin levels but low transferrin saturation levels.^{16,18}

Anemia of Chronic Disease in IBD

IBD condition resulting in chronic inflammation state does not only occur locally in the intestinal mucosal but also as chronic systemic inflammation. A study showed that IBD patients had elevated circulating levels of proinflammatory cytokines and chemokines also related to local intestinal inflammation and tissue damage.¹⁹ A study from Antunes et al. showed that higher C-reactive level protein (CRP) levels were found among anemic IBD patients and increased CRP by each 1 mg/L increased the risk for anemia.⁴ Elevated inflammatory cytokines may result in the suppression of erythropoiesis. Interleukin 1 (IL-1) and Tumor Necrosis Factor (TNF) inhibit the response of bone marrow to anemia by reducing erythropoietin production. Interleukin 6 (IL-6) increased hepcidin production, therefore inhibiting the iron absorption and transportation of iron from iron storage.^{17,18,20} Chronic inflammatory conditions also shorten erythrocyte survival, which is mainly caused by increased activation of macrophage and increased erythrocyte destruction rate in the reticuloendothelial system.²⁰

Erythropoietin and IBD

Erythropoietin (EPO), the primary regulator of erythropoiesis, a hormone secreted by the kidney, has blunted response in chronic inflammatory conditions such as IBD. Inflammatory cytokines also may impair the production of EPO. Some studies showed higher EPO levels among IBD patients, possibly due to part of the compensation to the anemic condition. However, the elevated production may still be inadequate according to anemia condition, which is termed relative EPO deficiency.²¹ The role of EPO stimulating agent (ESA) usage in anemia IBD management may be beneficial when IBD patients had been adequately repleted but still had anemia, possibly due to EPO deficiency. ESA

was used to overcome this problem.²²

IBD Drug-induced Anemia

Common IBD medications such as sulfasalazine, azathioprine, or 6-mercaptopurine have a side effect of myelosuppression which manifests as anemia, leucopenia, thrombocytopenia, or pancytopenia. The incidence of bone marrow suppression due to thiopurines and azathioprine are reported ranging from 2-5% and 2-4%, respectively.²³

Micronutrient Deficiency and Anemia

Patients with IBD are at higher risk for malnutrition and especially micronutrient deficiency. This condition may be related to the malabsorptive condition caused by IBD, persistent inflammation, chronic gastrointestinal bleeding post-surgery, and decreased oral intake. Deficiency of iron, folate, and vitamin B12 may also lead to anemia. A study by Park et al.²⁴ showed that 39% of IBD patients had either folate, vitamin B12, or 25-OH-vitamin D deficiency. Study by Huang et al.²⁵ showed the prevalence of vitamin B12 deficiency was 14.9% and folate deficiency was 13.3%. Vitamin B12 and Folate deficiency may lead to the development of macrocytic Anemia.

DIAGNOSTIC APPROACH

The basic understanding of interpreting laboratory results of anemia workup is essential for diagnosing anemia in IBD. Sometimes, it may be challenging to determine the actual cause of anemia in IBD due to overlapping anemia mechanisms and contributing factors.²⁶

World Health Organization (WHO) defined the hemoglobin (Hb) cut-off for Anemia in the general population as lower than 12 g/dl for women, lower than 11 g/dl for pregnant women, and lower than 13 g/dl for men. Several studies and consensus by European Crohn's and Colitis Organisation (ECCO) used the same Hb cut-off for anemia diagnosis in IBD.²⁷

ECCO recommended anemia workup should be commenced whenever hemoglobin level was below normal. History taking should be done carefully, consisting of questions regarding the history of disease activity, recent gastrointestinal bleeding, medication being taken, dietary

history, anemia symptoms, occupation, travel and family history, menstruation cycle, alcohol consumption, history of bleeding disorders, or excessive bruising, and surgical history. A comprehensive physical examination is warranted to find the sign of anemia, bleeding disorder, organomegaly, and tumor.^{27,28}

ECCO differentiated anemia laboratory workup into two categories, basic and extensive workup. Basic workup was determined as the minimum examination in order to evaluate the cause of anemia in IBD. Minimum workup includes Hb, red blood cell count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), reticulocytes count/production index, red cell distribution width (RDW), different blood cell count, serum ferritin, transferrin saturation, CRP concentration, reticulocyte count, thrombocyte count, and leucocyte count. Extensive workup is warranted to further determine the cause of IBD anemia after basic examination, including serum concentrations of vitamin B₁₂, folic acid, haptoglobin, the percentage of hypochromic red cells, reticulocyte hemoglobin, lactate dehydrogenase, soluble transferrin receptor, creatinine, and urea. ECCO recommended having hematological consultation if the cause of anemia is still unknown despite extensive workup.²⁷

Interpretation of anemia laboratory workup result may begin from the interpretation of MCV and MCH to categorize the type of Anemia into microcytic/normocytic/macrocyclic anemia. A common anemia diagnosis algorithm may be applied in evaluating anemia in IBD (**Figure 1**). Microcytic anemia in IBD may have resulted from iron deficiency and chronic disease. Normocytic anemia may have resulted from chronic disease or other hematological disorders. Macrocytic anemia may be related to folate acid and vitamin B12 deficiency due to malabsorption or strict dietary restriction, IBD drugs usage such as azathioprine, 6-mercaptopurine, sulfasalazine.²⁷

Reticulocyte count may represent the erythropoiesis compensation response due to low Hb level. Low reticulocyte count may indicate inappropriate erythropoiesis condition,

which occurred in IDA whilst high reticulocyte count may be related to the hemolytic process. Reticulocyte production index (RPI) is more useful than absolute reticulocyte count. RPI can easily be calculated using the formula: Reticulocyte percent x Hematocrit/45 x 1/correction factor.^{26,29} RDW is reflecting the broadness of erythrocyte size distribution or anisocytosis.³⁰ High RDW value is found among patients with IDA.³¹ Disease activity and inflammation degree in IBD related to CRP level. High CRP levels may indicating ongoing inflammation process that may inhibit the erythropoiesis in IBD patients.⁴ Platelet and white blood cell count is helpful to determine whether the anemia was part of pancytopenia or not. Pancytopenia may be caused by bone marrow failures such as aplastic anemia, myelodysplastic anemia, or other hematological malignancies.³²

Iron Studies

Iron studies are mandatory for evaluating the iron status of anemic IBD patients, which is most commonly caused by iron deficiency. Ferritin is a laboratory examination to evaluate body iron storage. While low ferritin serum level is a marker of functional iron deficiency, ferritin is also categorized as an acute phase reactant protein, which may be elevated in inflammatory conditions such as IBD. Therefore, interpretation of ferritin should be made carefully, as the measured serum ferritin in inflammatory conditions may not be well correlated with the actual iron storage condition.^{26,33} ECCO recommended using ferritin cut-off level of 30 µg/L to be used in diagnosing iron deficiency anemia in IBD and using the cut-off level of 100 µg/L if the IBD patients are in an active inflammatory condition.²⁷ Transferrin is a glycosylated protein that functions as a transport for iron to the usage and storage sites. Transferrin saturation (TSAT) is reflecting the percentage of binding sites on all transferrin molecules occupied with iron molecules. TSAT is simply calculated by dividing iron serum with total iron-binding capacity (TIBD). A low level of TSAT is consistent with the iron deficiency condition. Therefore, TSAT is a helpful marker for the diagnosis of IDA. However, transferrin may be

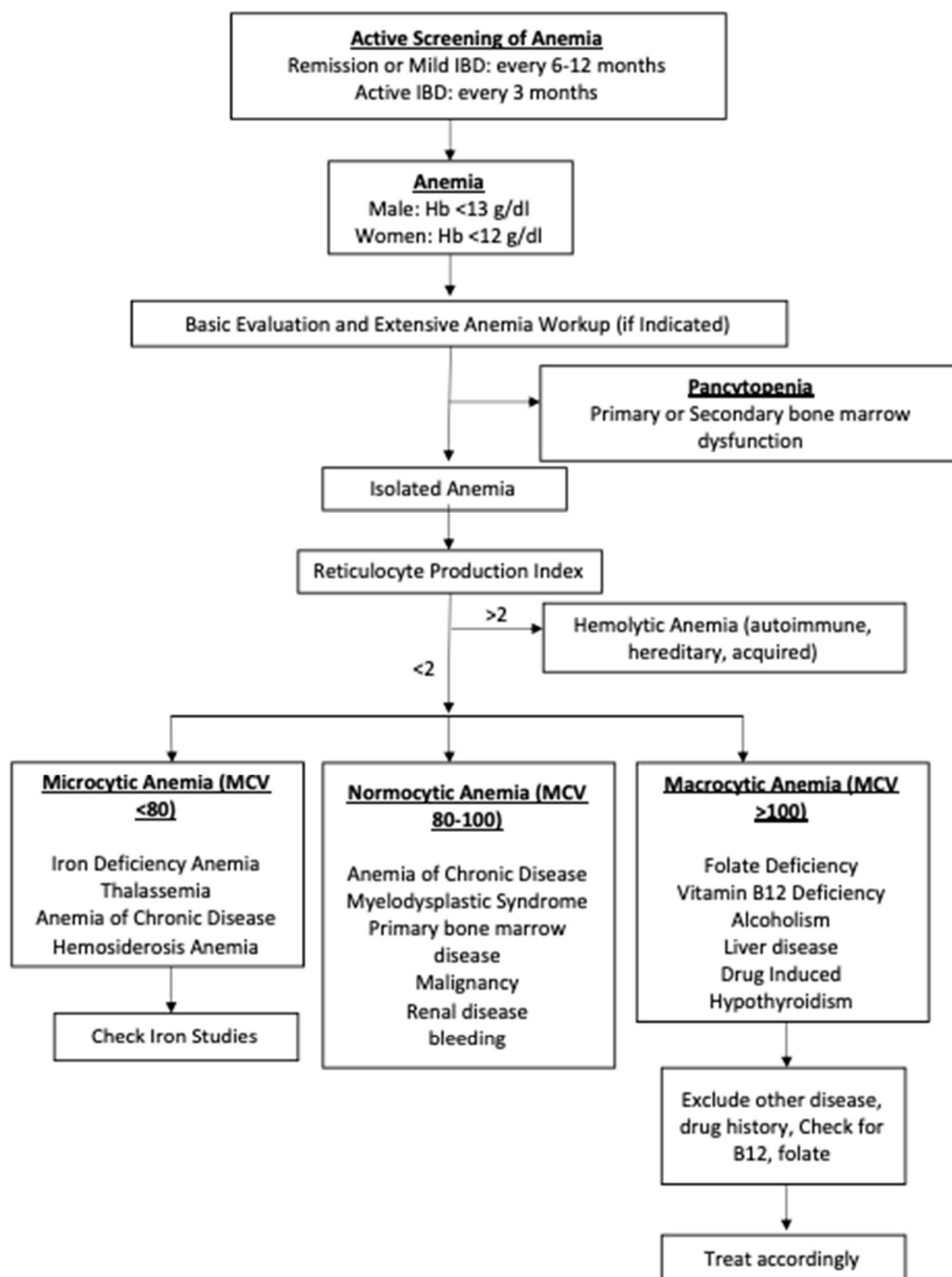


Figure 1. Approach to Anemia Screening and Evaluation in IBD patients^{26,27,32}

downregulated in inflammation conditions.^{26,34} Soluble transferrin receptors (sTfR), a novel biomarker for iron studies, is found to be unaffected by inflammation condition or concomitant chronic disease. Therefore, it may serve to be a valuable diagnostic tools

to differentiate iron deficiency and chronic disease anemia, especially IBD. Further studies are needed to validate the reference range in IBD.^{31,35,36}

According to ECCO in their European Consensus on the Diagnosis and Management of

Iron Deficiency and Anemia in IBD, diagnostic criteria for IDA should be differentiated according to the status of inflammation of IBD patients. In non-active IBD patients who are defined as having no active clinical symptoms (diarrhea, hematochezia), no active endoscopic inflammation lesions, and no elevation of inflammatory biochemical marker (CRP, WBC); serum ferritin $<30 \mu\text{g/L}$ may be indicative for IDA. While serum ferritin $< 100 \mu\text{g/L}$ cut-off should be used if there is active inflammation. Anemia of chronic disease in IBD can be diagnosed if there is biochemical or clinical evidence of active inflammation and ferritin level $> 100 \mu\text{g/L}$ and TSAT $< 20\%$. A ferritin level of $30\text{-}100 \mu\text{g/L}$ may indicate the presence of a combination between IDA and ACD.^{26,27}

MANAGEMENT

Management of anemia in IBD patients should be comprehensive and targeted to the underlying etiology, as outlined above. The stabilization of the IBD disease course, in conjunction with dietary regulation, should be optimized. All patients with IBD should be actively screened for anemia. Screening of anemia can be employed every 6-12 months for patients in remission and every three months in active IBD patients.²⁶

Management of IDA

ECCO recommended that IDA should be treated with iron supplementation in all IBD patients. The goal of treatment should be normalization of the Hb value and restore iron stores. ECCO recommended Hb improvement should be at least 2 gr/dl within four weeks of treatment.²⁷

Iron supplementation may be given orally or intravenously. ECCO recommends intravenous iron supplementation as the first-line treatment, especially in patients with active IBD, documented previous oral iron intolerance, Hb below 10 gr/dl, and patients in ESA therapy. However, in some developing countries, this recommendation may not be applicable due to the higher cost of the intravenous iron regimen, required administration by a healthcare professional, and required close monitoring

due to the possibility for iron overload and anaphylactic reactions. Intravenous iron has some advantages over oral supplementation, especially in IBD patients, which is faster repletion of iron stores and effective even with impaired intestinal absorption. On the other hand, oral iron supplementation becomes a more feasible option for developing countries, as iron supplementation is inexpensive and can be self-administered by the patients at home. Several issues are related to the oral iron supplementation in IBD patients, which may create issues in attaining Hb goal and maintaining patient compliance, such as the impaired intestinal absorption and gastrointestinal side effects (abdominal pain, nausea, constipation).^{27,28} Oral iron supplementation was also found to cause gut bacterial diversity shifting in IBD patients.³⁷ Real-world data from Germany, including more than 1000 IBD patients with IDA, showed that IBD patients who received intravenous iron supplementation had less hospitalization rate and lower total healthcare costs than patients receiving oral iron.³⁸ The safety and effectiveness of oral iron supplementation in IBD patients have been shown by several studies. Therefore, oral iron supplementation is still recommended by ECCO for inactive IBD, no history of previous oral iron intolerance, and patient with mild anemia (Hb 11.0-11.0 gr/dl for nonpregnant women and 11.0-12.9 gr/dl in men).^{26,27} Several newer oral and intravenous iron supplementation have been developed in order to minimize gastrointestinal side effects and anaphylactic reaction. It is also noted that oral iron side effects are dose-dependent.²⁸ ECCO recommended no more than 100 mg elemental iron per day should be given in patients with IBD. Estimation of iron needs may be based on baseline Hb and body weight. For patients with Hb 10-13 gr/dl the estimated total iron need is 1000 mg and 1500 mg, respectively for bodyweight $<70 \text{ kg}$ and $\geq 70 \text{ kg}$. For patients with Hb 7-10 gr/dl and body weight $< 70 \text{ kg}$ required 1500 mg total iron, while patients with similar Hb and body weight $\geq 70 \text{ kg}$ required 2000 mg.^{27,39} List of oral and intravenous iron supplementation can be seen in **Table 1**.^{28,40}

Blood transfusion is generally not

Table 1. List of Oral and Intravenous Iron Preparations^{28,40}

Preparations	Elemental iron (mg)	Dose
Oral		
Ferrous Sulfate	65	325 mg tid
Ferrous Gluconate	36	300 mg tid
Ferrous Fumarate	33	100 mg tid
Iron Polysaccharide Complex	150	150 mg tid
Carbonyl iron	50	50 mg bid
Intravenous		
Iron Sucrose		100-200 mg over 2-5 minutes OR infusion over 15 minutes
Iron Dextran		100 mg over 2 minutes
Ferric Gluconate		125 mg over 10 minutes
Ferumoxytol		510 mg over 15 minutes
Sodium Ferric Gluconate Complex		62.5-125 mg over 1 hour
Ferric Carboxymaltose		750 mg over 15 minutes

recommended for first-line treatment of IDA due to several possible side effects. A blood transfusion may be given for patients with active bleeding with unstable hemodynamics, critical anemia (Hb <7 gr/dl), having significant cardiovascular comorbidity, which warrants a higher hemoglobin level. Packed red cell transfusion should be considered as temporary management only in iron deficiency anemia management. The hemoglobin threshold for initiating blood transfusion may vary between healthcare facilities.^{26, 28,41}

ESA usage in managing anemia in IBD still require further studies. The non-optimal response of Hb normalization after iron correction therapy may indicate concurrent ACD. ESA should be given after restoration of iron level. Possible risks of ESA administration in IBD patients are the risk of venous thromboembolism and the possibility of the response of EPO receptors that may be expressed in several malignant cells.²⁶ ECCO recommended ESA initiation for patients with ACD with an insufficient response to iron correction and after optimization of IBD therapy. Hemoglobin target with ESA therapy should not higher than 12 g/dl.²⁷

Monitoring of IDA treatment should be performed every three months for at least a year after correction and periodically 6 and 12 months after. Recurrent anemia after correction warrants further evaluation as it may indicate persistent intestinal disease activity. Maintenance therapy may be needed in order to maintain the adequacy

of iron stores and Hb. Iron supplementation can be resumed if the ferritin level was lower than 100 µg/L.^{26,28} Summary of IDA management in IBD patients can be seen in **Figure 2**.

Management of Non-Iron Deficiency Anemia

Dietary supplementation with cobalamin and folic acid should be given to correct whenever folate and vitamin B12 deficiency is present. Correction of malnutrition may be needed. Guidelines from The Asian Working Group regarding diet and inflammatory bowel disease, published in 2019 or practical guidance of clinical nutrition in IBD by the European Society for Clinical Nutrition and Metabolism, may be used as dietary guidance.^{42,43} Optimization of IBD treatment in order to reduce inflammation or to achieve remission should be emphasized, as high inflammatory cytokines level may inhibit erythropoiesis, leading to refractory ACD. Drug-induced Anemia such as azathioprine and 6-mercaptopurine may be managed by switching to other drugs if possible or to have the dose adjusted. Cooperation with a hematologist may be required in managing anemia in IBD. Other causes of non-iron deficiency anemia should be evaluated and excluded, such as infections and malignancies, and managed concomitantly.²⁷

CONCLUSION

Anemia in IBD is often a neglected health issue in IBD management. Anemia in IBD is highly correlated with a worse prognosis and lower quality of life. Active screening of anemia

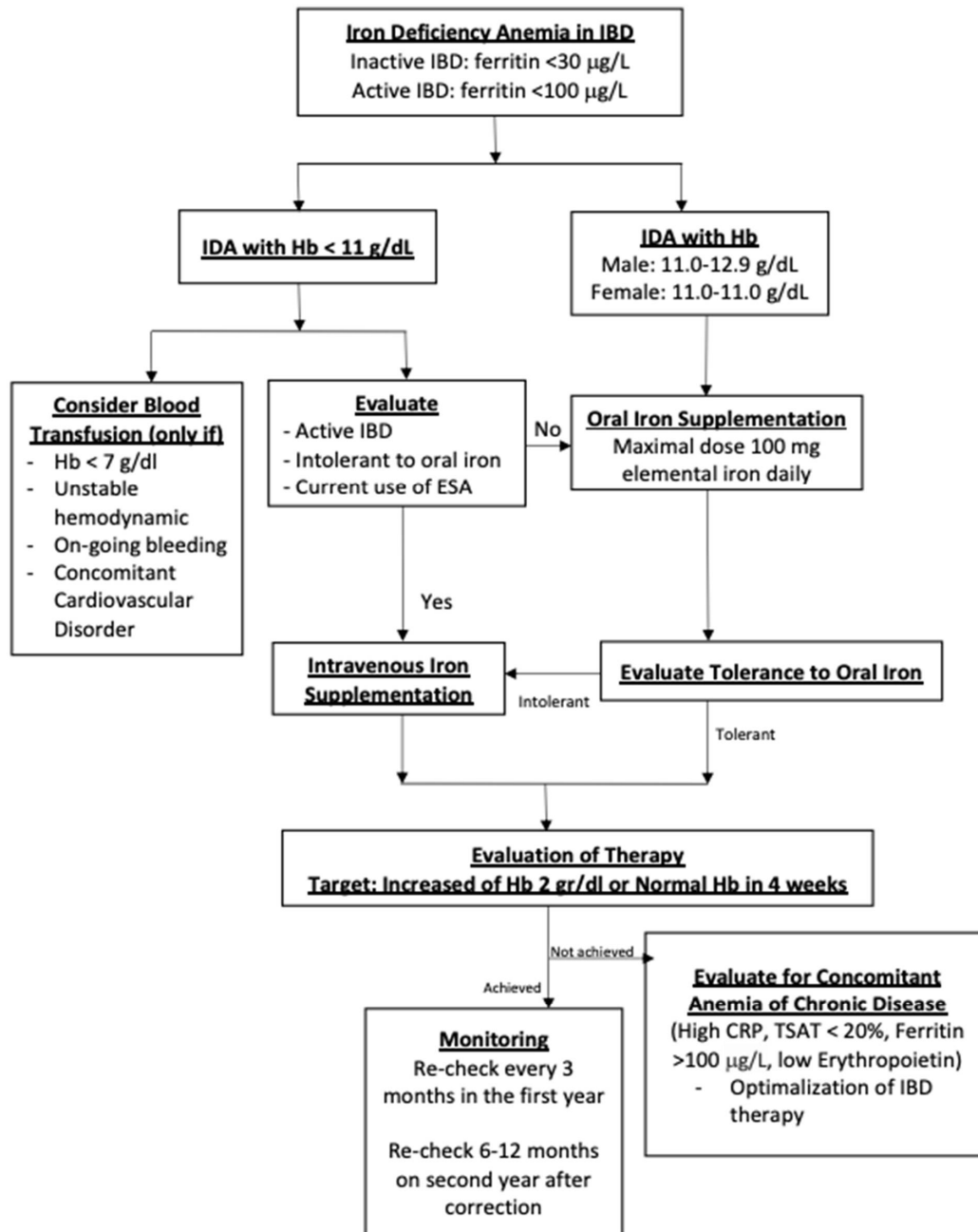


Figure 2. Management of Iron Deficiency Anemia in IBD Patients^{26,27}

in IBD, structured evaluation, comprehensive management, and multidisciplinary collaboration are needed.

REFERENCES

1. Guan Q. A comprehensive review and update on the pathogenesis of inflammatory bowel disease. *J Immunol Res*. 2019;2019.
2. Weiss G, Gasche C. Pathogenesis and treatment of anemia in inflammatory bowel disease. *Haematologica*. 2010;95(2):175–8.
3. Qureshi T, Peter NT, Wang R, et al. Improving anemia in inflammatory bowel disease: Impact of the anemia care pathway. *Dig Dis Sci*. 2019;64(8):2124–31.
4. Antunes CVDA, Neto AEH, Nascimento CRDA, et al. Anemia in inflammatory bowel disease outpatients: Prevalence, risk factors, and etiology. *Biomed Res Int*. 2015;2015.
5. Mücke V, Mücke MM, Raine T, et al. Diagnosis and treatment of anemia in patients with inflammatory bowel disease. *Ann Gastroenterol*. 2017;30(1):15–22.
6. Koutroubakis IE, Ramos-Rivers C, Regueiro M, et al. Five-year period prevalence and characteristics

- of anemia in a large us inflammatory bowel disease cohort. *J Clin Gastroenterol.* 2016;50(8):638–43.
7. Filmann N, Rey J, Schneeweiss S, et al. Prevalence of anemia in inflammatory bowel diseases in European countries: A systematic review and individual patient data meta-analysis. *Inflamm Bowel Dis.* 2014;20(5):936–45.
 8. Burisch J, Vegh Z, Katsanos KH, et al. Occurrence of anaemia in the first year of inflammatory bowel disease in a European population-based inception cohort-An ECCO-EpiCom study. *J Crohn's Colitis.* 2017;11(10):1213–22.
 9. Chaubal A, Pandey V, Choksi D, et al. Anemia in patients with ulcerative colitis in remission: A study from western India. *Indian J Gastroenterol.* 2017;36(5):361–5.
 10. Atıf Ö, Kani HT, Banzragch M, et al. Incidence rate of anemia in inflammatory bowel diseases. *Turkish J Gastroenterol.* 2016;27(2):143–8.
 11. Parra RS, Feitosa MR, Ferreira S, et al. Anemia and iron deficiency in inflammatory bowel disease patients in a referral center in brazil: Prevalence and risk factors. *Arq Gastroenterol.* 2020;57(3):272–7.
 12. Wells CW, Lewis S, Barton JR, et al. Effects of changes in hemoglobin level on quality of life and cognitive function in inflammatory bowel disease patients. *Inflamm Bowel Dis.* 2006;12(2):123–30.
 13. Michailidou M, Nfonsam VN. Preoperative anemia and outcomes in patients undergoing surgery for inflammatory bowel disease. *Am J Surg.* 2018;215(1):78–81.
 14. Gafter-Gvili A, Schechter A, Rozen-Zvi B. Iron Deficiency Anemia in Chronic Kidney Disease. *Acta Haematol.* 2019;142(1):44–50.
 15. Wallace DF. The Regulation of Iron Absorption and Homeostasis. *Clin Biochem Rev.* 2016;37(2):51–62.
 16. Gasche C. Anemia in IBD: The overlooked villain. *Inflamm Bowel Dis.* 2000;6(2):142–50.
 17. Pagani A, Nai A, Silvestri L, et al. Hepcidin and Anemia: A Tight Relationship. *Front Physiol.* 2019;10:1294.
 18. Kaitha S, Bashir M, Ali T. Iron deficiency anemia in inflammatory bowel disease. *World J Gastrointest Pathophysiol.* 2015;6(3):62.
 19. Singh UP, Singh NP, Murphy EA, et al. Chemokine and cytokine levels in inflammatory bowel disease patients. *Cytokine.* 2016;77:44–9.
 20. Nemeth E, Ganz T. Anemia of inflammation. *Hematol Oncol Clin North Am.* 2014;28(4):671–81.
 21. Tsiolakidou G, Koutroubakis IE. Stimulating erythropoiesis in inflammatory bowel disease associated anemia. *World J Gastroenterol.* 2007;13(36):4798–806.
 22. Christodoulou DK, Tsianos EV. Anemia in inflammatory bowel disease - The role of recombinant human erythropoietin. *Eur J Intern Med.* 2000;11(4):222–7.
 23. McLean LP, Cross RK. Adverse events in IBD: To stop or continue immune suppressant and biologic treatment. *Expert Rev Gastroenterol Hepatol.* 2014;8(3):223–40.
 24. Park YE, Park SJ, Park JJ, et al. Incidence and risk factors of micronutrient deficiency in patients with IBD and intestinal Behçet's disease: folate, vitamin B12, 25-OH-vitamin D, and ferritin. *BMC Gastroenterol.* 2021;21(1):1–9.
 25. Huang S, Ma J, Zhu M, et al. Status of serum vitamin B12 and folate in patients with inflammatory bowel disease in China. *Intest Res.* 2017;15(1):103–8.
 26. Niepel D, Klag T, Malek NP, et al. Practical guidance for the management of iron deficiency in patients with inflammatory bowel disease. *Therap Adv Gastroenterol.* 2018;11:1756284818769074.
 27. Dignass AU, Gasche C, Bettenworth D, et al. European consensus on the diagnosis and management of iron deficiency and anaemia in inflammatory bowel diseases. *J Crohn's Colitis.* 2015;9(3):211–22.
 28. Jimenez K, Kulnigg-Dabsch S, Gasche C. Management of iron deficiency Anemia. *Gastroenterol Hepatol.* 2015;11(4):241–50.
 29. Buttarello M. Laboratory diagnosis of anemia: are the old and new red cell parameters useful in classification and treatment, how? *Int J Lab Hematol.* 2016;38:123–32.
 30. Fava C, Cattazzo F, Hu Z-D, et al. The role of red blood cell distribution width (RDW) in cardiovascular risk assessment: useful or hype? *Ann Transl Med.* 2019;7(20):581–581.
 31. Oustamanolakis P, Koutroubakis IE, Kouroumalis EA. Diagnosing anemia in inflammatory bowel disease: Beyond the established markers. *J Crohn's Colitis.* 2011;5(5):381–91.
 32. Gnanaraj J, Parnes A, Francis CW, et al. Approach to pancytopenia: Diagnostic algorithm for clinical hematologists. *Blood Rev.* 2018;32(5):361–7.
 33. Dignass A, Farrag K, Stein J. Limitations of Serum Ferritin in Diagnosing Iron Deficiency in Inflammatory Conditions. *Int J Chronic Dis.* 2018;2018:1–11.
 34. Pfeiffer CM, Looker AC. Laboratory methodologies for indicators of iron status: Strengths, limitations, and analytical challenges. *Am J Clin Nutr.* 2017;106(Suppl 6):1606S-1614S.
 35. Peng YY, Uprichard J. Ferritin and iron studies in anaemia and chronic disease. *Ann Clin Biochem.* 2017;54(1):43–8.
 36. Abitbol V, Borderie D, Polin V, et al. Diagnosis of iron deficiency in inflammatory bowel disease by transferrin receptor-ferritin index. *Med (United States).* 2015;94(26).
 37. Lee T, Clavel T, Smirnov K, et al. Oral versus intravenous iron replacement therapy distinctly alters the gut microbiota and metabolome in patients with IBD. *Gut.* 2016;66(5):863–71.
 38. Stein J, Haas JS, Ong SH, et al. Oral versus intravenous iron therapy in patients with inflammatory bowel disease and iron deficiency with and without anemia

- in Germany - A real-world evidence analysis. *Clin Outcomes Res.* 2018;10:93–103.
39. Avnit T, Bieber A, Steinmetz T, et al. Treatment of anemia in inflammatory bowel disease- systematic review and meta-analysis. *PLoS One.* 2013;8(12):e75540.
 40. Pergola PE, Fishbane S, Ganz T. Novel oral iron therapies for iron deficiency anemia in chronic kidney disease. *Adv Chronic Kidney Dis.* 2019;26(4):272–91.
 41. Cotter J, Baldaia C, Ferreira M, et al. Diagnosis and treatment of iron-deficiency anemia in gastrointestinal bleeding: A systematic review. *World J Gastroenterol.* 2020;26(45):7242–57.
 42. Sood A, Ahuja V, Kedia S, et al. Diet and inflammatory bowel disease: The Asian Working Group guidelines. *Indian J Gastroenterol.* 2019;38(3):220–46.
 43. Bischoff SC, Escher J, Hébuterne X, et al. ESPEN practical guideline: Clinical Nutrition in inflammatory bowel disease. *Clin Nutr.* 2020;39(3):632–53.