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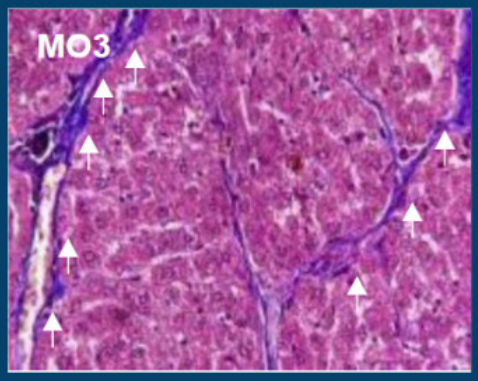

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Assessing the impact of antimicrobial stewardship programs in tertiary hospitals for UTI: does it work alone?

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ABSTRACT

Antibiotic resistance is currently an emergent global challenge. Urinary tract infection (UTI), one of the most commonly reported infections, are is becoming a difficult case to treat considering the increasing prevalence of antimicrobial resistance (AMR). The objective of this study was to assess the impact of a hospital antibiotic stewardship program on AMR in managing UTI at a tertiary referral hospital in Yogyakarta, Indonesia, while considering the absence of the program in lower-tier referral hospitals. A retrospective cross-sectional study was conducted from January 2017 to December 2020, classified into pediatric and adult samples. Urine samples were collected and cultured from all patients with UTI hospitalized in the Dr. Sardjito General Hospital, Yogyakarta. The UTI causative bacteria and antibiotic susceptibilities were investigated in the comparison of the first 2 years (2017-2018, prior to the hospital antibiotic stewardship program) and the last 2 years (2019-2020, following the implementation of the hospital antibiotic stewardship program). The isolates from 717 adult urine samples were cultured. *Escherichia coli* (39.1%), *Acitenobacter baumanii* (9.3%), and *Pseudomonas aeruginosa* (8.5%) were identified as the most common bacteria prior to the hospital antibiotic stewardship program. Extended-spectrum β-lactamase-producing *E. coli* and *Burkholderia cepacia* were still increasing in the following the implementation of the hospital antibiotic stewardship program. Our study indicated that the stewardship program does not exhibit a significant change during the first two years considering the absence of the program in lower-tier referral hospitals.

Keywords:
antimicrobial stewardship;
urinary tract infection;
antibiotic resistance;
Indonesia;
extended-β-lactamase

ABSTRAK

Resistensi antibiotik saat ini merupakan tantangan global yang muncul. Infeksi saluran kemih (ISK), merupkan salah satu infeksi yang paling umum dilaporkan, menjadi kasus yang semakin sulit untuk di atasi mengingat prevalensi resistensi antimikroba yang meningkat. Tujuan dari penelitian ini adalah untuk menilai dampak dari program *stewardship on antibiotic* di rumah sakit terhadap resistensi antimikroba dalam mengelola UTI di rumah sakit rujukan tersier di Yogyakarta, Indonesia, tanpa kehadiran program yang sama di RS referral lebih rendah lainnya. Penelitian potong lintang dilakukan dari Januari 2017 hingga Desember 2020, diklasifikasikan menjadi sampel pediatrik dan dewasa. Sampel urin dikumpulkan dan dibiakkan dari semua pasien dengan ISK yang dirawat di Rumah Sakit Umum Pusat Dr. Sardjito, Yogyakarta. Bakteri penyebab infeksi saluran kemih dan kerentanannya terhadap antibiotik dievaluasi dengan membandingkan 2 tahun pertama (2017-2018, sebelum program pengelolaan antibiotik rumah sakit) dan 2 tahun terakhir (2019-2020, setelah penerapan program pengelolaan antibiotik rumah sakit). Dari 717 sampel urin dewasa telah dibiakkan *Escherichia coli* (39.1%), *Acitenobacter baumanii* (9.3%), dan *Pseudomonas aeruginosa* (8.5%) diidentifikasi sebagai bakteri paling umum sebelum program pengelolaan antibiotik rumah sakit. *Escherichia coli* yang memproduksi β-laktamase spektrum luas dan *Burkholderia cepacia* masih meningkat setelah penerapan program pengelolaan antibiotik rumah sakit. Penelitian ini menunjukkan bahwa program pengelolaan tidak menunjukkan perubahan nyata selama dua tahun pertama mengingat ketidakhadiran program tersebut di rumah sakit rujukan tingkat lebih rendah.

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INTRODUCTION

Urinary tract infection (UTI) is one of the most common bacterial infections. Globally, 150 million people were estimated to be affected by UTI,[1] with all age groups being susceptible. The pattern of infection differs between gender and age groups and patients with predisposing factors.[2] Alarmingly, standard antibiotic treatments have shown decreasing success in UTI management due to increasing antimicrobial resistance (AMR). This problem has been reported globally. According to the World Health Organization (WHO) in 2014, antimicrobial resistance is increasing all over the globe, adding the threat of further complications and mortality from UTI.[3] However, these studies are mostly from developed countries. There have been few reports from Indonesia, the world's fourth-largest population. Some reports have evaluated AMR in UTI from Indonesia in both population-based and laboratory-based research. Sugianli *et al*.[4] assessed the difference in resistance prevalence between laboratory-based and population-based surveillance (PBS) among uropathogens in Indonesia. Sugianli *et al*.[5] also assessed the prevalence of AMR to commonly used antimicrobial drugs in *Escherichia coli* and *Klebsiella pneumoniae* isolated from patients with community- or healthcare-associated UTIs in Indonesia.

Appropriate empirical treatment for UTI plays a key role in treatment success and the prevention of complications. Understanding the demographics and resistance profiles of uropathogens is crucial for determining the appropriate antibiotics.[6,7] However, the rise of AMR has made selecting appropriate empirical antibiotics more challenging. Current microbial susceptibility patterns must be known to treat UTI cases empirically. Bryce *et al*.[8] reported that the global prevalence of AMR in pediatric UTI is largely due to *E. coli* and that the routine use of antibiotics in primary care contributes to AMR in pediatric UTI. In this study, we evaluated the presence of UTI-causative bacteria and their susceptibility patterns in Yogyakarta after the antibiotic stewardship program (ASP) (initiated in the middle of 2018) over four year to establish an appropriate empirical therapy and evaluate our ASP, after classifying the data into pediatrics and adults and comparing with epoch changes as well (2017-2018: before ASP vs 2019-2020: after ASP).

MATERIALS AND METHODS

Study design

This was a hospital-based cross-sectional study. The data were retrospectively gathered from electronic medical records in Dr. Sardjito General Hospital, Yogyakarta a tertiary referral hospital in Yogyakarta, Indonesia, which is also the largest hospital in Central Java with over 813 beds and 23 departments. Antibiotic stewardship program were established at the middle 2018. Antibiotics were grouped into three groups in our ASP using the Carmeli scoring system.[9]-

Antibiotic stewardship program teams closely regulate certain antibiotics, which are grouped in lines 2-3 displayed in Supplemental TABLES 1-3, pre-authorized antibiotic prescriptions are required on line 3, while line 2 items are monitored and evaluated consecutively by ASP teams. The aforementioned groups of antibiotics were only prescribed with approval by the members of antibiotic stewardship boards. This means the physicians cannot use these kinds of broad-spectrum antibiotics by their own judgments from the middle of 2018. To assess how ASP works, this study analysed UTI data from before and after ASP beginning. In the region, the implementation of ASP is not yet widely adapted. Our hospital initiated

an ASP during the time of this study, but lower-tier referral hospitals have not yet implemented the program.

The data were collected consecutively from the laboratory data on electronic medical records of hospitalized UTI or UTI-suspected patients from the internal medicine, surgery, and pediatric departments. Microbiology and antibiotic susceptibility data were collected for all patients with clinical signs and symptoms of UTI, pyuria, at least 50,000 bacterial cfu/mL as well as diagnostic codes for suspected UTI or UTI from January 2017 to January 2020. All urine was collected mid-stream or by catheter. To determine changes over time, we divided the data into four groups, the first 2 years (January 2017- December 2018: prior ASP, pediatrics and adults) and the second 2 years (January 2019- December 2020: following ASP initiation, pediatrics and adults). This is because of the deference between pediatric and adults UTI the underlying disease and history of antibiotic exposures. Furthermore, these four categories for ASP evaluation may aid in providing more precise data on which half (paediatrics or adults) is better controlled. The Medical and Health Research Ethics Committee of Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada/Dr. Sardjito General Hospital, Yogyakarta (KE/0395/04) approved this study.

Sample collection

Clinical symptoms of UTI including fever, dysuria, urinary frequency, hematuria, abdominal pain, pain in the bladder area, back pain, and new daytime incontinence were used as criteria for requesting a urinalysis and culture. Urine cultures were performed just after the aseptic urine collection. Patients were selected according to their coding systems that indicate UTI. Bacterial counting, identification, and susceptibility test was done by both conventional methods and the Vitek 2 automated system (bioMerieux, France). Incubate 1 µL of well-mixed urine on cystine-lactose-electrolyte-deficient (CLED) and MacConkey agar plates in an aerobic environment at 37°C for 24 hr. Bacterial growth was observed and the colonies were counted as cfu/mL. Colonies on both types of plates were identified and the resistance pattern were examined based on the minimal inhibitory concentration (MIC) using VITEK 2 automated system as well as the inhibition diameter zone using disc diffusion method.[10]

Antimicrobial susceptibility tests

The antimicrobial susceptibilities of isolates were tested by the micro dilution broth using Vitek 2 automated system as well as the disk diffusion method using Mueller-Hinton medium according to the Clinical and Laboratory Standards Institute (CLSI) recommendations. The antimicrobial agents tested were amoxicillin (AMX), ampicillin-sulbactam (SAM), ceftazidime (CAZ), nitrofurantoin (NIT), trimethoprim-sulfamethoxazole (SXT), ampicillin (AMP), piperacillin-tazobactam (TZP), Ciprofloxacin (CIP), amoxicillin-clavulanic acid (AMC), and tigecycline (TGC). Extended-spectrum β-lactamase (ESBL)-producing bacteria were diagnosed with a positive ESBL test.

RESULTS

Bacteria isolated from pediatric patients

A total 487 samples of urine, and 455 were positive cultures from pediatric patients (93.01%) were collected. *Escherichia coli* (35.1%) was the major causative microbe for UTI, with *E. faecalis* (15.2%) as the second and *K. pneumoniae* (14.9%) the third most causative UTI microbes' prior ASP (2015-2017) (TABLE 2). In the second

period, among 341 samples that were collected, 279 shown positive cultures (81.8%). Although causative bacterial resistance rates remained similar in the second 2-year period the proportion of ESBL increased. Susceptibility tests of *E. coli* ESBL improved toward NIT (96.2%), CIP (37.7%), SXT (24.5%), and TGC (24.5%) after ASP, compared to before ASP. *Escherichia faecalis* had less susceptible to some of tested antibiotics such as AMX, AMC, or TZP but was more susceptible to CAZ, SXT, AM, and TGC. The susceptibilities test result on both *E. coli* ESBL (+) and *K. pneumonia* ESBL (+) were observed steady on this study.

TABLE 1. Antimicrobial susceptibility of isolated bacteria from adult patients

Microbes	n	AMX		SAM		CAZ		NIT		SXT		AMP		TZP		CIP		AMC		TGC	
		%	p	%	p	%	p	%	p	%	p	%	p	%	p	%	p	%	p	%	p
E. coli (2017-2018)	197	-		42.1		46.7		91.9		30.5		14.7		79.7		29.9		1.5		98.0	
			N/A		0.438		0.002		0.000		0.004		0.074		0.000		0.004		0.637		0.000
E. coli (2019-2020)	149	-		46.3		63.1		72.2		45.6		22.1		60.4		45.0		0.7		79.1	
E. coli ESBL (+) (2017-2018)	83	-		31.3		8.4		96.4		15.7		0		77.1		12.0		-		98.8	
			N/A		0.409		0,000		0.000		0.026		0.549		0.023		0.040		N/A		0.000
E. coli ESBL (+) (2019-2020)	161	-		36.6		36.0		62.7		28.6		1.2		62.7		23.0		-		74.2	
K. pneumoniae (2017-2018)	25	-		44.0		56.0		64.0		64		4		60		52.0		-		76.0	
			N/A		0.234		0.753		0.146		0.924		0.287		0.589		0.974		N/A		0.887
K. pneumoniae (2019-2020)	62	-		58.1		59.7		46.8		62.9		0		66.1		51.6		-		77.4	
K. pneumoniae ESBL (+) (2017-2018)	23	-		0.0		8.7		52.2		26.1		0		56.5		30.4		-		78.3	
			N/A		0.553		0.06		0.97		0.857		1.000		0.797		0.957		N/A		0.918
K. pneumoniae ESBL (+) (2019-2020)	57	-		5.3		28.1		52.6		28.1		1.8		59.6		29.8		-		77.2	
P. aeruginosa (2017-2018)	61	1.6		1.6		59.0		0.0		1.6		1.6		63.9		49.2		1.6		0.0	
			0.430		1.000		0.859		0.000		0.000		1.000		0.315		0.981		0.430		0.000
P. aeruginosa (2019-2020)	81	0		2.5		60.5		23.5		29.6		1.2		55.6		49.4		0		30.9	
E. faecalis (2017-2018)	3	-		100		66.7		66.7		66.7		0		0		66.7		-		0.0	
			N/A		0.143		1.000		0.107		0.464		1.000		1.000		0.464		N/A		0.464
E. faecalis (2019-2020)	5	-		20.0		60.0		0.0		20		20		20		20.0		-		40.0	
E. cloacae (2017-2018)	20	89.5		5.0		50.0		50.0		65		5		65		70.0		-		86.4	
			0.002		0.317		0.337		0.337		0.171		0.317		0.087		0.024		N/A		0.268
E. cloacae (2019-2020)	43	25.0		0.0		37.2		62.8		46.5		0		41.9		39.5		-		74.4	

Extended-spectrum β-lactamase; Chi Square analysis ($p < 0.05$); Fisher test analysis ($p<0.05$); amoxicillin (AMX); ampicillin-sulbactam (SAM); ceftazidime (CAZ); nitrofurantoin (NIT), trimethoprim-sulfamethoxazole (SXT); ampicillin (AMP); piperacillin-tazobactam (TZP); ciprofloxacin (CIP); amoxicillin-clavulanic acid (AMC); tigecycline (TGC).

TABLE 2. Antimicrobial susceptibility of isolated bacteria from pediatric patients

Microbes	n	AMX		SAM		CAZ		NIT		SXT		AMP		TZP		CIP		AMC		TGC	
		%	p	%	p	%	p	%	p	%	p	%	p	%	p	%	p	%	p	%	p
E. coli (2017-2018)	107	-	N/A	23.4	0.002	46.7	0.000	96.3	0.100	32.7	0.186	9.3	0.004	50.5	0.001	49.5	0.013	1.9	1.000	60.7	0.000
E. coli (2019-2020)	48	-		47.9		89.6		89.6		43.8		27.1		79.2		70.8		0.0		94.0	
E. coli ESBL[1)] (+) (2017-2018)	53	-	N/A	17.0	0.360	7.5		96.2		24.5		-		18.9		37.7		-	N/A	24.5	
							0.079		0.648		0.092		N/A		0.000		0.137				0.000
E. coli ESBL[1)] (+) (2019-2020)	64	-		25.0		18.8		95.3		17.2		-		75.0		25.0		-		98.4	
K. pneumoniae (2017-2018)	30	-	N/A	23.3	0.08	40.0		60.0		40.0		-		30.0		60.0		80.0	0.478	56.7	
							0.114		0.825		0.825		N/A		0.000		0.825				0.004
K. pneumoniae (2019-2020)	19	-		47.4		63.2		63.2		36.8		-		84.2		63.2		71.4		94.7	
K. pneumoniae ESBL (+) (2017-2018)	37	-	N/A	-	N/A	0.0		75.7		27.0		-		10.8		64.9		-	N/A	18.4	
							0.054		0.051		0.032		N/A		0.000		0.000				0.000
K. pneumoniae ESBL (+) (2019-2020)	37	-		-		13.5		54.1		8.1		-		64.9		21.6		-		91.9	
Enterococcus faecalis (2017-2018)	69	59.4	0.033	1.4	0.310	2.9	0.094	84.1		5.8		62.3		27.5		37.7		59.4	0.033	0.0	
									0.143		0.252		0.290		0.567		1.000				0.190
E. faecalis (2019-2020)	4	0.0		0.0		25.0		50.0		25.0		100		0.0		25.0		0		7.7	
Pseudomonas aeruginosa (2017-2018)	33	-	N/A	-	N/A	57.6	0.009	0.0		0.0		-		21.2		75.8		-	N/A	47.8	
									0.256		0.441		N/A		0.000		0.093				0.122
P. aeruginosa (2019-2020)	26	-		-		88.5		3.8		3.8		-		76.9		92.3		-		0.0	
Enterobacter cloacae (2017-2018)	13	-	N/A	-	N/A	15.4	1.000	84.6		30.8		-		15.4		84.6		-	N/A	53.3	
									0.202		1.000		N/A		0.645		0.202				0.043
E. cloacae (2019-2020)	6			-		16.7		58.3		33.3		-		25.0		58.3		-		91.7	

Extended-spectrum β-lactamase; Chi Square analysis (p< 0.05); Fisher test analysis (p<0.05); amoxicillin (AMX); ampicillin-sulbactam (SAM); ceftazidime (CAZ); nitrofurantoin (NIT), trimethoprim-sulfamethoxazole (SXT); ampicillin (AMP); piperacillin-tazobactam (TZP); ciprofloxacin (CIP); amoxicillin-clavulanic acid (AMC); tigecycline (TGC).

Antimicrobial susceptibility

The results of antimicrobial susceptibility test were confirmed positive with the formation of an inhibition zone by clavulanic acid (CLA) on the middle ceftazidime (CAZ)/CLA disc gert-year period (90,3%). Among the isolates, *E. coli* (n=280, 39.1%), *Acinetobacter baumanii* (n=67, 9.3%), and *Pseudomonas aeruginosa* (n=61, 8.5%) were identified as the most common bacteria. In the second two-year period (following ASP), approximately 943 samples were collected, and 882 samples shown positive cultured (93.5%). The changes over time showed that *E. coli* and *K. pneumoniae* significantly declined as causative microbes for UTI Following ASP Program (2019-2020) compared to the prior ASP program (2017-2018). The proportion of ESBL-producing *E. coli* increased from 11.6% prior ASP to 18.5 % following ASP, and *P. aeruginosa* increased from 8.5% to 9.2% (TABLE 1). *Escherichia coli* showed better susceptibility to CIP (29.9% in 2017-2018 and 45% in 2019-2020, p= 0.004) as well as ESBL-producing *E. coli* (12% before ASP and 24% after ASP). We also found *Enterococcus faecalis* resistance to most antibiotics increased after ASP-compared to prior ASP (p < 0.05).

Susceptibility test results for *P. aeruginosa* remained steady over the entire study period, with susceptibility tests showing favorable results for CAZ compared to SXT, TZP, and CIP satisfactory susceptibility (TABLE 3).

TABLE 3. Isolated bacteria

Microbes	Adults				Pediatrics			
	2017-2018		2019-2020		2017-2018		2019-2020	
	n	%	n	%	n	%	n	%
E. coli	197	27.5	149	16.9	107	23.5	50	18.0
E. coli ESBL[1)] (+)	83	11.6	163	18.5	53	11.6	64	23.0
K. pneumoniae	25	3.5	62	7.0	30	6.6	19	6.8
K. pneumoniae ESBL (+)	23	3.2	57	6.5	38	8.3	37	13.2
E. gallinarum	9	1.25	11	1.2	2	0.4	3	0.6
P. aeruginosa	61	8.5	81	9.2	33	7.2	26	9.3
Candida tropicalis	79	11.2	80	9.0	18	3.9	6	2.1
E. faecalis	3	0.4	5	0.6	69	15.2	4	1.4
Sphingomonas paucimobilis	1	0.1	3	0.3	2	0.4	0	0.0
Acinetobacter baumannii	67	9.3	88	10.0	16	3.5	4	1.4
Citrobacter freundii	9	1.3	8	0.9	2	0.4	1	0.4
E. cloacae	22	3.1	43	4.9	15	3.2	12	4.3
Staphylococcus haemolyticus	24	3.3	15	1.7	23	5.0	12	4.3
Proteus mirabilis	15	2.1	12	1.4	5	1.0	4	1.4
Burkholderia cepacia	22	3.1	24	2.7	8	1.7	19	6.8
S. aureus	17	2.4	19	2.1	6	1.3	2	0.7
C. krusei	3	0.4	4	0.4	3	0.7	0	0.0
S. epidermidis	4	0.6	2	0.2	3	0.7	4	1.4
K. oxytoca	2	0.3	3	0.3	1	0.2	0	0.0
C. lusitaniae	17	2.4	12	1.4	1	0.2	3	1.0
C. parapsilosis	10	1.7	13	1.5	9	1.8	2	0.7
S. hominis	4	0.5	3	0.3	2	0.4	2	0.7
P. putida	7	1.0	11	1.2	1	0.2	0	0.0
Aeromonas hydrophila/caviae	3	0.4	1	0.1	2	0.4	0	0.0
C. koseri	2	0.3	1	0.1	2	0.4	2	0.7
E. casseliflavus	3	0.4	3	0.3	0	0.0	2	0.7
S. saprophyticus	4	0.6	8	0.9	0	0.0	1	0.4
Salmonella enterica	1	0.1	1	0.1	0	0.0	0	0.0
Total	717	100	882	100	453	100	279	100

DISCUSSION

Microbial drug resistance is common worldwide and is increasing year after year. Our hospital implemented Rational Use of Medicine and ASP to address increased antibiotic resistance, but the high AMR has not changed in first two years. After the implementation of the national universal health coverage, infection management in Indonesia strategically focused on the secondary and tertiary health centers. Patients with more complicated or unresolved infections, such as ESBL or antibiotic-resistant infections, are more likely to be referred to tertiary hospitals, thus registering as an increase in antibiotic-resistant infections despite the current ASP. Pre-authorization and formulary restrictions of antibiotics are part of the strategy that has been implemented in

our hospital. These strategies involve several antibiotics listed in lines 2 and 3 according to Carmeli scoring systems which cannot be prescribed without permission and approval from the ASP team, and prescriptions are reviewed case by case. ASP has been set up in a limited number of regional hospitals with various difficulties, and our hospital has been the program initiator in our region.

Antibiotic resistance is caused in part by over-prescription and inappropriate usage of broad-spectrum antibiotics such as fluoroquinolones.[11-13] The increasing proportion of ESBL is an alarming sign of the imminent threat of untreatable UTI. The rise of UTI caused by ESBL-producing bacteria has been observed in both developed and developing countries.[14,15] Bacterial susceptibility to AMP, AMX, and AMC was also decline in this study.

Unnecessary prescriptions due to the lack of properly administered ASP may contribute to declines in bacterial susceptibility.[11,13,16] The high rate of NIT TZP TGC resistance in *E. coli* spp. in this study is not surprising in the adult population, since the intrinsic resistant phenotypes of these bacteria have been widely reported.[17,18] In contrast, AMS, CAZ, and SXT had good susceptibility for non-ESBL-producing *E. coli*[14] and remained around 45% in ESBL-producing *E. coli* over the study period. Similar results were also reported by Cantas *et al.*[15] who found that ESBL-producing *E. coli* were highly susceptible to ERT and MER. Another report in Surabaya Indonesia, found that susceptibility to AMI was 100% for ESBL-producing *E. coli*. Our results in Yogyakarta Indonesia, were lower, with a susceptibility to AMI for ESBL-producing *E. coli* of 90.9%,[14] which may indicate the need for caution in UTI treatment, considering the antibiograms in each institution.

Nitrofurantoin is not commonly available at primary health centers in Indonesia and is seldom used in secondary health centers. Sekyere *et al.*[19] discussed the vertical spread of nitrofurantoin resistance genes through clonal and multiclonal expansion, commonly found locally. Abia *et al.*[20] also reported *E. coli* resistance to nitrofurantoin genes in South Africa. Our results are consistent with Procop *et al.*[21] and Garau *et al.*[22] demonstrating that ESBL spp. have lower susceptibility to nitrofurantoin than non-ESBL, as seen now in Indonesia. Our results showed that some bacteria such as *E. coli* and *E. coli*-producing ESBL, increased susceptibilities to nitrofurantoin after ASP initiation.

Susceptibility tests for *K. pneumoniae* showed a more alarming result. We found high rates of resistance toward a wide variety of antibiotics. In this study, *K. pneumoniae* showed lower susceptibility levels to most available antibiotics for both the adult and pediatric age groups. High incidence rates of ESBL and extended resistance were found in Australia, Israel, Greece, Colombia, and China, but susceptibilities consistently remained high.[23-25] Cautious usage and close observation are essential to maintain the efficacy of AMI, ERT, and MER.

Enterococcus faecalis is a common cause of UTI and forms a biofilm responsible for persistent UTI and treatment resistance.[25,26] In this study, *E. faecalis* showed significant changes of increasing resistance to most of the antimicrobials tested. Living in a competitive environment and exposed to a myriad of antibiotics, this bacterium has developed a variety of responses and considerable genetic plasticity to cope with threats.[27,28] Our susceptibility tests showed that SAM (100%), CAZ (66.7%), NIT (66.7%), SXT (66.7%), and CIP (66.7%) were initially suitable for *E. faecalis* UTI in adults before ASP, and rapidly declined after the ASP. Other antibiotics had similar results, with similar patterns for both pediatric and adult patients despite the implementation of ASP in

our hospital. This may indicate that ASP need to be implemented at a region-wide level as part of a comprehensive policy.

This study showed good satisfactory susceptibility of *P. aeruginosa* to CAZ (88.5%) and CIP (92.3%) in pediatric patients before the implementation of ASP, and a decline to 57.6% (TAZ) and 75.8% (CIP) after implementation of ASP, which was alarming even though it did not reach statistical significance. Our study found favorable susceptibilities of FTN and TGC for ESBL-producing *E. coli* in pediatric patients. Favorable susceptibilities of CAZ and TGC were also found in the adult population.

This study has several limitations, including the use of retrospective cross-sectional design and potential selection bias. We were also unable to exclude cases caused by anatomical abnormality or distinguish between nosocomial and non-nosocomial UTI. Our data also did not include complete antibiotic dosing history or intrinsic antibiotic resistance data. In addition, resistance patterns of bacteria in water and sludge were not investigated previously; this may be a confounding factor in this study. We collected whole-hospital data consecutively without distinguishing between clinical departments. Lastly, we lacked the evaluation data of ASP performance in detail, for instance, how much such intervention was done or what was the physicians' response or patients' outcome, and the link with bacterial data. Further studies are needed to determine the best alternative treatments after obtaining and applying antibiotic susceptibility results. The study provides valuable insights into the effectiveness of ASPs and emphasizes the need for a collaborative and integrated approach to antimicrobial stewardship across the healthcare continuum.

CONCLUSION

In conclusion, we found that our ASP the implementation of ASPs in tertiary hospitals is significantly unable to overcome the effects of antibiotic use in lower-referral hospitals. The findings of this study have important implications for the design and implementation of ASPs. It suggests that ASPs should be complemented by additional interventions, such as enhancing antibiotic prescribing in lower-referral hospitals, in order to be effective in modifying antibiotic susceptibility pattern. We highlight the importance of a comprehensive and coordinated approach to antimicrobial stewardship across all healthcare settings.

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The curative effect of ethanolic extract of *Moringa oleifera* leaf in an animal model of liver fibrosis

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ABSTRACT

Studies about the prevention effect of *Moringa oleifera* on liver fibrosis has been reported. However, its curative effect has not been reported, yet. This study was conducted to evaluate the curative effect of *M. oleifera* leaf extract on liver fibrosis. It was a laboratory experimental study with a post-test-only control group design. Rats were divided into 5 groups i.e. normal control which received intraperitoneally injections of 1 mL/kg BW of 0.9% NaCl solution twice a wk for 11 wk. Liver fibrosis control which received intraperitoneally injections of 1 mL/kg BW of 10% CCl_4 solution twice a wk for 11 wk. Three *M. oleifera* treatment group which received intraperitoneally injections of 1 mL/kg BW of 10% CCl_4 solution twice a wk for 11 wk continued by *M. oleifera* leaf ethanolic extract at dose of 600 mg/kg BW daily for 3 (MO3), 6 (MO6), and 10 (MO10) wk, respectively. The liver fibrosis level was assessed based on the METAVIR score. Histopathological analysis of liver tissues demonstrated that the 11-week CCl_4 induction successfully resulted in liver fibrosis in rats (F3 and F4). The administration of *M. oleifera* leaf ethanolic extract decreased METAVIR scores ranged from F3 to F1. The optimal reduction of the METAVIR scores (F1) was observed in MO3 group after 6 wk administration ($p<0.05$). It was indicated that *M. oleifera* leaf ethanolic extract ameliorated liver fibrosis. In conclusion, *M. oleifera* leaf ethanolic extract has a curative effect against liver fibrosis.

ABSTRAK

Penelitian tentang efek pencegahan *Moringa oleifera* pada fibrosis hati telah banyak dilaporkan. Namun, penelitian tentang efek kuratifnya belum banyak dilaporkan. Penelitian ini dilakukan untuk mengevaluasi efek kuratif ekstrak daun *M. oleifera* terhadap fibrosis hati. Penelitian ini merupakan penelitian eksperimental laboratoris dengan desain *post test only control group*. Tikus dibagi menjadi 5 kelompok yaitu kontrol normal yang menerima suntikan intraperitoneal 1 mL/kg BB larutan NaCl 0,9% dua kali seminggu selama 11 minggu. Kontrol fibrosis hati yang menerima suntikan intraperitoneal 1 mL/kg BB larutan CCl_4 10% dua kali seminggu selama 11 minggu. Tiga kelompok perlakuan *M. oleifera* yang mendapat suntikan intraperitoneal 1 mL/kg BB larutan CCl_4 10% dua kali seminggu selama 11 minggu dilanjutkan dengan pemberian ekstrak etanol daun *M. oleifera* dengan dosis 600 mg/kg BB setiap hari selama masing-masing 3 (MO3), 6 (MO6), dan 10 (MO10) minggu. Derajad fibrosis hati dinilai berdasarkan skor METAVIR. Analisis histopatologi jaringan hati menunjukkan bahwa induksi CCl_4 selama 11 minggu berhasil menyebabkan fibrosis hati pada tikus (F3 dan F4). Pemberian ekstrak etanol daun *M. oleifera* menurunkan skor METAVIR berkisar antara F3 sampai F1. Penurunan optimal skor METAVIR (F1) diamati pada kelompok MO3 setelah pemberian selama 6 minggu ($p<0,05$). Diindikasikan bahwa ekstrak etanol daun *M. oleifera* dapat memperbaiki fibrosis hati. Kesimpulannya, ekstrak etanol daun *M. oleifera* mempunyai efek kuratif terhadap fibrosis hati.

Keywords:
antifibrotic;
curative effect;
liver fibrosis;
Moringa oleifera;
extract

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INTRODUCTION

Liver fibrosis is a condition characterized by an aberrant wound-recovery process reaction that often represents the final stage in the progression of chronic liver disease.[1] As liver disease advances, the progression of fibrosis becomes a crucial factor influencing the prognosis of liver disease and the risk of hepatocellular carcinoma (HCC).[2] Recently, no standard treatment for liver fibrosis is available. Therefore, the management of liver fibrosis usually focuses on eliminating the etiology and complications.[3]

The most effective treatment for liver fibrosis is elimination of the etiology, however it still faces many obstacles. Reversal fibrosis by eliminating of the causative agent occurs too slowly or too rarely to avoid life-threatening complications, especially in advanced fibrosis.[4] The pathogenesis of liver fibrosis is very complex, involving the interaction of various cells and cytokines in liver tissue.[5] Hepatic stellate cells are the cells that play the most role in the progression of liver fibrosis.[6] Therefore, inhibiting HSC activation and inducing a-HSC apoptosis has promise in inhibiting liver fibrosis.

Potent herbal medicines for the management of liver fibrosis have been reported. *Moringa oleifera*, locally name as *kelor*, is one of the herbal medicines that has been used for the treatment various diseases including liver fibrosis. *Moringa oleifera* contains various active constituents such as alkaloids, saponins, tannins, steroids, phenolic acids, terpenes, and flavonoids. The flavonoid of *M. oleifera* includes myricetin, quercetin, kaempferol, isorhamnetin, and rutin.[7-9] A previous study reported that *M. oleifera* leaf extract administration can accelerate liver cell regeneration of rats after CCl4 induction.[10] *Moringa oleifera* leaf extract was also reported to have hepatoprotective effect on CCl_4-induced in mice through various pathways and targets including control of oxidative stress, modulation of TLR4/NF-κB, inhibition of α-smooth muscle actin, inhibition of metalloproteases-1, and collagen-1.[11-13] This study aimed to evaluate the curative effect of ethanolic extract of *M. oleifera* leaf on liver fibrosis rats chronically induced by CCl_4.

MATERIAL AND METHODS

Extract and samples preparation

The ethanolic extract of *M. oleifera* was prepared by cold maceration using 96% ethanol as solvent. Briefly, 100 g of dried *M. oleifera* leaves powder was macerated with 900 mL of 96% ethanol. The maceration process was conducted for 24 hr at 37 ^{o}C with stirred occasionally. The homogenized mixture was filtered using Whatman filter paper and the residue left behind was subjected to two identical maceration. Subsequently, the filtrates obtained from the three macerations were combined and evaporated using a vacuum rotary evaporator until a crude extract was obtained. The extract tested at dose of 600 mg/kg BW was than prepared by diluting the crude extract with aquadest.[12,13]

Experimental design and animal treatment

The curative effect of ethanolic extract of *M. oleifera* leaf in rats model of liver fibrosis was conducted according a method developed by Li *et al.*[14] It was part of a larger study concerning the effects of *M. oleifera* on liver fibrosis of rats conducting at the Laboratory of Pharmacology, Faculty of Medicine, Universitas Brawijaya, Malang. This experimental study used a post-test only control group design conducting on male Wistar rats weighing 150-250 g and 10-12 wk old with 3 rats in each group. The rats were acclimatized to animal house conditions for a period of 7 d before experimental and then intraperitoneal

injected by 0.9% CCl_4 in corn oil (1:9) for 11 wk except normal control rats which intraperitoneal injected 0.9% NaCl. Followed after injection of 0.9% CCl_4, the rats were administered *M. oleifera* leaf extract at dose of 600 mg/kg BW daily for 3 (MO3), 6 (MO6), and 10 (MO10) wk, respectively (TABLE 1). The rats were sacrificed 48 hr following the last NaCl or CCl4 or *M. oleifera* leaf extract administration. The liver tissue was isolated for subsequent histopathological analysis.

The protocol of the study was approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia (No. 09/EC/KEPK/01/2023).

Liver morphological examination

The photograph of liver tissue was taken and its visual characteristics including the color, surface texture, and freshness were assessed. The liver tissue was then weighed and its volume was measured. The volume of liver tissue was measured by calculating the difference of the volume between before and after submerging the liver tissue in 10% formaldehyde.

Liver pathological analysis

Liver tissue samples were preserved in 10% neutral-balance formalin solution, followed by rehydration through a series of ethanol with different concentrations. Subsequently, the liver tissues were embedded in paraffin blocks and sliced into sections that were approximately 5 μm thick using a microtome. These sections were then placed onto glass slides. The liver sections underwent a dewaxing process using xylene and rehydration through graded ethanol for 2-3 min. After rinsing each of the sections with distilled water, they were subsequently dyed using Masson's trichrome reagent. An Olympus BX51 light microscope was employed to observe the liver tissue images.

The liver fibrosis level was assessed by anatomical pathologist based on the METAVIR score. The liver fibrosis level was categorized as no fibrosis with score of 1 (F0); portal fibrosis without septa with score of 2 (F1); portal fibrosis with a few septa with score of 4 (F2); numerous septa without cirrhosis with score of 8 (F3); and cirrhosis with score of 16 (F4). The ordinal METAVIR score data was presented in ratio data.[16]

TABLE 1. Treatment of rats

Groups	Treatment
Normal control	Injection of 0.9 % NaCl (i.p) for 11 wk.
Fibrosis control	Injection of 10 % CCl_4 (i.p) for 11 wk.
MO3	Injection of 10 % CCl_4 (i.p) for 11 wk, followed by oral administration of *M. oleifera* leaf extract at dose of 600 mg/kg BW daily for 3 wk.
MO6	Injection of 10 % CCl_4 (i.p) for 11 wk, followed by oral administration of *M. oleifera* leaf extract at dose of 600 mg/kg BW daily for 6 wk.
MO10	Injection of 10 % CCl_4 (i.p) for 11 wk, followed by oral administration of *M. oleifera* leaf extract at dose of 600 mg/kg BW daily for 10 wk.

Statistical analysis

Data were presented as the mean ± standard error of the mean (SEM). Comparisons among multiple groups were conducted by one-way analysis of variance (Anova) continued by the post hoc Tukey method using IBM SPSS version 23 software. A p value less than 0.05 was considered as statistically significant.

RESULTS

Characteristic of liver

The general condition of the rats in all groups were good. No cases died were observed or all rats were alive in this study. The liver weight in the fibrosis control group and the treatment groups (MO3, MO6, and MO10) were higher than the normal control. However, it was not statistically significant ($p>0.05$). The liver volume in the fibrosis control group and the treatment groups, except MO6, were also higher than the normal control. However, it was also not statistically significant ($p>0.05$) (TABLE 2).

Macroscopically, the color, surface texture, and freshness of the liver organs of each group were different (TABLE 3). The liver of the fibrosis control groups appeared pale with a wrinkled surface texture resembling an orange peel. In contrast, the liver organ in the normal control groups exhibited a fresh and smooth surface. The livers of MO3 and MO10 treatment groups appeared smoother and darker brown than the fibrosis control groups. Meanwhile, the liver of MO6 rat groups displayed a macroscopically glossy and fresh appearance, resembling the liver of the normal control rats.

TABLE 2. The weight and liver volume

Liver chacteristics (mean ± SE)	Normal control	Fibrosis control	MO3	MO6	MO10
Weight (g)	7.90 ± 0.34	9.13 ± 1.09	10.06 ± 0.77	8.17 ± 0.70	9.37 ± 0.70
Volume (mL)	7.13 ± 0.55	9.33 ± 120	8.73 ± 0.63	6.10 ± 1.52	9.40 ± 0.70

TABLE 3. The macroscopic appearance of the rat liver organs in the normal control, fibrosis control, MO3, MO6, and MO10 groups.

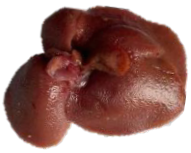
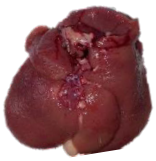
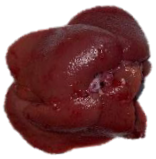
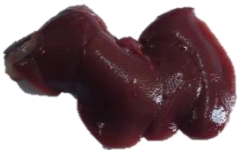
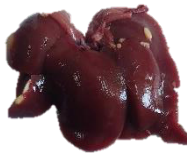
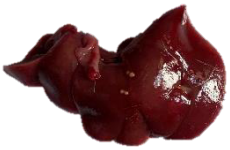
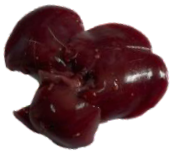
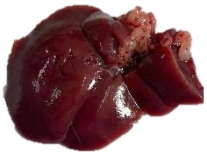
Groups	Liver		
	Rat 1	Rat 2	Rat 3
Normal control			
Fibrosis control			
MO3			
MO6			
MO10			

TABLE 4. Fibrosis degree based on METAVIR score

Groups	n	METAVIR score					Fibrosis score (mean ± SE)	p
		F0 (1)	F1 (2)	F2 (4)	F3 (8)	F4 (16)		
Normal control	3	2	1	-	-	-	1.33 ± 0.33	-
Fibrosis control	3	-	-	1	-	2	12.00 ± 4.00	<0.05[a]
MO3 treatment	3	-	-	2	1	-	5.33 ± 1.33	<0.05[a,b]
MO6 treatment	3	-	3	-	-	-	2.00 ± 0.00	<0.05[a,b,c]
MO10 treatment	3	-	2	1	-	-	2.67 ± 0.67	<0.05[a,b,c]

Note: [a]significantly different vs. normal control; [b]significantly different vs. fibrosis control; [c]significanlty different vs. MO3

Histopathological changes in the liver

The histopathological analysis of liver tissue resulted in METAVIR scores, which were transformed into ratio data to calculate the average fibrosis scores, as presented in TABLE 4. The table reveals that the administration of CCl4 for 11 wk resulted in two model rats with liver fibrosis, with METAVIR scores of F4, and one rat with a score of F2. MO administration led to a decrease in METAVIR scores, ranging from F3 to F1. A 6 wk treatment with MO successfully reduced the METAVIR scores to F1 in all rats ($p<0.05$). It indicates that the 6 wk MO treatment significantly produced fibrosis scores approaching the conditions observed in the control group.

The liver histological examination used Masson's Trichrome staining (FIGURE 1). This image represents a typical example of the METAVIR scores for each rat group, as presented in Table 2. In the control group, no fibrotic tissue was detected (F0), whereas the Model rats exhibited cirrhosis (F4). In the MO3 group, fibrotic tissue was visible in the portal triad, extending into the central vein and forming portal-central fibrosis (septal fibrosis/F3). The MO10 rats showed fibrotic tissue in several portal triads, resulting in portal fibrosis with a few septa (F2). Fibrotic tissue in the MO6 rats was limited to the portal triad and perisinusoidal regions (portal fibrosis/ F1).

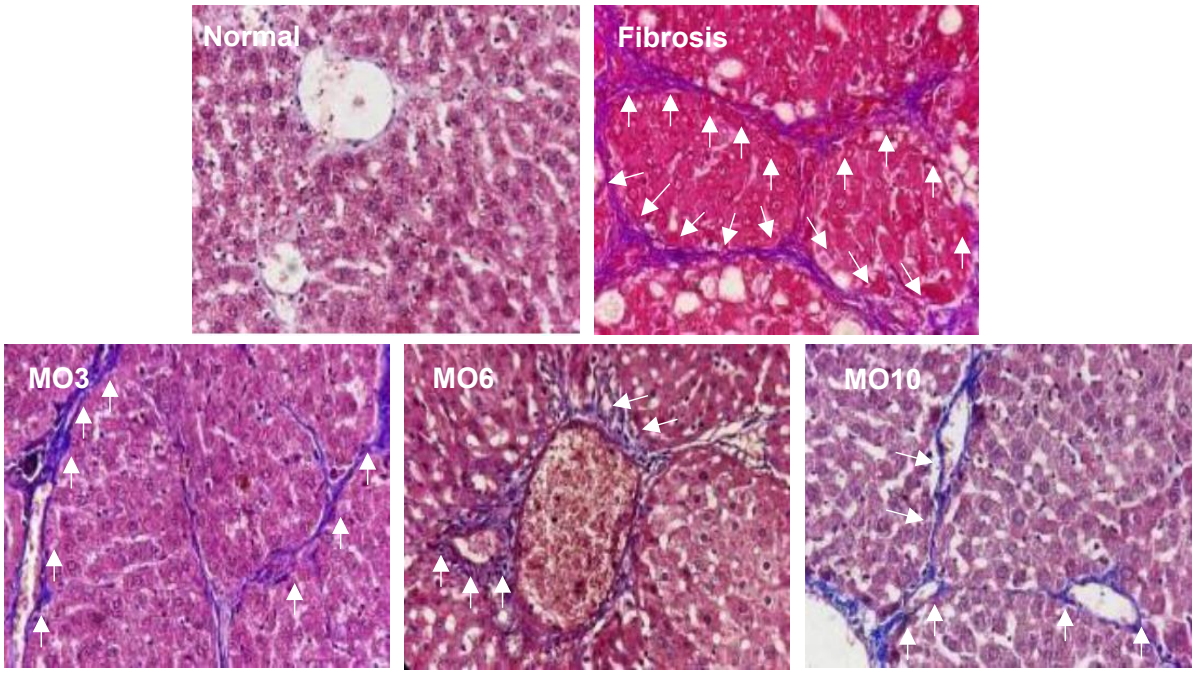


FIGURE 1. Histological liver tissue structure by Masson's Trichrome staining. The images were obtained using an Olympus BX51 microscope with a 400x magnification. The white arrow indicates the fibrosis area.

DISCUSSION

Chemically induction by CCl4 is the most commonly used for animal models of liver fibrosis.[17] The CCl_4 generates free radicals that cause lipid peroxidation, oxidative stress, membrane integrity loss, and hepatocyte apoptosis/ necroptosis.[18] It leads to Kupffer cell and hepatic stellate cell activation, resulting in an abundance of inflammatory mediators and collagen production, culminating in long-term liver fibrosis formation.[19] In this study, intraperitoneal injections of CCl_4 for 11 wk successfully induced fibrosis, even cirrhosis (F4) in the fibrosis control. In addition, severe fibrosis was more observed compared to the normal control. These findings were also supported by the macroscopic appearance of the liver, in which the liver in the fibrosis control appeared pale and wrinkled.-

The liver weight and volume were also more remarkable in the fibrosis control than in the normal control, although these differences were not statistically significant ($p>0.05$). It suggests that liver fibrosis does not significantly affect liver weight and volume. It was consistent with previous study reporting that fibrosis and cirrhosis did not substantially impact liver weight.[20] In addition, the correlation between fibrosis and rat's weight and volume might be biased due to the necessity of considering the rat's body weight in this scenario.

The objective of this study is to evaluate the curative effects of *M. oleifera* against liver fibrosis. Histologically, the liver tissues of rats treated with *M. oleifera* leaf extracts appeared better than those in the fibrosis control (FIGURE 1). Histological observations revealed that the presence of scattered fibrotic tissue around portal triads and septa of the liver fibrosis rats reduced after administration of *M. oleifera* leaf extract, especially for 6 and 10 wk administrations ($p<0.05$). The administration of *M. oleifera* leaf extract for 3 wk also demonstrated enhancement, although it was not statistically significant ($p>0.05$). It indicated that 6 wk administration of the *M. oleifera* leaf extract has a curative effect against rat liver fibrosis. Susanto *et al.*[21] reported that administration of *M. oleifera* leaf extract for 6 wk significantly decrease in TNF-β expression and prevent hepatocellular carcinoma progression.

The findings of this study supported previous studies in which *M. oleifera* leaf extract had a preventive effect against rat liver fibrosis. Administration of *M. oleifera* leaf extract and induction of CCl_4 simultaneously, could reduce liber fibrosis degree in rats by inhibiting HSC activation and promoting these a-HSC apoptosis.[12-13] Aly *et al.*[22] reported that *M. oleifera* leaf extract has an hepatoprotective effect by reducing liver enzymes, TNF-α, TNF-β, and degree of liver fibrosis in acetaminophen-induced liver fibrosis in rats.[23] These mechanisms[12,13,22] are believed can explain the curative effect of *M. oleifera* leaf extract.

The curative effect of *M. oleifera* against liver fibrosis is due to its active secondary metabolites especially quercetin and kaempferol. Quercetin was reported ameliorate liver inflammation and fibrosis by regulating hepatic macrophages activation and polarization.[23] Furthermore, quercetin reduces hepatic fibrogenesis by inhibiting TGF-β/Smad3 signaling pathway.[24] Whereas, kaempferol was reported inhibit a-HSC by regulating miR-26b-5p/Jag1 axis and notch pathway.[25] It was also reported ameliorate hepatic fibrosis by suppressing NF-κB pathway and promoting HSC apoptosis.[26]

The management of liver fibrosis remains a challenge for medical practice due to the unavailability of the standard treatment. Development of alternative treatment from medical plants is urgently needed. *Moringa oleifera* is

one of the potential medicinal plants for the treatment of liver fibrosis. Several studies in animal model of liver fibrosis have proven the protective and curative effect of *M. oleifera* leaf against liver fibrosis.[10-13,22-25] The toxicology was also reported that *M. oleifera* leaf can be categorized not toxic with a lethal dose 50% (LD_{50}) of 5,585 mg/kg BW.[27] However, further studies are needed including sub chronic or chronic toxicology, and development of formulation before clinical study conducted.

CONCLUSION

In conclusion, the administration of ethanolic extract of *M. oleifera* leaf at dose of 600 mg/kg BW daily for 6 wk has an effective curative effect against liver fibrosis in rat model fibrosis. Further studies will be conducted to evaluate the chronic toxicity, and to develop the formulation before clinical study conducted.

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The effect of infection on mortality in acute coronary syndrome patients at Dr. Sardjito General Hospital, Yogyakarta

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ABSTRACT

Ischemic heart disease is the second most significant health burden in Indonesia and the world. The prevalence of coronary heart disease patients in Yogyakarta is predicted to experience a continuous increase. In Sardjito Hospital, mortality rate of acute coronary syndrome (ACS) patients reaches 15%, with pneumonia infection identified as one of the predictors. Despite this high mortality rate, there is a lack of studies addressing the contribution of infectious comorbidities to mortality incidence among ACS patients. This study aimed to investigate the e ffect of infectious comorbidities on the incidence of mortality among ACS patients and its mortality rate in Sardjito Hospital. This study used a cross-sectional design in 794 patients diagnosed with ACS and registered in the SCIENCE registry from January to December 2022 at Sardjito Hospital. The analysis was conducted using the Chi-square method to determine the effect of infectious comorbidities on mortality among ACS patients and a logistic regression test to evaluate the correlation between variables. Based on bivariate analysis, it was found that infectious comorbidities increased mortality rate among ACS patients (p<0.001, OR=2.22[1.46-3.38]), reaching 5.2%. The bivariate analysis between confounding factors and outcome of patients showed that obesity, dyslipidemia, and revascularization significantly influenced the results of ACS patients. Based on multivariate analysis, it was discovered that infectious comorbidities, obesity, diabetes, dyslipidemia, and revascularization had a significant association with mortality of patients with ACS. Furthermore, infectious comorbidities increased the odds of mortality for ACS patients by 2.04 times. Infectious comorbidities increased the incidence of mortality in ACS patients by 2.04 times with mortality rate of 5.2%.

ABSTRAK

Penyakit jantung iskemik merupakan beban penyakit kedua di Indonesia dan dunia. Angka pasien penyakit jantung koroner di Yogyakarta pun diprediksikan akan terus meningkat. Tingkat mortalitas pasien sindrom koroner akut (SKA) di RSUP Dr.Sardjito mencapai 15% dengan salah satu prediktor mortalitasnya adalah infeksi pneumonia. Dari angka kematian yang cukup besar tersebut, belum ada studi yang membahas mengenai kontribusi komorbid infeksi terhadap kejadian mortalitas pasien sindrom koroner akut. Mengetahui pengaruh komorbid infeksi terhadap kejadian mortalitas pasien SKA dan mengetahui tingkat mortalitasnya di RSUP Dr.Sardjito. Penelitian ini menggunakan desain studi uji potong lintang (*cross sectional*) pada 794 pasien yang terdiagnosis sindrom koroner akut dan terdaftar di registri SCIENCE periode Januari-Desember 2022 RSUP Dr.Sardjito. Penelitian dilakukan menggunakan metode *Chi-square* untuk melihat pengaruh komorbid infeksi terhadap mortalitas pasien SKA dan uji regresi logistik untuk mengetahui korelasi antarvariabel. Berdasarkan analisis bivariat ditemukan bahwa komorbid infeksi meningkatkan kejadian mortalitas pasien SKA (p<0,001, OR=2,22[1,46-3,38]) dengan tingkat mortalitas mencapai 5,2%. Berdasarkan hasil analisis bivariat antara faktor perancu dengan luaran pasien, ditemukan bahwa riwayat obesitas, dislipidemia, dan revaskularisasi berpengaruh terhadap luaran pasien sindrom koroner akut secara signifikan. Sementara itu, berdasarkan analisis multivariat ditemukan bahwa komorbid infeksi, obesitas, diabetes, dislipidemia, dan revaskularisasi memiliki hubungan dengan kejadian mortalitas pasien SKA secara signifikan. Komorbid infeksi meningkatkan peluang kejadian mortalitas pasien SKA sebesar 2,04 kali. Komorbid infeksi meningkatkan kejadian mortalitas pasien SKA sebanyak 2,04 kali secara dependen dengan tingkat mortalitas sebesar 5,2%.

Keywords:
ischemic heart disease;
acute coronary syndrome;
comorbid infection;
in hospital mortality;
SCIENCE registry

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INTRODUCTION

Cardiovascular disease is the leading cause of death globally, followed by ischemic heart disease, particularly in developing countries,[1] including in Indonesia.[2] Meanwhile, the prevalence of coronary heart disease patients in Yogyakarta is predicted to experience a continuous increase.[3] Ischemic heart disease initiates with an imbalance between the supply and demand of oxygen to the heart due to occlusion of atherosclerotic plaque, spasm of blood vessel muscles, blockage by embolism, or arterial thrombus.[4] Consequently, acute coronary syndrome (ACS) arises, presenting a spectrum of clinical symptoms ranging from STEMI (ST-segment elevation myocardial infarction), NSTEMI (Non ST-segment elevation myocardial infarction), to UAP (unstable angina pectoris).[4]

Approximately 11.1% of ACS patients had nosocomial infection and 7% of patients suffered from sepsis.[5,6] Infection, such as pneumonia play a significant role in increasing mortality risk of cardiovascular disease patients admitted to the Cardiac Intensive Care Unit (CICU). Consequently, infectious comorbidities are included in the components of the mortality of patients risk scoring system.[7,8] When patients develop infection after ACS event, infection-causing pathogens, such as bacteria, can invade the ruptured plaque.[9] This results in an increased inflammatory response causing the infarct area to expand, leading to sepsis in patients.[6] Mortality rate of ACS patients is significantly high, reaching 52%. Meanwhile, in Dr. Sardjito General Hospital, Yogyakarta mortality rate of patients with cardiovascular disease reaches 15%, with pneumonia being one of the predictors.[7,9] Despite these high mortality rates, there is a lack of studies investigating the contribution of infectious comorbidities to mortality rate of ACS patients.

MATERIAL AND METHODS

Study design

This study used an analytical observational method with a cross-sectional design to evaluate the comparison of mortality rates of ACS patients accompanied by infectious comorbidities at Dr. Sardjito General Hospital, Yogyakarta from January to December 2022. The secondary data were obtained from the SCIENCE registry (Sardjito Cardiovascular Intensive Care) of the Dr. Sardjito General Hospital.

Protocol of study

This study followed the protocol in the SCIENCE registry and the protocol has received ethical approval from the Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada with ethical clearance number KE/FK/0311/EC/2023.

The inclusion criteria were patients diagnosed with ACS, while those aged <18 y.o. with incomplete data were excluded. The data collected included demographic (age, gender, and patients risk factors), clinical (diagnosis of ACS, diagnosis of infection, pneumonia, urinary tract infection, and sepsis), and outcome of patients (mortality).

The dependent and independent variables in this study were mortality of patients and infectious comorbidities, respectively. Meanwhile, the confounding variables were smoking, dyslipidemia, hypertension, obesity, diabetes mellitus, and revascularization.

Statistical analysis

The data collected were processed with IBM SPSS and bivariate analysis using the Chi-square method was performed to determine the effect of infectious comorbidities on mortality of patients. Chi-square analysis was also carried out to determine the effect of confounding variables on mortality of patients. A $p < 0.05$ was considered as statistically significant. All bivariate analysis results with a $p < 0.25$ were subjected to further multivariate analysis in the form of regression analysis to determine the likelihood of mortality in ACS patients based on predictor variables such as infectious comorbidities and other confounding factors.

RESULTS

The secondary data in the SCIENCE registry showed that there were 797 patients diagnosed with ACS between January and December 2022. A total of 3 patients with incomplete data were excluded from this study, resulting in a final sample size of 794 patients.

Analysis of baseline characteristics in TABLE 1 showed that the sample was predominantly male (76.2%), aged ≥ 60 years (53.7%), classified as obese based on body mass index (39.9%), diagnosed with IMAEST (70%), received revascularization procedures (84%), and experienced cardiogenic shock (19.4%). The mean age of the sample was 60 years with a standard deviation of 11.57 and a variance of 133.97, showing that the age variation of the sample was wide. Additionally, only 18.6% of patients in the population had infectious comorbidities with the most common infection being pneumonia (14.1%). Based on risk factors for heart disease, more than half of the sample had a history of hypertension (61.1%) and smoking (59.7%).

Bivariate analysis showed that mortality rate of ACS patients with infectious comorbidities was 5.2% (TABLE 2). It was indicated that infectious comorbidities significantly increased mortality rate of ACS patients [$p<0.001$, OR=2.22 (1.46-3.38)].

Bivariate analysis was also performed between confounding variables and mortality of outcomes of patients. The results showed that history of obesity ($p=0.011$), dyslipidemia ($p=0.019$), and revascularization ($p=0.002$) significantly influenced the outcomes of ACS patients (TABLE 3).

The results of bivariate analysis with a significance value <0.25 were further examined for correlation with the dependent variable using logistic regression analysis. This was carried out to determine whether infectious comorbidities served as an independent factor influencing mortality incidence of ACS patients. Based on bivariate analysis, the variables of obesity ($p=0.011$), diabetes ($p=0.074$), dyslipidemia ($p=0.019$), revascularization ($p=0.002$), and the presence of comorbid infection ($p<0.001$) met the criteria for logistic regression analysis to determine the correlation with the dependent variable.

TABLE 1. Baseline characteristics of patients (n=794)

Variable	n (%)
Sex	
• Male	605 (76.2)
• Female	189 (23.8)
Age [(mean ± SD); variance]	[(60.68 ± 11.57); 133.97]
• < 60	368 (46.3)
• ≥ 60	426 (53.7)
BMI Classification	
• Underweight	37 (4.7)
• Normal	265 (33.4)
• Overweight	175 (22.0)
• Obesity	317 (39.9)
Risk factors	
• History of coronary heart disease (MI, CABG, angioplasty)	164 (20.7)
• Smoking	474 (59.7)
• History of ischemic heart disease	88 (11.1)
• Hypertension	485 (61.1)
• Diabetes	230 (29.0)
• Dyslipidemia	101 (12.7)
Type of ACS Disease	
• STEMI	556 (70.0)
• NSTEMI	185 (23.3)
• UAP	53 (6.7)
• Infectious Comorbidities	148 (18.6)
• Pneumonia	112 (14.1)
• UTI	29 (3.7)
• Sepsis	6 (0.8)
• Mortality of patients	136 (17.1)
Revascularization	
• Yes	667 (84.0)
• No	127 (16.0)
• Presence of shock	168 (21.1)
• Cardiogenic shock	154 (19.4)
• Septic shock	13 (1.6)
• Hypovolemic shock	1 (0.1)

TABLE 2. Chi-square analysis of the presence of infectious comorbidities on outcome of patients

	Outcome of patients		p	OR (95%CI)
	Mortality [n (%)]	Alive [n (%)]		
Infectious comorbidities				
• Yes	41 (5.2)	107 (13.5)	<0.001	2.22 (1.46-3.38)
• No	95 (12.0)	551 (69.4)		
Total	136 (17.1)	658 (82.9)		

TABLE 3. Chi-square analysis of confounding factors on mortality of ACS patients

Variable	Outcome of patients		p
	Mortality [n (%)]	Alive [n (%)]	
Obesity	41 (5.2)	276 (34.8)	0.011
Smoking	79 (9.90	395 (49.7)	0.674
Hypertension	81 (10.2)	404 (50.9)	0.689
Diabetes	48 (6.0)	182 (22.9)	0.074
Dyslipidemia	9 (1.1)	92 (11.6)	0.019
Revascularization	102 (12.8)	565 (71.2)	0.002
Total	136 (17.1)	658 (82.9)	

TABLE 4. Results of binary logistic regression analysis on mortality of ACS patients

Variable	OR (95% CI)	p
Infectious comorbidities	2.04 (1.32-3.14)	0.001
Obesity	0.65 (0.43-0.98)	0.042
Diabetes	1.52 (1.01-2.27)	0.044
Dyslipidemia	0.43 (0.21-0.89)	0.024
Revascularization	0.59 (0.37-0.94)	0.025

Multivariate analysis showed that all variables, namely infectious comorbidities(p=0.001),obesity(p=0.042), diabetes (p=0.044), dyslipidemia (p=0.024), and revascularization (p=0.025) had a significant association with mortality incidence of ACS patients (TABLE 4). However, only infectious comorbidities (OR=2.04) and diabetes (OR=1.52) could increase the chance of mortality for ACS patients. Based on the results, in ACS patients with infectious comorbidities, the chance of mortality was 2.04 times higher than in ACS patients without infection.

DISCUSSION

This study showed that there were 148 patients (18.6%) who had infectious comorbidities with mortality rate of 5.2%. Infectious comorbidities had the significant effect on increasing mortality incidence of ACS patients with mortality rate of 41 patients (p<0.001). Several mechanisms were identified through which infection increased incidence of mortality in ACS patients. This included an increase in systemic inflammatory conditions that have previously occurred in ACS patients, affecting the elevation of pro-inflammatory cytokines, hypercoagulation status, and excessive vasodilation. Hypercoagulable status in patients is caused by increased interaction between immune cells and tissue factors capable of causing DIC (disseminated intravascular coagulation).[10] Furthermore, excessive vasodilation is triggered by the inflammatory response, which is associated with cardiogenic shock, a condition prone to occur in myocardial infarction patients as a form of body decompensation to the shock.[11] Both DIC and excessive vasodilation were found to cause impaired hemodynamics and perfusion to organs resulting in organ dysfunction and shock.[11]

Mortality in ACS patients are usually caused by complications such as acute heart failure, cardiogenic shock, sepsis shock, and arrhythmias.[12] Myocardial infarction causes failure to the heart's pumping function, so the heart cannot pump blood adequately to the rest of the body, hence hypoperfusion occurs.[13] Initially, the body will compensate for the hypoperfusion condition by activating sympathetic responses, which can later lead to an increase in pressure in the left ventricle, followed by pulmonary vascular congestion, then an increase in right ventricular pressure followed by vascular congestion throughout the body.[13] This leads to heart failure, hemodynamic dysfunction (cardiogenic shock) and damage to various organs.[20] Comorbid infections in ACS patients will worsen the complications of cardiogenic shock by 20-30% due to hyperinflammation that causes excessive vasodilation and worsening of hemodynamic disturbance in the body.[11]

Impaired perfusion to various organs can also occur due to infection, which is called sepsis shock.[11] As many as 7% of patients with a primary diagnosis of acute myocardial infarction develop sepsis.[6] Comorbid infection, which is the cause of sepsis, affects the outcome of acute coronary syndrome patients through the increased of systemic inflammatory response (hyperinflammation), enlargement of myocardial infarction area, activation of coagulation, and invasion of atherosclerotic plaque by infectious bacteria.[6]

In this study, obesity significantly influenced mortality of ACS patients (p=0.011) and was a protective factor for mortality of patients (OR: 0.65; 95% CI: 0.43-0.98). Generally, obesity causes various changes in cardiac work and endothelial function due to increased oxidative stress from the release of pro-inflammatory cytokines by adipose tissue.[14] These changes in endothelial and cardiac function lead to increased atherosclerosis formation, resulting in

a higher risk of ACS in obese patients.[14] Although the risk of ACS is increase in obese patients, mortality rate among patients is lower compared to normal and underweight BMI group.[15] Further studies found that the obesity paradox phenomenon only occurred in short-term outcome of patients. This showed that obesity would reduce the risk of poor prognosis in the short term (30 d), while long-term (30 d to 1 yr) prevalence is characterized by a worse prognosis than patients without obesity.[16]

Obesity is associated with various conditions that pose a risk for cardiovascular disease such as dyslipidemia.[17] Dyslipidemia is characterized by an imbalance in lipid profile of a person, where HDL levels in the blood are low, while LDL and triglyceride levels are high.[17] In this study, there were 101 patients (12.7%) who had dyslipidemia, and the incidence of mortality was relatively low, occurring in 9 out of the total sample (1.1%). The condition of dyslipidemia influenced mortality incidence of ACS patients significantly (p=0.019) and was a protective factor for mortality of patients with ACS (OR: 0.43; 95% CI: 0.21-0.89). Although obesity and dyslipidemia are different, 60-70% of obese patients have dyslipidemia.[18]

A total of 84% of patients in this study received revascularization, which affected mortality incidence of ACS patients (p=0.002), acting as a protective factor (OR: 0.59; 95% CI: 0.37-0.94). Since almost all patients received revascularization procedures, the risk of associated infection increased. Post-revascularization infection arise due to instrumentation when performing PCI (Percutaneous Coronary Intervention) procedures. This includes access to the vasculature, repetitive injections in the same location, and the duration of the procedure, contributing to the risk of infection to increase.[19]

In this study, 230 patients (29%) were identified with diabetes. The results showed that diabetes did not affect the incidence of mortality in ACS patients (p=0.074). However, multivariate analysis showed that diabetes increased the odds of mortality in ACS patients by 1.52 times. In diabetic patients, vascular endothelial dysfunction occurs due to decreased synthesis of potent vasodilators, namely nitrogen monoxide (NO), caused by reduced eNOS (endothelial nitric oxide synthase). This phenomenon led to impaired vasodilatory function of coronary vessels, resulting in high smooth muscle cell proliferation, leukocyte adhesion, and platelet aggregation in coronary vessels.[18] The condition of insulin resistance that appeared in type two DM patients also caused the energy efficiency of the heart to decrease.[20] This study has a limitation, such as not being able to examine the effect of specific infections on the mortality of ACS patients.

CONCLUSION

In conclusion, infectious comorbidities increased mortality rate of ACS patients by 2.04 times (p=0.001; 95%CI: 1.32-3.14) with mortality rate of 5.2%. It is recommended, ACS patients with comorbid infections should receive more attention considering the influence of comorbid infections on patient mortality.

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Laboratory findings of postoperative venous thromboembolism (VTE) in Dr. Sardjito General Hospital, Yogyakarta

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ABSTRACT

Venous thromboembolism (VTE) is a significant risk, especially for older individuals. Indonesian studies found 37.1% VTE incidence in bedridden patients over 40 and 2.1% in major surgeries. Surgery, like hip fractures, raises the risk temporarily. Diagnosis relies on tests like ultrasound with Doppler, Wells score, and neutrophil-to-lymphocyte ratio (NLR). This study aimed to evaluate the laboratory findings of postoperative VTE. A retrospective analysis was conducted in Dr. Sardjito General Hospital, Yogyakarta using medical record data of VTE patients who underwent major surgery. The laboratory data, including complete blood count characteristics for every month for three months after postsurgery and DVT presentation when it occurred on the diagnostic day were collected. In total of 27 patients involved in this study, VTE cases were more common in digestive (41.2%) and obstetric gynecology surgeries (29.4%) for females, and nervous (44.4%) and cardiovascular surgeries (22.2%) for males. Females had a higher prevalence of Well Score ≥3 (82.4% vs 40%; p=0.058) and longer VTE therapy durations (65.50 ± 46.51 vs 39.60 ± 41.04 d; p=0.172). Males had more unilateral VTE occurrences (90.9 vs 56.3%; p=0.070) and a higher proportion of total occlusion cases (60 vs 37.5%; p=0.422). NLR exhibited a significant decrease from the 1st to the 2nd month (10.52 vs 3.64; p=0.009), followed by an insignificant increase in the 3rd month (3.64 vs 3.98; p=0.878). Notably, NLR trended downward in the 2nd month examination. In conclusion, VTE occurs in 0.21% of postoperative patients, with the highest incidence observed in post-gynecological surgery patients. The NLR can serve as a diagnostic tool for VTE in extremities, as an elevated NLR indicates the presence of a more proximal thrombus.

ABSTRAK

Tromboemboli vena (VTE) merupakan risiko nyata, terutama pada orang lanjut usia. Penelitian di Indonesia menemukan 37,1% kejadian VTE pada pasien berusia di atas 40 tahun yang terbaring di tempat tidur dan 2,1% pada pasien operasi besar. Pembedahan, seperti patah tulang pinggul, meningkatkan risiko untuk sementara. Diagnosis bergantung pada tes seperti USG dengan Doppler, skor Wells, dan rasio neutrofil-limfosit (NLR). Penelitian ini bertujuan untuk mengevaluasi temuan laboratorium pasca operasi VTE. Analisis retrospektif dilakukan di RSUP Dr. Sardjito Yogyakarta dengan menggunakan data rekam medis pasien VTE yang menjalani operasi besar. Data laboratorium dikumpulkan, termasuk karakteristik hitung darah lengkap setiap bulan selama tiga bulan setelah pasca operasi dan gambaran DVT yang terjadi pada saat diagnosis. Sebanyak 27 pasien yang terlibat dalam penelitian ini, kasus VTE lebih banyak terjadi pada operasi pencernaan (41,2%) dan obstetri-ginekologi (29,4%) pada wanita serta operasi saraf (44,4%) dan kardiovaskular (22,2%) untuk pria. Perempuan memiliki prevalensi skor Wells ≥3 yang lebih tinggi (82,4% vs 40%; p=0,058) dan durasi terapi VTE yang lebih lama (65,50 ± 46,51 vs 39,60 ± 41,04 hari; p=0,172). Laki-laki memiliki lebih banyak kejadian VTE unilateral (90,9 vs 56,3%; p=0,070) dan proporsi total kasus oklusi yang lebih tinggi (60 vs 37,5%; p=0,422). NLR menunjukkan penurunan nyata pada bulan ke-1 hingga ke-2 (10,52 vs 3,64; p=0,009), diikuti peningkatan tidak nyata pada bulan ke-3 (3,64 vs 3,98; p=0,878). Khususnya, NLR cenderung menurun pada pemeriksaan bulan ke-2. Kesimpulannya, VTE terjadi pada 0,21% pasien pasca operasi, dengan insiden tertinggi terjadi pada pasien pasca operasi ginekologi. NLR dapat berfungsi sebagai alat diagnostik untuk VTE pada ekstremitas, karena peningkatan NLR menunjukkan adanya trombus yang lebih proksimal.

Keywords:
postoperative; venous thromboembolism; neutrophil to lymphocyte ratio; complete blood count; pulmonary embolism

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INTRODUCTION

Venous thromboembolism (VTE) is a disease that includes pulmonary embolism (PE) and deep vein thrombosis (DVT). Every year, about 900,000 people in the United States are estimated to suffer from VTE, and up to 100,000 of them may not survive. The probability of developing VTE increases with age, with 60% of all cases occurring in individuals aged 70 years and above.[1] VTE incidence varies by race, with higher incidence in African Americans and lower incidence in Asians, Asian Americans, and Native Americans. Age is a significant factor in VTE incidence, with both men and women experiencing higher rates of DVT and PE as they age. Men have a higher overall incidence rate of VTE compared to women, but women have slightly higher rates during their childbearing years. Men are also at a higher risk of VTE recurrence than women, due in part to the low recurrence risk in women with hormone-related index thrombosis. Women are at an increased risk of VTE during their fertile years, with additional burden associated with hormonal contraceptives or pregnancy, and in later life due to hormone therapy for menopause. Therefore, gender, age, and exposure to sex-specific triggers all play a role in the incidence of VTE.[2,3] In Indonesia, a prospective observational registry study found that the incidence of DVT was 37.1% in eligible patients and 40.3% in evaluable patients who were over 40 years old and bedridden due to acute medical illnesses.[4] Meanwhile, another cross-sectional study found that the incidence of VTE was 2.1% among patients who underwent major orthopedic or abdominal surgery. In their report, DVT and PE accounted for 1.3% and 0.8% of cases.[5]

Virchow's triad, comprising venous stasis, blood hypercoagulability, and vascular wall injury, is the basis for venous thrombosis. There are some risk factors contribute for DVT, which can be provoked or unprovoked, transient or persistent. Major surgery are transient risk factors for DVT; however, the risk varies depending on procedure-based factors, including patient age, prior DVT, malignancy, surgical procedure, and duration of bed rest. For instances, patients with hip fractures and long bone fractures have higher risk of DVT compared to other surgical procedures. The Caprini assessment model can be used to predict higher-risk patients based on specific patient characteristics and medical history.[1,6]

To diagnose DVT, objective tests are required due to the nonspecific symptoms of DVTs and PEs. Although D-dimer is sensitive, it has low specificity and cannot be used alone. Less invasive diagnostic tests include compression ultrasound, computed tomography pulmonary angiography, and ventilation perfusion scans. The Wells score can be used to stratify clinical presentation and probabilities, and ultrasound with Doppler is the imaging test of choice. Additionally, the ratio of neutrophils to lymphocytes (NLR) can be used as an additional diagnostic tool.[7,8] This study will assess laboratory findings of DVT after major surgery and provide DVT epidemiology data in Indonesia.

MATERIAL AND METHODS

Subjects and design

This retrospective analysis was conducted using data collected from medical records at Dr. Sardjito General Hospital, Yogyakarta, Indonesia. The study was approved by the Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada/Dr. Sardjito General Hospital, Yogyakarta with reference number KE/FK/0612/EC/2022.

Procedure

The medical records with ICD10 codes for venous thromboembolism (VTE) were selected. Patients who underwent major surgery and diagnosed with VTE from 2016 to 2021 were included. The exclusion criteria were patients with incomplete medical records, those who had a history of anticoagulant prophylaxis before and after surgery, those with disseminated intravascular coagulation (DIC), and those with DVT diagnosed more than 12 mo after cardiac and orthopedic prosthesis placement and more than one month after other major surgeries (FIGURE 1). The subjects were then grouped based on the type of surgery, and data was collected for each patient, including the length of stay, mortality (all causes of death), DVT onset, wells score, location of thrombus, laterality of thrombus, and degree of occlusion. Laboratory data, including complete blood count characteristics monthly after post-surgery day and DVT presentation when it occurred at the diagnostic day were also collected.

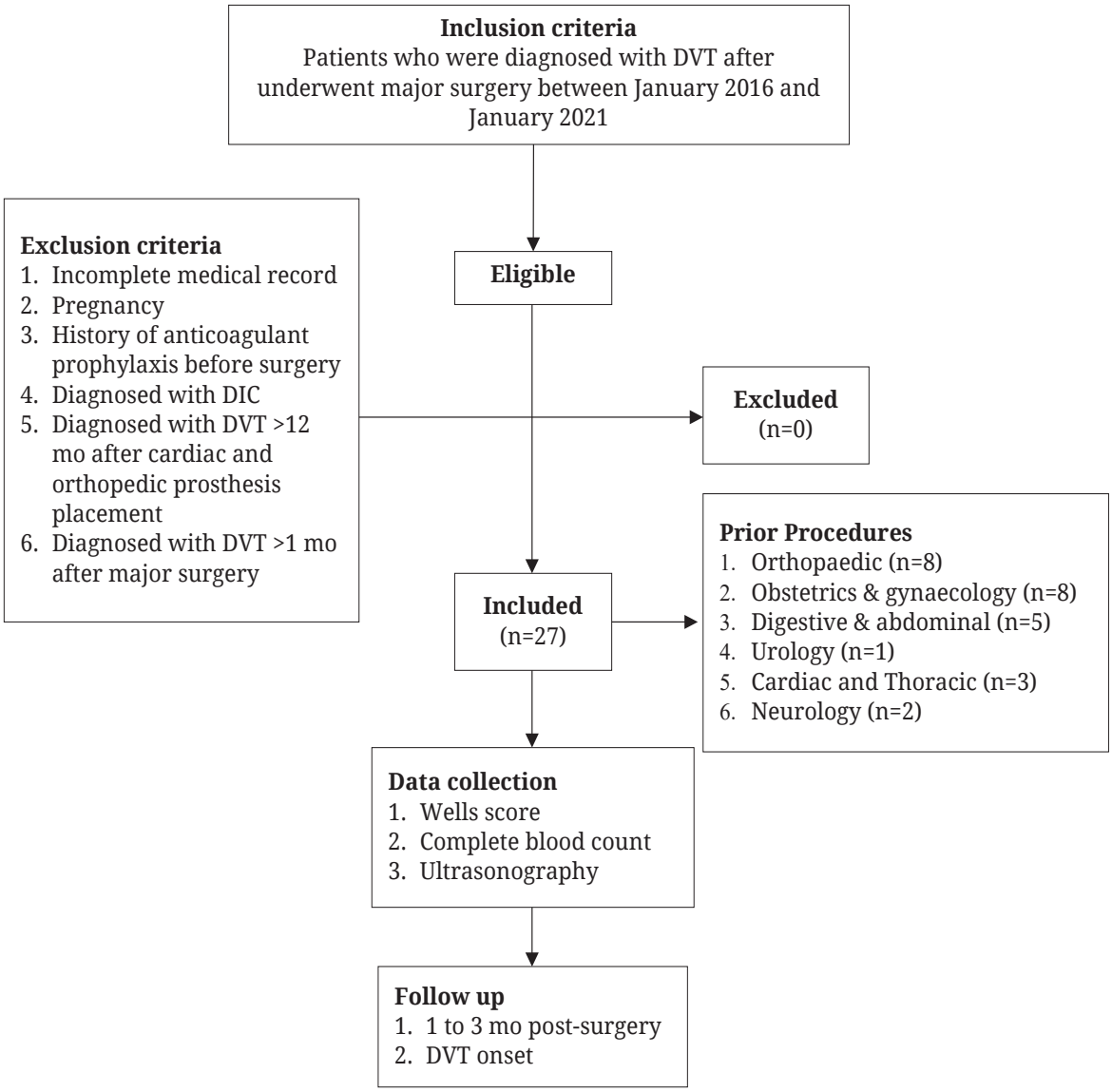

FIGURE 1. Selection criteria for eligible patient

Statistical analysis

Data were presented as mean ± standard of deviations (SD) or frequency or median (min. – max.). Kruskal Wallis or Mann Whitney tests were applied to analyze differences among groups. A p value of <0.05 was considered significant. All statistical analyses were performed using MedCalc software (version 19.6).

RESULTS

A total of 27 subjects were included in this study. The demographic information of the subjects are presented in TABLE 1. The mean age of male subjects is higher than that of female subjects (54.71±8.72 vs 51.10±20.15 y.o.). Risk stratification for surgery indicates that a greater proportion of female subjects are classified as grade 3 compared to male subjects (64.7 vs 22.2%; p=0.060). When stratified by organ systems, the majority of post-surgery VTE cases in female subjects occurred in surgeries involving the digestive and obstetric gynecology system (41.2 and 29.4%), while in male subjects, most post-surgery VTE cases were associated with surgeries involving the nervous and cardiovascular system (44.4 and 22.2%). The percentage of subjects with a Well Score ≥3 was higher in female subjects than in male subjects (82.4 vs 40%; p=0.058). The duration of VTE therapy was longer in female subjects compared to male subjects (65.50±46.51 vs 39.60±41.04 d; p=0.172). Regarding the presentation of VTE, a higher proportion of cases were unilateral in male subjects compared to female subjects (90.9 vs 56.3%; p=0.070). Meanwhile, the location of thrombus formation indicates a higher proportion of proximal thrombus in female subjects compared to male subjects (87.5 vs 80%; p=0.625). Male subjects also exhibit a higher proportion of total occlusion compared to female subjects (60 vs 37.5%; p=0.422).

The haematological laboratory characteristics 3 months post-surgery are presented in TABLE 2. The complete blood examination in the first month post-surgery showed a median erythrocyte count of 3.79 (2.77-5.19) $x10^3$ cells/mL, median hemoglobin of 10.70 (7.80-14.30) g/dL, and median hematocrit of 32.40% (23.60-44.40). The D-dimer result in the first month post-surgery shows a median value of 5269.50 (709-11320) ng/mL (TABLE 2). There is a significant difference in the results of the leucocyte differential count (p<0.05), despite the leucocyte count showing no significant difference (p=0.133). The platelet count demonstrates a median increase from the first to the second month (215.5 vs 401.0 $x10^3$ cells/mL; p=0.061), followed by an insignificant median decrease from the second to the third month (401.0 vs 266.5 $x10^3$ cells/mL; p=0.508). The neutrophil to lymphocyte ratio (NLR) exhibits a notably high median value in the first month, which decreases significantly in the second month (10.52 vs 3.64; p=0.009), followed by an insignificant increase in the third month (3.64 vs 3.98; p=0.878). Additionally, it is noteworthy that there is a significant difference in erythrocyte count, which was higher in unilateral compared to bilateral VTE (4.02 ± 0.61 vs 3.51 ± 0.41 $x10^3$ cells/mL; p=0.031). This pattern is mirrored in hemoglobin value (11.21 ± 1.71 vs 9.84 ± 1.34 g/dL; p=0.051), hematocrit value (34.22 ± 5.02 vs 29.88 ± 3.98%; p=0.039), and lymphocyte count, which was higher in unilateral compared to bilateral VTE (11.52 ± 7.13 vs 6.91 ± 2.73 $x10^3$ cells/mL; p=0.045), as presented in TABLE 3.

TABLE 1. Patient baseline characteristics and demographic data

Variables	Quantity
Age (mean ± SD yr)	53.37 ± 13.80
Sex [n (%)]	
• Male	10 (37.0)
• Female	17 (63.0)
Risk stratification [n (%)][a]	
• 1	1 (3.7)
• 2	12 (44.4)
• 3	14 (51.9)
Surgery classification [n (%)]	
• Endocrine	1 (3.7)
• Reproductive	8 (29.6)
• Digestive	5 (18.6)
• Cranial and nerve system	2 (7.4)
• Cardiovascular	2 (7.4)
• Musculoskeletal	8 (29.6)
• Renal and urinary system	1 (3.7)
Post-surgery length of stay (mean ± SD d)	12.15 ± 11.04
DVT onset (d)	20 ± 18.30
Well score [n (%)]	
• 1	3 (11.1)
• 2	6 (22.2)
• 3	12 (44.4)
• 4	4 (14.8)
• 5	2 (7.4)
Therapy duration (mean ± SD d)	54.71 ± 45.29
DVT presentation [n (%)]	
• Unilateral	18 (69.23)
• Bilateral	8 (30.77)
Location of thrombus [n (%)]	
• Proximal	22 (84.62)
• Distal	4 (15.38)
Degree of occlusion [n (%)]	
• Partial	14 (53.85)
• Total	12 (46.15)

During the initial one-month follow-up of laboratory patients, the NLR was found to be significantly higher in proximal thrombus compared to distal thrombus (14.82 ± 9.77 vs 3.15 ± 0.86; p<0.0001). Additionally, the neutrophil count demonstrated an elevated value in proximal thrombus compared to distal thrombus (85.99 ± 5.69 vs 66.63 ± 4.65 $x10^3$ cells/mL; p=0.007). Conversely, the lymphocyte count was lower in proximal thrombus compared to distal thrombus (7.95 ± 3.99 vs 21.87 ± 3.97 $x10^3$ cells/mL; p=0.015). Furthermore, the eosinophil count was reduced in proximal thrombus compared to distal thrombus (0.91 ± 1.59 vs 2.17 ± 0.64 $x10^3$ cells/mL; p=0.044), as presented in TABLE 4.

TABLE 2. Hematological laboratory characteristics 3 months post-surgery

Variable	1st month	2nd month	3rd month	p
Leucocyte (x10³ cells/mL)	12.15 (3.02-25.55)	8.15 (1.15-28.16)	9.74 (5.66-20.55)	0.133
Erythrocyte (x10³ cells/mL)	3.79 (2.77-5.19)	4.01 (2.77-5.21)	3.775 (2.37-4.84)	0.362
Platelet (x10³ cells/mL)	215.5 (62-745)	401.0 (16-707)	266.5 (116-657)	0.103
Hemoglobin (g/dL)	10.7 (7.8-14.3)	10.9 (7.4-14)	10.85 (6.4-12.5)	0.677
Hematocrit (%)	32.4 (23.6-44.4)	33.7 (22.5-43.3)	32.5 (18.9-39.2)	0.413
Neutrophil (%)[a,b]	85.65 (63.1-94.9)	67.9 (30.7-91.3)	70.15 (59.9-87.4)	0.001
Lymphocyte (%)[a,b]	8 (2.5-25)	19.2 (5.2-43.9)	17.95 (7.1-28.4)	0.001
Monocyte (%)[b]	5.65 (1.4-11.4)	7 (1.5-18.1)	8 (3.1-17.5)	0.029
Eosinophil (%)[a,c]	0.35 (0-6.4)	2.2 (0-8.5)	0.85 (0.1-4.5)	0.008
Basophil (%)[a]	0.2 (0.1-0.8)	0.4 (0.2-1.9)	0.45 (0-0.9)	0.014
NLR[a,b]	10.52 (2.59-38.31)	3.64 (0.7-17.27)	3.98 (2.14-12.35)	0.001

Note: p-value was analyzed using Kruskal-Wallis and Wilcoxon test for post hoc; [a] significantly different between 1st and 2nd month ($p<0.05$); [b]significantly different between 1st and 3rd month ($p<0.05$); [c]significantly different between 2nd and 3rd month ($p<0.05$); NLR: neutrophil to lymphocyte ratio

TABLE 3. Hematological laboratory characteristics based on VTE presentation in the first month

Variable	Unilateral	Bilateral	p
Leucocyte (x10³ cells/mL)	10.76 (3.02-25.55)	12.98 (6.11-16.48)	0.839
Erythrocyte (x10³ cells/mL)	3.91 (3.11-5.19)	3.52 (2.77-4.04)	0.031*
Platelet (x10³ cells/mL)	215.50 (62-745)	261.50 (183-635)	0.593
Hemoglobin (g/dL)	11.05 (8.10-14.30)	10.35 (7.80-11.20)	0.051*
Hematocrit (%)	33.70 (25-44.40)	31.45 (23.60-34.10)	0.039*
Neutrophil (%)	83 (63.10-92)	87.40 (81.50-94.90)	0.059*
Lymphocyte (%)	10.65 (2.50-25)	6.60 (2.50-10.90)	0.045*
Monocyte (%)	6.20 (1.40-11.40)	4.65 (2.50-8.20)	0.373
Eosinophil (%)	0.45 (0-6.40)	0.25 (0-2.30)	0.200
Basophil (%)	0.20 (0.10-0.80)	0.20 (0.10-0.70)	0.802
NLR	7.98 (2.59-36.46)	13.34 (7.56-38.31)	0.381

Note: number of sample p-value was analyzed using Mann-Whitney; *significantly different ($p < 0.05$); NLR: neutrophil to lymphocyte ratio.

TABLE 4. Hematological laboratory characteristics based on thrombus location in the first month

Variable	Proximal	Distal	p
Leucocyte (x10³ cells/mL)	12.52(3.02-25.55)	9.96 (6.24-11.33)	0.131
Erythrocyte (x10³ cells/mL)	3.79(2.77-5.19)	3.89 (3.33-4.36)	0.937
Platelet (x10³ cells/mL)	214(62-745)	220 (216-252)	0.231
Hemoglobin (g/dL)	10.60(7.80-14.30)	11.80 (9.90-12.20)	0.459
Hematocrit (%)	32.30(23.60-44.40)	34.90 (28.90-39.10)	0.594
Neutrophil (%)	86.80(74.80-94.90)	64.90 (63.10-71.90)	0.007*
Lymphocyte (%)	6.90(2.50-15.90)	23.20 (17.40-25)	0.015*
Monocyte (%)	5.20(1.40-8.20)	8.70 (6.40-11.40)	0.100
Eosinophil (%)	0.30(0-6.40)	1.80 (1.80-2.90)	0.044*
Basophil (%)	0.20(0.10-0.70)	0.50 (0.20-0.80)	0.272
NLR	12.55(5.05-38.31)	2.72 (2.59-4.14)	<0.0001*

Note: number of sample p-value was analyzed using Mann-Whitney; *significantly different ($p < 0.05$); NLR: neutrophil to lymphocyte ratio.

TABLE 5. Hematological laboratory characteristics based on VTE occlusion degrees

Variable	Partial	Total	p
Leucocyte (x10^3 cells/mL)	11.30 (3.02-25.55)	12.52 (9.82-16.48)	0.618
Erythrocyte (x10^3 cells/mL)	3.79 (2.77-4.75)	3.80 (3.20-5.19)	0.196
Platelet (x10^3 cells/mL)	252 (62-745)	192 (98-583)	0.219
Hemoglobin (g/dL)	10.30 (7.80-14.20)	11.10 (7.80-14.30)	0.283
Hematocrit (%)	32.20 (23.60-40.50)	32.70 (24-44.40)	0.278
Neutrophil (%)	86.10 (63.10-94.90)	85.20 (64.90-92.30)	0.986
Lymphocyte (%)	9 (2.50-23.20)	7.70 (4-25)	0.820
Monocyte (%)	5.70 (1.40-11.40)	5.60 (2.50-8.20)	0.867
Eosinophil (%)	0.30 (0-3.40)	0.60 (0-6.40)	0.517
Basophil (%)	0.20 (0.10-0.70)	0.20 (0.10-0.80)	0.564
NLR	9.79 (2.72-38.31)	11 (2.59-22.44)	0.648

Note: p-value was analyzed using Mann-Whitney; NLR: neutrophil to lymphocyte ratio.

The hematological laboratory characteristics based on VTE occlusion degrees were not significantly different (TABEL 5). The erythrocyte count was found to be lower in cases of partial occlusion when compared to total occlusion VTE, with values of 3.68 ± 0.49 vs 4.05±0.69 x10^3 cells/mL, respectively (p=0.196). Similarly, hemoglobin levels exhibited a decrease in instances of partial occlusion compared to total occlusion VTE, registering values of 10.38 ± 1.63 vs 11.20 ± 1.76 g/dL, respectively (p=0.283). Additionally, the hematocrit also demonstrated a lower value in partial occlusion VTE cases compared to total occlusion, with measurements of 31.58 ± 4.36 vs 34.17 ± 5.82%, respectively (p=0.278).

The median of neutrophil differential count significanlty decreased in the 2nd month (85.65 vs. 67.9%; p=0.003), however it not significanlty increased in the 3rd month (67.9 vs. 70.15%; p=0.386). In contrast, the median of lymphocyte differential count significanlty increased in the 2nd month (8% vs. 19.2%; p=0.006), and not significantly decreased in the 3rd month (19.2% vs. 17.95%; p=0.919). The monocyte differential count also significantly increased in the 2nd month (5.65% vs. 7%; p=0.05), followed by a non significantly increased in the 3rd month (7% vs. 8%; p=0.878). Similarly, the eosinophil differential count it significantly increased in the 2nd month (0.35% vs. 2.2%; p=0.003), followed by a significantly decrease in the 3rd month (2.2% vs. 0.85%; p=0.013). Lastly, the basophil differential count significantly increased in the 2nd month (0.2% vs. 0.4%; p=0.001), however not significantly increased in the 3rd month (0.4% vs. 0.45%; p=0.235), as presented in FIGURE 2.

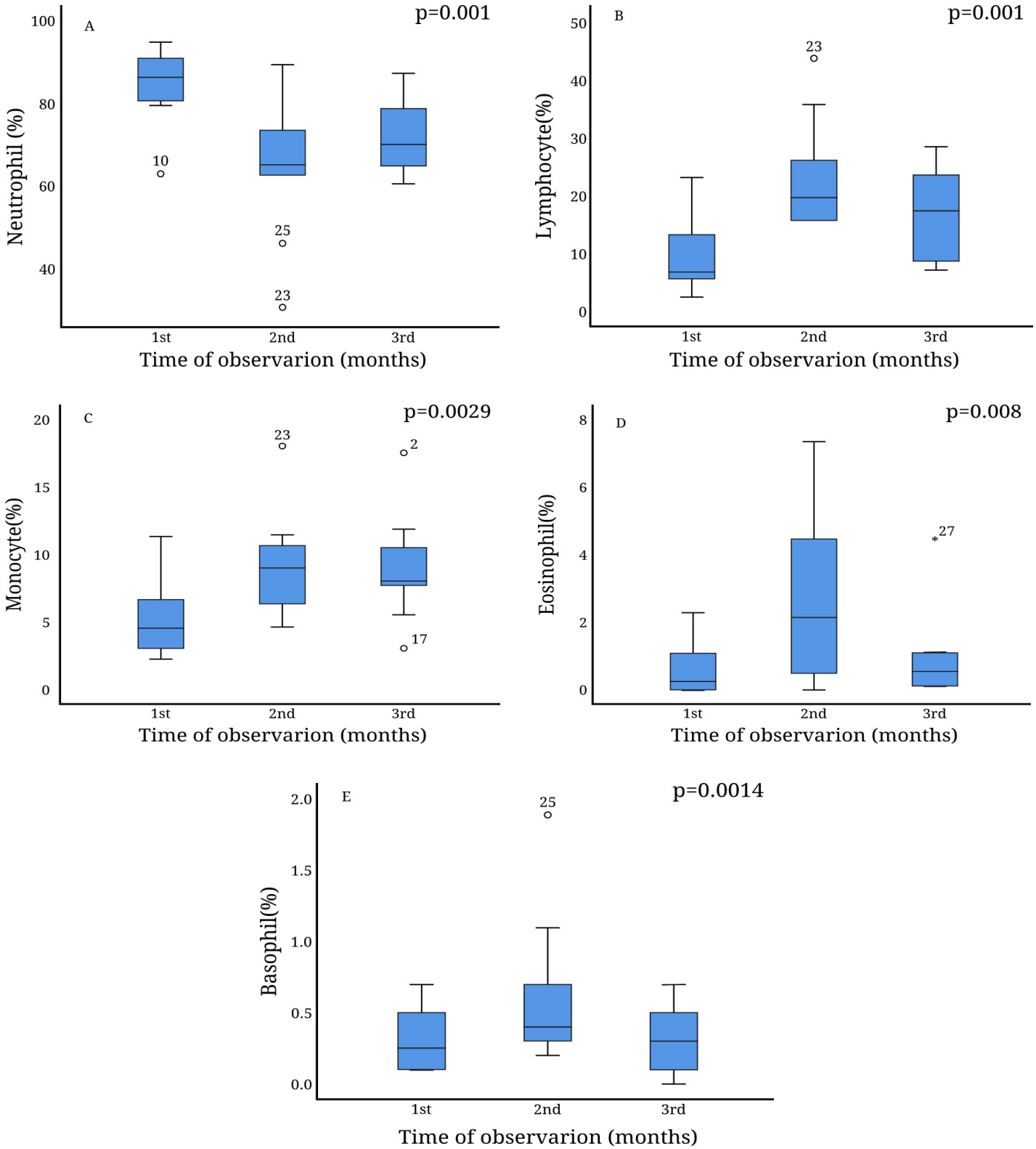

FIGURE 2. Box plot comparison of differential leucocyte count between months. A) neutrophil [n (%)]; B) lymphocyte, [n (%)]; C) monocyte [n (%)]; D) eosinophil [n (%)]; E) basophil [n (%)].

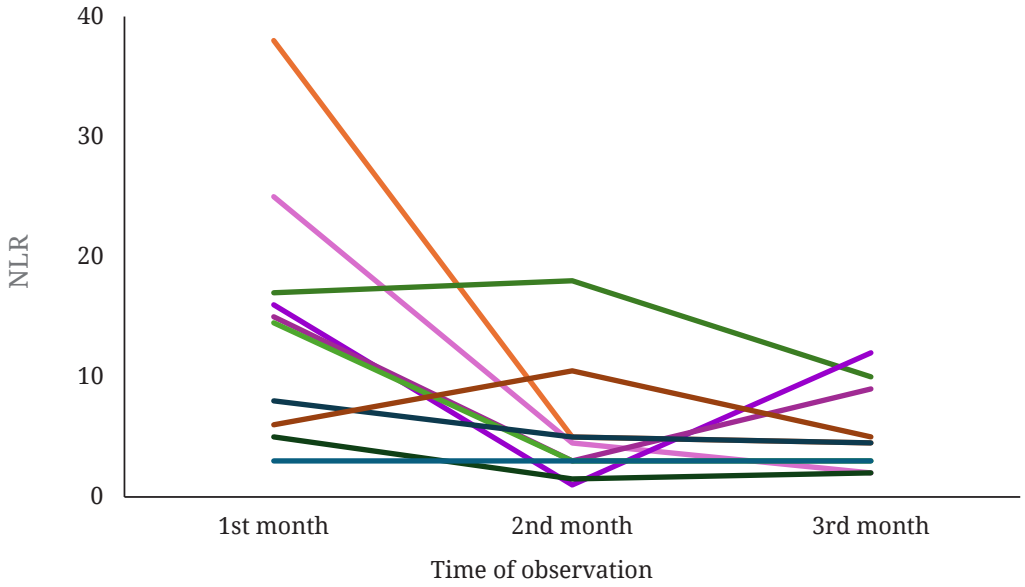

FIGURE 3. NLR kinetic between subjects

Moreover, among all study subjects, only two individuals—those who underwentsurgeryonthegastrointestinal and urinary systems—experienced an increase in NLR during the 2nd month of post-surgery examination. Despite these isolated cases, overall the NLR decresed in the 2nd month examination (FIGURE 3).

DISCUSSION

In this study, 27 (0.21% of cases) out of 12681 postoperative patients since 2016 were diagnosed with DVT. This result is consistent with a previous cross-sectional study that reported a VTE incidence of 2.1% among patients who underwent major orthopaedic or abdominal surgery, with DVT and PE accounting for 1.3% and 0.8% of cases, respectively.[5] On patients with acute medical illness in Indonesia, the rate of DVT was 37 - 40%.[4] Another Asian study revealed that after total knee replacement and total hip replacement, the incidence of asymptomatic DVT was 17 and 24%, respectively, which is comparatively lower than the incidence rates of 36-84% in Western populations. The study also found that the overall postoperative DVT rate without pharmacologic thromboprophylaxis was 7.5% in Asia, where thromboprophylaxis is less commonly used due to the lower probability of VTE development among Asians compared to Caucasians and African Americans. Additionally, racial and ethnic differences in incidence rates have been observed, with Hispanics and Asians having significantly lower rates than Caucasians or African Americans.[9] Another study found that VTE tended to develop after a median of 16 d following non-oncologic general surgery, with a higher incidence of events occurring within the first week compared to those occurring after 30 d. This delayed presentation may increase the likelihood of symptomatic PE, which was observed in half of the patients with VTE, and lead to unfavourable outcomes.[10]

Although age is a major risk factor for VTE and incident VTE is more common in older individuals due to higher blood coagulability potency, as

well as a higher prevalence of provoking risk factors for VTE such as cancer, immobility, hospitalization, and surgery, this study did not find age and sex to be significantly related to VTE incidences. However, gender influences VTE risk, with men having a higher age-adjusted incidence rate of VTE than women (130 per 100,000 and 100 per 100,000 population, respectively). Nevertheless, in young adulthood, women have a slightly higher annual incidence of VTE due to hormonal exposures such as pregnancy, the postpartum period, and oral contraceptive use. The use of exogenous hormones such as oral contraceptive therapy has been positively linked with a 1.5-fold greater risk of incident VTE in women.[11,12] Besides, surgery duration, perioperative immobilization, and the development of postoperative complications may impact the risk of VTE in patients after surgery.[13]

Majority of patients who develop VTE after surgery are women, and 29.4% of them undergo obstetric and gynecologic surgeries. The incidence of VTE following gynecologic surgery depends on whether the patient has benign or malignant disease, with hysterectomy being the most commonly performed major gynecologic surgical procedure. The prevalence of DVT after gynecologic surgery varies, with reported rates of 14% for benign gynecologic and 38% for malignant disease. The surgical approach may also impact the rate of VTE, as more women undergo minimally invasive surgery. Minimally invasive surgery is thought to offer benefits such as early ambulation and decreased VTE risk.[14] Patients with gynecologic cancer have an estimated VTE risk that is approximately 14 times higher than those without cancer. Studies have reported DVT incidence ranging from 17-40% and PE incidence ranging from 1-2.6% in these patients.[15] This is suspected to be related to the decrease in antithrombin III, protein C, and protein S activity· post-open surgery patients are also at a higher risk due to increased dysfibrinogenemia.[16]

Venous thromboembolism in this study occurred on average 20 d after surgery. This result is consistent with previous research indicating that VTE events generally occur within the first 4 wk after surgery. The influence of preoperative factors is a major predisposition to postoperative VTE events. Endothelial damage commonly occurs during surgery, suspected to increase procoagulant activity in patients with high initial risk.[16,17]

The majority of subjects had a Well score of 3, and only 6 subjects had a Well score >3 (22.2%). A higher Well score is associated with a higher risk of VTE events. However, the sensitivity and specificity of the Well score in predicting VTE events are uncertain[19,20] Some researchers have attempted to find other indices to predict VTE events, including hematological results. A study showed that red blood cell count, hemoglobin, and hematocrit are risk factors for VTE events in the general population. Hematocrit is one of the determinants of blood viscosity, and an increase in hematocrit may trigger clot formation by increasing the contact time between platelets and coagulation factors in circulation, which is adjacent to dysfunctional endothelium. Increased hematocrit can also promote platelet transport to the vessel wall, thereby increasing interaction with the vascular wall and raising the risk of VTE.[16,17]

The relationship between the NLR and thromboembolic events is believed to be influenced by various systemic factors such as inflammation, endothelial dysfunction, and oxidative stress. Inflammation causes damage to the endothelial layer through interactions between neutrophils and the endothelium. During ischemic conditions, inflammation becomes a dominant process, and leukocytes play a crucial role in this process.[18]

Inflammation triggers the release of chemical compounds from damaged cells or tissues, including prostaglandins, leukotrienes, histamine, bradykinin, and other pro-inflammatory compounds. This leads to the infiltration of leukocytes, particularly neutrophils and lymphocytes. Neutrophils and macrophages are the first types of leukocytes to appear in the inflammatory response. They release pro-inflammatory cytokines and chemokines, which stimulate T cells, a type of lymphocyte.[19]

During the acute phase of inflammation, the number of lymphocytes decreases due to the release of cortisol from the adrenal cortex. Conversely, in chronic inflammation, impaired cortisol function leads to an increase in lymphocyte count. In the acute phase, stress responses during inflammation activate the HPA axis, leading to the release of corticotropin-releasing hormone (CRH) from the hypothalamus. This stimulates the release of adrenocorticotropic hormone (ACTH), which triggers the release of cortisol. Cortisol inhibits lymphocytes, resulting in a decrease in their count during the acute phase. In the chronic phase, excessive cortisol levels bind to glucocorticoid receptors, causing dysregulation and glucocorticoid receptor resistance, leading to decreased cortisol levels and an increase in lymphocyte count.[20]

Neutrophil to lymphocyte ratio, which can be easily calculated from a complete blood count test, serves as a readily obtainable marker for indicating inflammation in the body. While the exact mechanism of using NLR as an inflammation marker is not fully understood, it is commonly employed for this purpose. Neutrophil to lymphocyte ratio reflects the balance between the innate immune response (neutrophils) and the adaptive immune response (lymphocytes). Severe systemic inflammatory conditions, such as sepsis and septic shock, result in increased NLR compared to mild systemic inflammation conditions.[18]

Otasevic *et al.*[21] reported that NLR, PLR, ESR, CRP, and LDH levels were significantly higher in lymphoma patients with VTE compared to those without VTE. Conversely, TP and albumin levels were significantly lower in lymphoma patients with VTE. Curve of ROC analysis indicated that NLR, PLR, and CRP demonstrated acceptable sensitivity and specificity in predicting VTE in lymphoma patients. The study also revealed that NLR and CRP were independent prognostic factors influencing the development of VTE in lymphoma patients. Previous studies have also suggested that NLR and PLR can be used as predictors of VTE development, although conflicting results have been reported. Additionally, the study found that lymphoma patients who did not respond well to therapy were more susceptible to VTE development, aligning with published data highlighting the association between aggressive lymphoma, advanced disease stage, lower survival rates, and higher mortality rates.[21]

Earlier research has demonstrated a significant increase in the number of neutrophils in the blood of patients with PE. Autopsy findings have also indicated neutrophil infiltration in most right ventricle samples from individuals who died from PE, suggesting a potential link between high NLR and VTE. This corresponds to the theory that neoplastic cells activate the coagulation and fibrinolysis systems to expedite angiogenesis, cell growth, and invasive properties of cancer cells.[22] As NLR rises, the risk of morbidity and mortality in patients experiencing organ damage and failure also increases.

Several previous studies have indicated that NLR exhibits moderate diagnostic accuracy for VTE, demonstrating moderate sensitivity and specificity. Meta-analyses conducted

earlier have shown that high NLR predicts short-term mortality. In another meta-analysis, NLR has been independently demonstrated to predict both overall mortality and short-term mortality. While D-dimer exhibits high sensitivity in diagnosing VTE, its specificity is low due to potential influences from various pathophysiological factors. Consequently, D-dimer is commonly utilized to rule out VTE diagnosis.[23]

Several laboratory tests, including the NLR, D-Dimer, and platelet to lymphocyte ratio (PLR), are used as predictors of thromboembolic events. Neutrophil to lymphocyte ratio is calculated by dividing the number of neutrophils by the number of lymphocytes. An increased neutrophil count indicates systemic inflammation, while a decreased lymphocyte count indicates sustained stress due to illness. Neutrophils have been implicated in blood clot formation through the release of neutrophil extracellular traps (NETs). This suggests the potential use of NLR as a biological marker for DVT.[24] Compared to D-dimer, NLR is easier to assess and widely available in healthcare facilities as it does not require specialized testing.

D-dimer is a commonly used marker for diagnosing DVT, and its measurement improves sensitivity and specificity in DVT and PE diagnosis. Guidelines from the American Society of Haematology recommend using D-dimer as an initial test to reduce the need for diagnostic imaging in low-risk VTE patients. D-dimer concentration increases shortly after surgery but returns to normal levels within a week. However, D-dimer tests are only useful for ruling out acute VTE and are not specific enough to confirm the diagnosis.[25,26]

D-dimer levels increase in patients with conditions such as heart attacks, pneumonia, sepsis, cancer, and post-surgery, as well as during the second or third trimester of pregnancy. As a result, D-dimer is not very useful in hospitalized patients since its levels often rise due to other systemic diseases. Therefore, it is important to incorporate other more specific markers in diagnostic assessments.[25,26]

In a study conducted by Rinaldi *et al*.[26], the diagnostic abilities of the NLR and D-dimer for DVT were compared. The study found that in individuals with a low probability of DVT based on the Wells score, NLR exhibited higher sensitivity than D-dimer (65 vs 60%). However, for individuals with a high probability of DVT, NLR showed higher specificity than D-dimer (69.2 vs 53.8%). Due to its superior sensitivity in the low probability group, NLR could serve as an additional and more effective screening tool. Additionally, NLR is easily accessible and does not require specialized testing, enabling quick evaluation of symptomatic patients suspected of having DVT and reducing the need for ultrasound in patients with a low probability of DVT.[26] These findings contrast with a study by Kadek, which reported that D-dimer had higher sensitivity than NLR, and NLR exhibited better specificity than D-dimer in DVT cases.[27]

Recent studies have also considered the PLR as a predictor of VTE in cancer patients and after surgery. According to Yamagata *et al.*[28] the platelet count is higher in the group with VTE compared to the group without VTE, and the platelet count is significantly associated with cancer patients. Platelet count is related to the risk of symptomatic VTE in cancer patients but not in subjects without cancer. Platelet to lymphocyte ratio is recommended for VTE diagnosis instead of relying solely on platelet count or lymphocyte count because it encompasses the inflammatory values of both blood cell types.[27]

Moreover, both thrombocytosis (elevated platelet count) and lymphocytopenia (low lymphocyte count) are associated with increased

systemic inflammation, and PLR has been identified as an independent risk factor for high inflammatory processes, serving as a novel marker that combines these two haematological indices.[27] Grilz *et al*.[29] observed a significant relationship between PLR and the occurrence of VTE in 1469 cancer patients. Kurtipek *et al*.,[19] reported higher PLR values in 71 patients with acute PE compared to healthy controls, suggesting that PLR may be linked to impaired arterial endothelial function in the lungs. Their study demonstrated that PLR values above 260 are an independent predictor of VTE cases in cancer patients.[19,30]

The combination of NLR and PLR yields sensitive, specific, and accurate results in identifying DVT events. Recent findings also indicate a moderate and significant association between the combination of NLR and PLR and the occurrence of DVT.[31] However, it is still recommended to use D-dimer followed by Doppler ultrasonography in the lower extremities as the primary diagnostic approach. This is because research results demonstrate that D-dimer has higher sensitivity compared to NLR, PLR, or the combination of NLR and PLR.[27]

Farah *et al.*[32] suggested that mean platelet volume (MPV) serves as an important inflammatory marker in lung and pancreatic cancer patients. Platelets play a significant role in clot formation, which is relevant to the development of VTE. Mean platelet volume, along with other markers related to platelet function and count, is commonly used in haematological assessments. Evidence indicates that MPV can reflect platelet turnover, as larger platelets tend to be younger and more reactive compared to smaller platelets. The association between increased MPV and the risk of VTE and cardiovascular risk has been well-established.[32]

McLeod *et al*.[33] reported that NLR levels at the time of DVT diagnosis are associated with a higher risk of thromboembolic and the occurrence of proximal DVT, which refers to thrombosis in veins closer to the heart. Proximal DVT includes thrombosis in the popliteal vein or above, while distal DVT refers to thrombosis in the calf veins.[33] These findings align with previous study, which indicated that NLR levels above the 95th percentile is associated with a 2.4 times higher risk of VTE.[34] Furthermore, the study suggests that NLR values in the highest quartile (>2.6) are linked to the development of post-thrombotic syndrome (PTS), supporting the notion that inflammation plays a significant role in the pathophysiology of PTS.[33]

Although the levels of white blood cells (WBC) were similar between the healthy control group and DVT patients, patients with DVT exhibited statistically higher levels of MPV, D-dimer, platelets, and NLR. Specifically, in the subgroup of patients with extensive DVT involving the iliac and femoral veins, there were significant increases in WBC levels, platelets, NLR, and the fibrinogen-to-albumin ratio (FAR). No significant differences were observed for other variables between the two groups. However, when considering the extent of DVT (proximal DVT and distal DVT), postoperative NLR and WBC levels were significantly higher in the proximal DVT group compared to the distal DVT group, which is consistent with previous research.[35] The underlying mechanism explaining this phenomenon is still unknown.

Kuplay *et al*.[36] showed that patients with distal thrombus had higher values of lymphocytes, monocytes, eosinophils, and basophils compared to proximal thrombus. Meanwhile, proximal thrombus had significantly higher neutrophil values compared to distal thrombus. As for NLR values, there was a significant difference, where proximal NLR was higher than distal NLR ($p<0.0001$). Previous research indicated that patients with proximal thrombus

had a higher mean NLR compared to patients with distal thrombus (4.40 ± 4.28 vs 3.54 ± 3.55; p=0.05). This indicates the presence of increased inflammation in patients with DVT with a more proximal thrombus location.[36]

This study brings forth several innovations, particularly in its thorough depiction and assessment of VTE occurrences in Indonesia. Notably, earlier research on VTE in the country concentrated solely on observing the incidence and characteristics of VTE patients over a two-year period, while this study extends this observation to encompass a five-year timeframe.[4] Furthermore, an analysis of NLR parameters and trends were conducted, serving as markers for VTE patients. In contrast to previous studies, which focused on the general VTE population, this study specifically addresses VTE events occurring post-surgery.[8] This study covers the period from 2016 to 2021, reflecting advancements and further developments in VTE detection in Indonesia. The limitations of this study stem from the reliance on electronic medical records, which were only widely implemented in this center from the beginning of 2016. Consequently, there is a possibility of overlooking some data. Researchers attempted to address this issue by expanding the scope of diagnosis codes searched (from I82 extended to I80), but the risk remains.

CONCLUSION

In conclusion, VTE occurs in 0.21% of post-operative patients in Dr. Sardjito General Hospital, with the highest incidence observed in post-gynecological surgery patients. The NLR can serve as a diagnostic tool for VTE in extremities, as an elevated NLR indicates the presence of a more proximal thrombus.

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Diagnostic performance of sex hormone binding globulin (SHBG) expression in predicting prostate cancer (PCa)

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ABSTRACT

Globally, 1.3 million new cases of prostate cancer (PCa) and 359,000 deaths were reported in 2018. Due to rapid population growth and increasing rates of aging worldwide, the PCa has become the 5th leading cause of death in men. Sex hormone binding globulin (SHBG) presents in both men and women and its expression is often associated with the development of PCa and breast cancers. This study was conducted to assess the diagnostic performance of SHBG expression in predicting PCa. A total of 31 patients with PCa and 14 patients with BPH as a control were involved in this study. The SHBG expression of formalin-fixed paraffin-embedded (FFPE) prostate tissue was examined by the quantitative reverse transcriptase polymerase chain reaction (RT-PCR). Receiver operator characteristic (ROC) curves were produced and the area under the curve (AUC) was calculated to evaluate the diagnostic performance of the SHBG expression in predicting the PCa. The AUC of SHBG expression was 0.868 (95% CI 0.764-0.971; p<0.001) which indicated good diagnostic performance. The cutoff value was 6.731 which corresponded to 80% accuracy, 71% sensitivity, and 100% specificity. In conclusion, SHBG expression in prostate cancer tissues could be a molecular marker in PCa diagnosis.

Keywords:
prostate cancer;
molecular marker;
sex hormone binding globulin;
mRNA expression;
diagnosis

ABSTRACT

Secara global terdapat 1,3 juta kasus baru kanker prostat (*prostate cancer*/PCa) dan 359.000 kematian dilaporkan pada tahun 2018. Karena pertumbuhan populasi yang cepat dan meningkatnya angka usia lanjut di seluruh dunia, PCa telah menjadi penyebab kematian utama ke-5 pada pria. *Sex hormone binding globulin* (SHBG) terdapat pada pria dan wanita dan ekspresinya sering dikaitkan dengan perkembangan PCa dan kanker payudara. Penelitian ini dilakukan untuk menilai potensi SHBG sebagai penanda molekuler dalam diagnosis PCa. Sebanyak 31 pasien PCa dan 14 pasien BPH sebagai kontrol dilibatkan dalam penelitian ini. Ekspresi SHBG dari jaringan prostat *formalin-fixed paraffin-embedded* (FFPE) diperiksa dengan *the quantitative reverse transcriptase polymerase chain reaction* (RT-PCR). *Receiver operator characteristic* (ROC) dihasilkan dan *the area under the curve* (AUC) dihitung untuk mengevaluasi kinerja diskriminatif ekspresi SHBG dalam diagnosis PCa. AUC ekspresi SHBG adalah 0,868 (95% CI 0,764-0,971; p<0,001) yang menunjukkan kinerja diskriminatif yang baik. Nilai *cutoff* adalah 6,731 yang berhubungan dengan akurasi 80%, sensitivitas 71% dan spesifisitas 100%. Kesimpulannya, ekspresi SHBG pada jaringan kanker prostat dapat menjadi penanda molekuler dalam diagnosis PCa.

INTRODUCTION

The incidence of prostate cancer (PCa) is the highest among other cancers in 100 countries in the world. It is the second-most prevalent malignancy in men worldwide and the fourth-most prevalent non-skin malignancy overall.[1] In 2018, up to 1.3 million new cases of PCa were reported causing 359,000 deaths globally. Early detection and treatment of the PCa are linked to lower mortality

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rates in many countries including the United States, North America, Oceania, Northern, and Western Europe, and developing countries in Asia.[2-5]

Different modalities, including surgery, chemotherapy, radiotherapy, immunotherapy and hormone therapy have been applied in cancer treatments. About 95% of PCa are adenocarcinomas that are treated with hormone therapy. Castration therapy, also known as androgen ablation, is a hormone therapy for PCa. It reduces the body's testosterone levels to inhibit the PCa cells growth. However, after 12 months castration therapy, 80% of patients with PCa developed resistance.[6,7] Studies are constantly conducted to investigate the factors leading to castration therapy resistance in patients with PCa. The characteristic genetics of the physiology and pathophysiology of PCa are still being studied.

Sex hormones present in both men and women produce the protein known as sex hormone binding globulin (SHBG). SHBG is a 90-kd glycoprotein that binds sex hormones such as testosterone, estradiol, and especially with a higher affinity for 5α-dihydrotestosterone (DHT). SHBG in humans is most abundantly expressed in hepatocytes and secreted into plasma. It is also expressed in several other tissues such as testis, breast and prostate. SHBG has also been found in human PCa tissue sections, where SHBG is locally produced and regulated. With disruption of fat metabolism linked to a risk of metabolic and cardiovascular illness as well as a function in malignancy, SHBG plays a crucial role in chronic disease.[8,9]

Androgens and estrogens bind to specific SHBG receptors (SHBG-R) on the membrane of selected cells. These binding stimulate cAMP and PKA in the PCa cell and the SHBG-R could connect to the G protein complex which may conversely bind androgens or influence the activity of a membrane androgen-binding indirect protein. The PCa cells will undergo cell cycle activity for cell regulation and proliferation when this G protein and PKA are activated and higher levels of SHBG expression were identified in PCa.[8,9]

The steroid-binding SHBG receptor complex stimulates intracellular cAMP, activates PKA, and G protein leading to PCa cell regulation and proliferation.[10] This study aimed to investigate the potency of SHBG as a molecular biomarker in PCa diagnosis in Indonesian population.

MATERIAL AND METHOD

Patient selection

The study was conducted in the Division of Urology, Department of Surgery, Dr. Sardjito General Hospital, Yogyakarta which has dealt with instances of PCa with or without concomitant disorders. A cluster random sampling was applied to formalin-fixed paraffin-embedded (FFPE) prostate tissue of patients from 2015-2020. The PCa was diagnosed based on the prostate-specific antigen (PSA) patient's serum examination. The PCa was also histopathologically diagnosed using prostate biopsy and/or transurethral to determine ISUP (International Society for Urological Pathology) and Gleason scores. Patients with benign prostatic hyperplasia (BPH) was selected as control. The SHBG expression was examined by the quantitative reverse transcriptase polymerase chain reaction (RT-PCR) on RNA at the Laboratory of Anatomical Pathology, Faculty of Medicine, Public Health, and Nursing, Yogyakarta.

Quantitative RT-PCR analysis

RNA was isolated from formalin-fixed paraffin embedded prostate cancer tissues using the FavorPrep™ Total RNA Plus Mini Kit according to the manufacturer's instructions. qRT-PCR was conducted on One-Step qRT-

PCR with KAPA SYBR FAST Universal according to the manufacturer's instructions. The PCR forward primer used was 5'-GCC CAG GAC AAG AGC CTA TC-3', whereas the primer used was 5'-CCT TAG GGT TGG TAT CCC CAT AA-3'. An individual reaction was performed using the Bioneer Exicycler™ 96 RealTime Quantitative 122 Thermal Block with reverse transcription at 42 °C for 5 min, followed by enzymatic activation at 95 °C for 3 min, denaturation for 1–3 sec at 95 °C, and elongation for up to 20 sec at 60°C. SHBG expression levels were calculated based on the cycle threshold (Ct) value of each reaction obtained by the StepOne qRT-PCR analysis software.

Data analysis

Data was presented as the mean ± standard deviation (SD) or as number (percentage). Shapiro Wilk test was applied to determine the data distribution of the study dataset. The difference in variables between the PCa and BPH patient groups was analyzed using independent t-test for normal data distribution and Mann-Whitney test for non-normal data distribution. Receiver operator characteristic (ROC) curves were produced by plotting sensitivity against (1-specificity) at each level. Area under the curve (AUC) was calculated to evaluate the diagnostic performance of the SHBG expression in predicting PCa. The discriminatory performance was classified as follows: AUC values closest 1 indicate a very good discrimination, AUC values above 0.80 are good discrimination or considered clinically useful, and AUC values below 0.80 are considered fair discrimination or limited clinical utility.[11] The ROC Youden index formula was used to determine the optimal SHBG cutoff value in predicting the PCa. p-value < 0.05 was considered significant.

RESULTS

Patient characteristics

The characteristics of patients are presented in TABLE 1. A total of 31 patients with PCa and 14 patients with BPH as a control were involved in this study. Mean age of the patients with PCa was 68.6 ± 9.0 years old, whereas the patients with BPH were 71.5 ± 7.4 years old. No significant difference in mean age between the both patient groups was observed (p=0.318). Mean PSA value of patients with PCa (855.5 ± 1217.1 ng/mL) was significantly higher than the patients with BPH (10.2 ± 18.0 ng/mL; p<0.001). Most of the patients with PCa had Gleason score of 9 with ISUP value >2. Twenty-one out of 31 PCa patients had metastasis (TABLE 1).

SHBG expression

The SHBG expression in the prostate tissue of patients with PCa (12.5 ± 9.0) was significantly higher than the patients with BPH (4.2 ± 1.4) as presented in FIGURE 1.

Diagnostic performance of the SHBG expression in predicting PCa

Receiver operating characteristic (ROC) curve of SHBG expression in predicting PCa is presented in FIGURE 2. The accuracy of the SHBG in predicting PCa, represented by AUC of SHBG expression was 0.868 (95% CI 0.764-0.971; p<0.001) which indicated that the SHBG has good diagnostic performance in predicting PCa. Youden index formula was then employed to optimize the both sensitivity and specificity of SHBG in predicting PCa using ROC. It provided a cutoff value of 6.731, which corresponded to 80% accuracy, 71% sensitivity and 100% specificity (TABLE 3).

TABLE 1. Patient characteristics

Variable	PCa (n=31)	BPH (n=14)	p
Age (mean ± SD yr)	68.6 ± 9.0	71.5 ± 7.4	0.318[a]
PSA (mean ± SD ng/mL)	855.5 ± 1217.1	10.2 ± 18.0	< 0.001[b]
Gleason Score [n (%)]			-
• 6	4 (12.9)	-	
• 7	3 (9.7)	-	
• 8	2 (6.5)	-	
• 9	13 (41.9)	-	
• 10	9 (29.0)	-	
ISUP [n (%)]			-
• ≤ 2	6 (19.4)	-	
• > 2	25 (80.6)	-	
Metastasis [n (%)]			-
• Yes	21 (67.7)	-	
• No	10 (32.3)	-	

Note: PCa=prostate cancer; BPH= benign prostatic hyperplasia; PSA=prostate-specific antigen; ISUP: International Society for Urological Pathology; SD=standard deviation; [a]Independent t-test; [b]Mann-Whitney test.

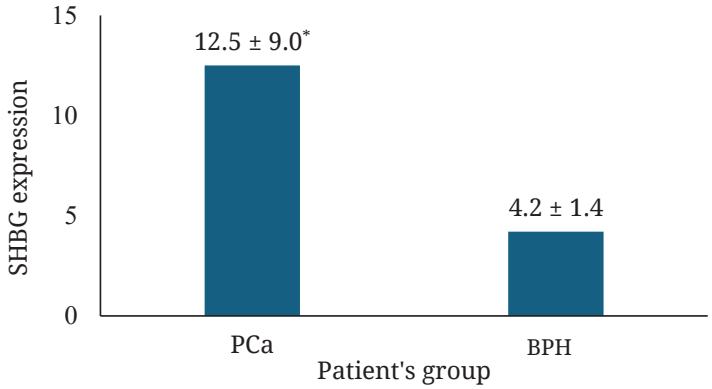

FIGURE 1. SHBG expression of the PCa and BPH groups (*p<0.001)

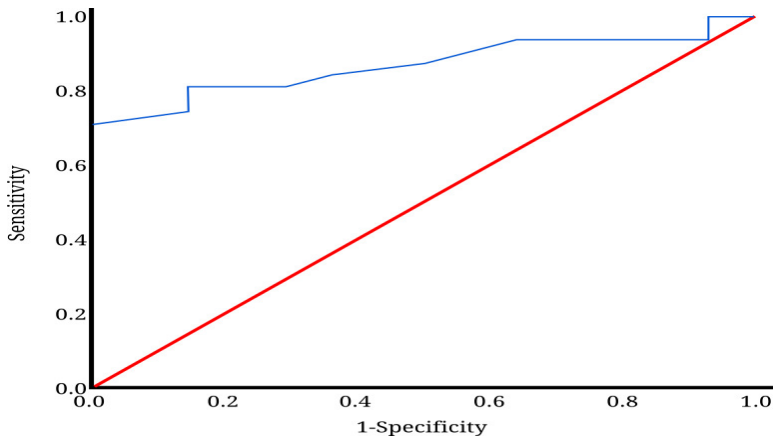

FIGURE 2. Receiver operating characteristic (ROC) curve of SHBG expression in predicting PCa

TABLE 3. Cross tabulation of PCa and BPH using SHBG best cutoff value

	PCa	BPH	p	Accuracy (%)	Sensitivity (%)	Specificity (%)
SHBG (using cutoff)						
≥ 6.731	22	0	<0.01	80	71	100
< 6.731	9	14				

DISCUSSION

This study demonstrated that SHBG has a good diagnostic performance in predicting PCa with the AUC value of 0.868 (95% CI = 0.764-0.971; p<0.001). Furthermore, it provided a cutoff value of 6.731, which corresponded to 80% accuracy, 71% sensitivity, and 100% specificity. Studies concerning SHBG as a diagnostic marker in predicting PCa have been reported from different countries. However, studies on Indonesian population are limited. This study is in line with previous studies reported by some authors. Watts *et al.*[12] reported a significant association between free testosterone with prostate cancer, melanoma, and SHBG with liver and prostate cancer. Furthermore, García-Cruz *et al.*[13] reported that low bioavailable testosterone levels and high SHBG levels are associated with a 4.9 and 3.2-fold increased risk of PCa. Xu *et al.*[14] reported that the later that testosterone levels dropped below 12.1 nmol/L in a man, the less the lifetime risk of PCa in that individual (HR= 0.68; 95%CI= 0.57-0.82). Whereas, Fard *et al.*[15] which stated that the average BMI, PSA, and SHBG have a significant relationship with PCa. Waldert *et al.*[16] reported that preoperative SHBG serum level is independently linked to biochemical recurrence following radical prostatectomy and enhances the predictive accuracy of a standard multivariable model.

However, some studies reported that there is no association between SHBG and PCa risk. Sawada *et al.*[17] reported that SHBG is not strongly associated with a risk for total PCa among Japanese men, although it is associated with an increased risk of PCa in younger men. A study among Italian people with PCa undergoing transrectal prostate biopsies also reported that SHBG is not predictive in diagnosing PCa and its aggressiveness.[18] Gann *et al.*[19] also reported that no evident links between the unadjusted levels of SHBG and the risk of PCa. Nonetheless, a strong correlation between testosterone and SHBG levels (r = 0.55), along with weaker correlations between testosterone and both estradiol (r = 0.28) and DHT (r = 0.32) levels was observed. A meta-analysis of 18 prospective studies conducted by the Endogenous Hormones and Prostate Cancer Collaborative Group reported that SHBG levels have significantly inverse relationship with the risk of PCa (RR = 0.86; 95% CI = 0.75 - 0.98) when comparing the highest quintile to the lowest quintile.[20]

In this study, SHBG level was demonstrated by SHBG mRNA expression in the prostate tissue of the PCa patients analyzed by qRT-PCR. It was reported that SHBG in the human prostate is locally synthesized and acts as an autocrine or paracrine effector.[21,22] This study is different with the previous studies which used serum samples to analyze SHBG levels.[18-20] In addition, the differences in lifestyle, and genetic characteristics between Indonesian (Mongoloid) and Caucasian may cause the SHBG serum levels. It was reported that alcohol consumption history is associated with serum SHBG levels.[23] Furthermore, it was reported that three coding region polymorphisms (rs6257, rs6258, rs6259) and variations in the TAAAA repeat in

the promoter region of the SHBG gene are associated with serum SHBG levels.[22] Another factor that influences the serum SHBG levels is PCa staging.[24] In this study most of PCa patients were already in metastatic state, whereas in the previous studies were non-metastatic state.[18-20]

This study was conducted with limited sample size in Indonesian population. Moreover, the SHBG expression was not confirmed by biopsy examination. Further study with larger sample size with biopsy confirmation is needed to obtain definitive association between SHBG expression and PCa.

CONCLUSION

In conclusion, the SHBG expression in prostate cancer tissues indicates good diagnostic performance in predicting PCa and it could be as molecular marker in PCa diagnosis in Indonesian population.

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Not applicable.

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Cytotoxic T lymphocyte associated antigen-4 (CTLA4) expression with renal cell carcinoma subtype and staging

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ABSTRACT

In Indonesia, approximately 45% of renal cell carcinoma (RCC) patients are at an advanced stage that requires checkpoint inhibition combination immunotherapy. Cytotoxic T lymphocyte associated antigen-4 (CTLA-4) is associated with poor prognosis of RCC and it is the first checkpoint developed in cancer immunotherapy. This study aimed to investigate CTLA-4 expression among RCC subtypes and staging. Formalin fixed paraffin embedded (FFPE) tissue of RCC patients from 2018-2020 were obtained from the Department of Anatomical Pathology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada/Dr. Sardjito General Hospital, Yogyakarta. Expression of CTLA-4 among RCC subtypes and stage was measured using quantitative reverse-transcription polymerase chain reaction (qRT-PCR) and compared. Among the 40 patients involved in this study, the CTLA-4 expression was higher in papillary RCC/pRCC (95.88 ± 31.58) compared to clear cell RCC/ccRCC (90.94 ± 43.05). However, no significantly different in CTL-4 expression based on histologic subtypes and tumor stage (p>0.05). In conclusion, neither the histologic subtype nor the tumor stage of RCC can be predicted by CTLA-4 expression.

ABSTRAK

Sekitar 45% pasien karsinoma sel ginjal (RCC) di Indonesia pada stadium lanjut yang memerlukan kombinasi imunoterapi dengan penghambat *check point*. Antigen *cytotoxic T lymphocyte associated antigen-4* (CTLA-4) berhubungan dengan prognosis buruk dari RCC dan penghambat *check point* pertama yang dikembangkan dalam imunoterapi kanker. Penelitian ini bertujuan untuk mengetahui ekspresi CTLA-4 pada subtipe dan stadium RCC. Jaringan formalin fixed paraffin embedded (FFPE) pasien RCC tahun 2018-2020 diperoleh dari Departemen Patologi Anatomi Fakultas Kedokteran Kesehatan Masyarakat dan Keperawatan Universitas Gadjah Mada/RSUP Dr. Sardjito Yogyakarta. Ekspresi CTLA-4 di antara subtipe dan stadium RCC diukur menggunakan *quantitative reverse transcription-polymerase chain reaction* (qRT-PCR) dan dibandingkan. Di antara 40 pasien yang terlibat dalam penelitian ini, ekspresi CTLA-4 lebih tinggi pada RCC/pRCC papiler (95,88 ± 31,58) dibandingkan dengan RCC/ccRCC *clear cell* (90,94 ± 43,05). Namun, ekspresi CTL-4 berdasarkan subtipe histologis dan stadium tumor tidak berbeda nyata (p>0,05). Kesimpulannya, subtipe histologis maupun stadium tumor RCC tidak dapat diprediksi dengan ekspresi CTLA-4.

Keywords:
renal cell carcinoma;
CLTA-4;
stage;
subtype;
Indonesia

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INTRODUCTION

Renal cell carcinoma (RCC) originates from the renal epithelium and accounts for more than 90% of renal cancers. There are wide variety of histopathological appearances and molecular subtypes with clear cell RCC (ccRCC) as the most common type of renal cancer (80%) and also the most common renal cancer-associated death.[1,2] In Indonesia, the incidence of renal cancer is 2.4-3 cases/100000 people with 45% of patients first discovered already at an advanced stage. Besides ccRCC, there are other RCC subtypes like papillary RCC (10-15%), and chromophobe RCC (5%).[3]

Diagnosis of RCC can be challenging because most RCC cases (85%) typically have unclear clinical presentations.[4] This causes a delay in diagnosis which explains why most RCC cases are diagnosed in advanced stages.[5] A checkpoint inhibitor (CI) combination immunotherapy showed better overall survival (OS) than tyrosine kinase inhibitor (TKI) monotherapy.[6] Cytotoxic T lymphocyte associated antigen-4 (CTLA-4) is a checkpoint that has been first studied and used in the management of melanoma and non-small cell lung cancer.[7] Renal cell carcinoma guidelines in Europe and Indonesia now include the combination of CIs in the management of advanced RCC.[8,2]

Identification the RCC subtype and stage is very important because it is associated with the treatment and prognosis of the tumor.[9] Five-year cancer-specific survival rates for clear cell RCC (ccRCC), papillary RCC (pRCC), and chromophobe RCC (chRCC) were 68.9%, 87.4%, and 86.7%, respectively. Clear cell RCC had shown a worse prognosis than another RCC subtype.[10] Stage T1 RCC has a survival rate of 83%, while the survival rate for stage T2 is 57%. For stage T3, the survival rate is 42%, and for stage T4, it drops further to 28%.[9]

The function of CTLA-4, which is a homologous protein of CD28, is to inhibit the costimulatory signals initiated by CD28-B7 through a competitive binding mechanism. This causes a decrease in the ability of T cells to interact with antigen presenting cell (APC).[10] CTLA-4 expression has a strong correlation with local recurrence, pathological stage, the degree of immune infiltration, lower overall survival, and cancer-specific survival rate.[11,12] While CTLA-4 expression increases with tumor severity, CTLA-4 should be high in ccRCC tumors as one of the RCC subtypes with a worse prognosis and late stage of RCC.[12] In this study, we compared the expression of CTLA-4 in RCC patients to determine the patterns and explored relationships between CTLA-4 and RCC subtype and stage.

There are several methods to detect gene or protein expression like polymerase chain reaction (PCR), Western blot or immunohistochemistry (IHC). PCR has become a crucial method in biochemistry and molecular biology, allowing for the quick detection of genetic material present in small quantities and the identification of individual copies of genomes. On the other hand, IHC uses monoclonal and polyclonal antibodies to identify specific tumor antigens expressed in tissue sections, serving as a valuable diagnostic tool for various medical conditions. However, not all proteins are uniformly well-preserved and detectable using this technique.[13] Furthermore, IHC demonstrates lower sensitivity and specificity compared to PCR-based molecular diagnostic methods. There is an alternative method called fluorescence in situ hybridization (FISH) which is cytogenetic technique that uses fluorescent probes that bind to only particular parts of a nucleic acid sequence with a high degree of sequence complementarity.[14]

CTLA-4 was detected using various methods at the mRNA level or the protein level. Examinations at the

mRNA level include Real-time PCR and quantitative reverse-transcription PCR (qRT-PCR), whereas at the protein level include Western blotting, immunohistochemistry, flow cytometry, ELISA, and fluorescence microscopy.[15] CTLA-4 protein has small structure which is approximately 24 kDa. This causes challenges associated with detecting the CTLA-4 protein. There is also possibility that potential protein damage can occur while preserving the tissue.[16]

It is believed that qRT-PCR is the best option for detecting CTLA-4 expression in RCC. This study aimed investigate the CTLA-4 expression in RCC patients by using qRT-PCR. Furthermore, the correlation between this expression with RCC was analyzed to assess the possibility of CTLA-4 in predicting subtype and stage.

MATERIAL AND METHODS

Subjects and data collection

Patients of RCC with formalin fixed paraffin embedded (FFPE) tissue from 2018-2020 in Pathology Anatomy Installation at Dr. Sardjito General Hospital, Yogyakarta were included in this study. Exclusion criteria for this study were the tissue findings outside ccRCC, pRCC and chRCC diagnosis. A total of 40 samples meeting the inclusion and exclusion criteria were selected, and patient data were subsequently gathered from the medical records. This study has been approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada/Dr. Sardjito General Hospital numbered KE/FK/0017/EC/2021.

While qRT-PCR amplification results can be compared with IHC, this study focused solely on the qRT-PCR procedure. The qRT-PCR procedure was conducted in the Department of Anatomical Pathology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta.

qRT-PCR analysis

The harvested tissue was extracted and then isolated in Mini Kit FavorPrep™. Total RNA followed by cDNA synthesis using AccuPower®Greenstar™ RT-qPCR Master Mix. Reverse transcription PCR was conducted using DT-lite real time PCR system with the following conditions: 95 °C for 1-3 sec (denaturation), 95 °C for 3 min (annealing), and 60 °C for ≥ 20 sec (extension). Qualitative PCR was done for CTLA-4 and GAPDH (as the internal control). The primer genes were summarized in TABLE 1. Details of qRT-PCR condition were denaturation, followed by up to 40 cycles: 95 °C for 1-3 sec, annealing, and 95 °C for 3 min, followed by elongation at 60 °C for more than 20 sec. Products of PCR were visualized in 2% agarose gel along with a 100-bp DNA ladder (Bioron, Germany, Cat. No. 306009) for RNA amplification process. Relative quantification by Livak method to determine CTLA-4 expression was used. Each of PCR signal from CTLA-4 and GADPH was measured. Delta cycle threshold (ΔCT value) was determined by subtracting the CTLA-4 signal and the GADPH. Tonsil as calibrator for this study was used. Delta ΔCT value (ΔΔCT value) was calculated by subtracting ΔCT value CTLA-4 with ΔCT value from tonsil. CTLA-4 expression value was obtained by using 2ΔΔCT value.

TABLE 1. Primer sequence of CTLA-4 and GADPH

Gene	Forward primer	Reverse primer
CTLA-4	GCTCTACCTCTTGAAGACCT	AGTCTCACTCACCTTTGCAG
GAPDH	ACCACAGTCCATGCCATCAC	TCCACCACCCTGTTGCTGTA

Statistical analysis

The statistical significance in CTLA-4 difference for tumor subtype was determined using independent t-test for normally distributed data, and Mann-whitney if only the data was not normally distributed. To determine CTLA-4 expression difference in tumor stadium, we used one-way Anova for the normally distributed data or Kruskal-Wallis test for normally undistributed data. The difference was significant if the p value < 0.05. Expression of CTLA-4 then was divided by median as cut-off with upper than median considered as high expression and lower than median considered as low expression. Level of CTLA-4 then was compared among RCC subtypes.

RESULTS

The RCC samples from 40 patients consisting of 31 ccRCC and 9 pRCC patients were included in this study. The patients consisted of 27 men and 13 women with mean age was 56.3 ± 11.7 y.o. The mean ages of the patients with ccRCC subtypes were 56.68 ± 12.3 y.o. and those pRCC subtypes were 55.33 ± 10.1 y.o. No significant difference in patients' age between tumor subtype was observed ($p>0.05$). The surgical approaches in this study included 18 open surgeries and 22 laparoscopic approaches. In total of 40 RCC, tumor ISUP grading for grade I, II, III and IV were 2, 13, 14 and 7, respectively were observed. Only 4 RCC cases with undefined grading were observed. No significant difference between ISUP grading and tumor subtypes was observed. In this study, 11 cases with vascular invasion and 29 cases without vascular invasion were found. Significant difference in vascular invasion between ccRCC and pRCC was observed ($p<0.05$). Thirty-nine patients with tissue invasion and only a patient without tissue invasion were found. In addition, 19 patients with tumor infiltrating lymphocyte (TIL) and 21 patients without TIL were found (TABLE 2).

TABLE 2. Characteristics of patients

Variable	Mean ± SD/ [n(%)]	p
Age (mean ± SD yr)	56.30 ± 11.7	
• Clear cell type	56.68 ± 12.3	0.767
• Papillary type	55.33 ± 10.1	
Gender [n (%)]		
• Male	27 (67.5)	0.624
• Female	13 (32.5)	
Histologic type [n (%)]		
• Clear cell type	31 (77.5)	N/A
• Papillary type	9 (22.5)	
TNM staging [n (%)]		0.307
• cRCC		
✓ I	4 (10.0)	
✓ II	11 (27.5)	
✓ III	3 (7.5)	
✓ IV	13 (32.5)	
• pRCC		
✓ I	3 (7.5)	
✓ II	2 (5.0)	
✓ III	2 (5.0)	
✓ IV	2 (5.0)	
Operation technique		
• Open Surgery	18 (45.0)	0.341
• Laparoscopic	22 (55.0)	
Vascular invasion		
• No	29 (72.5)	0.037
• Yes	11 (27.5)	
Tissue invasion		
• No	1(2.5)	0.225
• Yes	39(97.5)	
TIL		
• No	19 (47.5)	0.457
• Yes	21 (52.5)	
ISUP grading		
• I	2 (5.0)	
• II	13 (32.5)	0.821
• III	14 (35.0)	
• IV	7 (17.5)	
CTLA-4 (with median cut-off)		0.705
• ccRCC		
✓ >87.48	15 (37.5)	
✓ ≤87.48	16 (40.0)	
• pRCC		
✓ >87.48	5 (12.5)	
✓ ≤87.48	4 (10.0)	

The data showed the mean expression of CTLA-4 in all of our patients were 92.05 ±_40.43. Data of CTLA-4 expression were normally distributed proven by normality test (p=0.11). Subtype of pRCC (95.88 ± 31.58) had higher CTLA-4 expression compared to ccRCC (90.94 ± 43.05). However, it was not significantly different in CTLA-4 gene expression among RCC histologic subtype (TABLE 3).

Based on tumor stage, the mean of CTLA-4 expressions in ccRCC for stadium I, II, III, and IV were 85.99 ± 31.09, 88.31 ± 47.89, 80.59 ± 54.43, and 100.71 ± 41.14, respectively and in pRCC were 87.01 ± 12.13, 126.67 ± 59.71, 76.36 ± 29.20, and 98.89 ± 31.58 (TABLE 3 and FIGURE 1). No significantly different in CTLA-4 expression between each tumor stage was observed (p>0.05). Further classified of the tumor was performed to be early stage (stages I and II) and advanced stage (stages III and IV) and dichotomous data from median CTLA-4 expression was analyzed (TABLE 4). However, no significantly different in CTLA-4 expression between early stage and advanced stage was also observed (p>0.05).

TABLE 3. Means comparation between CTLA-4 expression on tumor subtype and stage

Variable	CTLA-4	> median	< median
ccRCC	90.94 ± 43.05	15	16
• I	85.99 ± 31.09	2	2
• II	88.31 ± 47.89	3	8
• III	80.59 ± 54.43	2	1
• IV	100.71 ± 41.14	8	5
pRCC	95.88 ± 31.58	5	4
• I	87.01 ± 12.13	2	1
• II	126.67 ± 59.71	1	1
• III	76.36 ± 29.20	1	1
• IV	98.89 ± 31.58	1	1

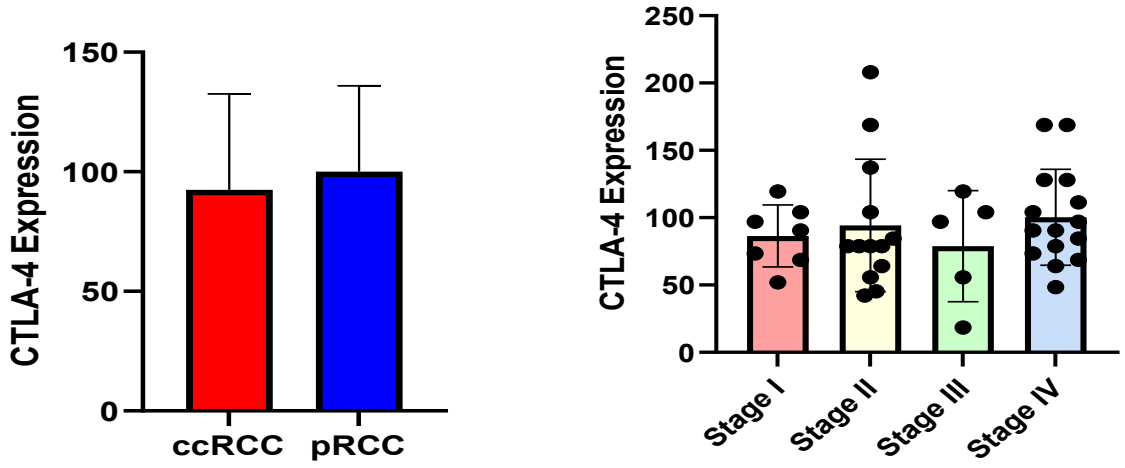

FIGURE 1. Expression of CTLA-4 in tumor subtype and stage1, stage2, stage3, stage4

TABLE 4. Comparing dichotomous data from median CTLA-4 expression and early and advanced or tumor stage

Variable	> median	< median
Advance stage	12	8
Early stage	8	12

DISCUSSION

In this study, CTLA-4 expression in pRCC was higher than ccRCC. However, it was not significantly different (p>0.05). It was in contrast to previous studies that CTLA-4 expression increases with tumor severity and it has a strong correlation with local recurrence, pathological stage, the degree of immune infiltration, lower overall survival (OS) and cancer-specific survival (CSS) rate.[11,12] It was also reported that CTLA-4 is more upregulated in ccRCC tissue and its expression was related to poorer prognosis.[17,18] Another study reported that polymorphisms of CTLA-4 gene are associated in higher risk for high-stage ccRCC.[19]

Although the availability is limited in Indonesia, CTLA-4 inhibitor (ipilimumab) in combination with nivolumab can be used for ccRCC as recommended by Indonesian Urological Association (IAUI) guideline for advance RCC.[2] Grimm *et al.*[20] reported that combination ipilimumab and nivolumab only increased 10% of objective response rate (ORR). Quite contrary from IAUI guideline and previous study, this study showed that CTLA-4 expression in pRCC might be as high as in ccRCC, this indirectly indicated that anti-CTLA-4 might have potential in management of pRCC. Park *et al.*[21] reported that renal metastatic adenocarcinoma cell line with systemic injection of JX-594 with ipilimumab and nivolumab reduced primary tumor burden and had stronger therapeutic effects both in early and advance model compared to JX-594 monotherapy alone. This also showed that recent discovery of anti-CTLA-4 efficacy in non-ccRCC.[21]

Cytotoxic T lymphocyte associated antigen-4 (CTLA-4) was known to attenuate T cell activation through cell intrinsic and extrinsic mechanisms.[22,23] Activation of CTLA-4 can inhibit IL-2 production, T cell proliferation, and arresting cell cycle. Regulatory T cell can inhibit activation of cytotoxic T cell through CTLA-4 which might cause T cell dysfunction.[17,24] Dysfunction of T cell characterized by reduced proliferative capacity, decreased effector function and overexpression of multiple inhibitor receptor especially CTLA-4.[24] Yang *et al.*[25] reported anti CTLA-4 could induce RCC regression by prevention of tumor immunosuppression mechanism. This explained CTLA-4 expression should be high in late-stage RCC.[25] In this study, there was no significant difference between CTLA-4 expression in tumor subtype and stage.

The association of PD-1/PD-L1 and CTLA-4 with RCC patient overall survival and cancer-specific survival was discovered and first reported by Kahlmeyer *et al.*[12] It was reported that the CTLA-4 expression is more marked in poorer prognosis. Furthermore, ccRCC had poorer prognosis among pRCC and ccRCC which indirectly implied CTLA-4 expression more likely to be high.[18,26,27] It is inconsistent with the results of this study, which found pRCC, a subtype with better prognosis, had higher CTLA-4 expression compared to ccRCC. This result suggest that CTLA-4 expression might be high in pRCC because most of

the pRCC cases included in this study were above stage II and had high ISUP grading. Antibody of CTLA-4 could also increase IFNγ-producing CD4+ cells in metastatic bladder cancer but not early stage of tumor.[28] This showed CTLA-4 role in the pathogenesis of advance staged tumor.

By using qRT-PCR, Klümper *et al.*[29] determined methylation of CTLA-4 and reported hypomethylation of CTLA-4 as a strong biomarker for poor prognosis in ccRCC. A hypomethylated DNA was associated with specific protein upregulation.[30] By using immunohistochemistry staining, Liu *et al.*[17] also reported CTLA-4 upregulation was associated with worse prognosis in ccRCC, whereas by using PCR, Tupikowski *et al.*[19] reported polymorphism of CTLA-4 genes was associated with its aggressive course in ccRCC. Conversely with the previous studies, this study showed CTLA-4 expression in less aggressive subtype (pRCC) might be as high as RCC.

Ipilimumab, a CTLA-4 checkpoint inhibitor, can be combined with nivolumab for treatment-naïve patients with advance ccRCC.[31] This indirectly indicated that CTLA-4 expression should be higher in late stage of tumor. Although not statistically significant, this was consistent with this study which stage IV ccRCC had highest CTLA-4 expression. There was toxicities regarding of nivolumab/ipilimumab usage but quality of life and overall survival (OS) for intermediate and poor-risk patients showed better result than sunitinib.[31]

Polymorphism of CTLA-4 +49 A/G decreased risk of cancer incidence in Asian population but not in Caucasian.[32] Different in genetic characteristics may contribute to divergent result because the distribution of the CTLA-4 allele frequency varies among Asians and Caucasians. The findings of this study might be applicable on Asian population because only Indonesian population were included. Only 40 samples had been used in this study which indirectly implied internal and external validity of this study may not be very good. It was the first study exploring CTLA-4 expression with tumor subtype and staging using Indonesian population. Less risk bias with large sample and heterogeneous ethnicity may be required to have definitive conclusion regarding CTLA-4 expression association with RCC tumor subtype.

CONCLUSION

In conclusion, the CTLA-4 expression in pRCC and ccRCC are not different. Late stadium of RCC does not always have high CTLA-4 expression. Neither the histologic subtype nor the stage of RCC can be predicted by CTLA-4 expression.

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A challenge in diagnosis and treatment in asthma patients with allergic bronchopulmonary aspergillosis: a review

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ABSTRACT

Allergic bronchopulmonary aspergillosis (ABPA) is a common form of fungal-related asthma disease mainly caused by *Aspergillus fumigatus*. The increasing prevalence of asthma globally and the characteristic of *Aspergillus* are very easily dispersed in the air to inhale, leading to increased cases of ABPA in asthma patients. Inhalation of conidia *Aspergillus* spp. can trigger asthma exacerbations due to poor mucociliary clearance. However, the exact pathogenesis is still unclear. Clinical features commonly found in ABPA patients are productive cough with dark green or brown mucus or even hemoptysis. Several criteria for establishing the diagnosis of ABPA can be based on clinical features, laboratory examinations, and imaging, but none has become the gold standard. However, the primary laboratory test utilized for ABPA screening is the measurement of serum-specific IgE levels to *A. fumigatus*, owing to its high sensitivity. Despite the challenges in finding the most fitting universal consensus, most clinicians still follow the criteria proposed by Rosenberg *et al.* in 1977. The recommended ABPA treatment is prednisone and/or azole antifungal agents such as itraconazole. In addition, the potential of monoclonal antibodies in ABPA therapy is still under further research. Long-term diagnosis and treatment delays can lead to complications such as bronchiectasis and fibrosis. This review aimed to highlight ABPA in asthma patients, from etiopathogenesis to managing the disease.

Keywords:
allergic bronchopulmonary aspergillosis;
Aspergillus fumigatus;
asthma;
diagnosis;
treatment

ABSTRACT

Aspergilosis bronkopulmoner alergika (ABPA) adalah salah satu bentuk umum dari penyakit asma yang berkaitan dengan infeksi jamur, utamanya *Aspergillus fumigatus*. Prevalensi asma yang meningkat di seluruh dunia dan karakteristik *Aspergillus* yang sangat mudah tersebar di udara dan mudah terhirup menyebabkan peningkatan kasus ABPA pada pasien asma. Inhalasi konidia *Aspergillus* spp. dapat menyebabkan eksaserbasi asma akibat klirens mukosilier yang buruk, namun patogenesisnya pastinya masih belum jelas. Gambaran klinis yang umumnya ditemukan pada pasien ABPA dengan asma adalah batuk produktif dengan sekret hijau tua atau coklat hingga hemoptisis. Kriteria penegakan diagnosis ABPA dapat didasarkan pada gambaran klinis, pemeriksaan penunjang, dan gambaran radiologi, namun belum ada yang menjadi standar baku emas. Meskipun demikian, pemeriksaan laboratorium yang sering digunakan untuk skrining ABPA adalah kadar IgE spesifik *A. fumigatus* pada serum karena sensitivitasnya yang tinggi. Terlepas dari kesulitan dalam menentukan konsensus universal, kebanyakan klinisi masih mengikuti kriteria yang disusun oleh Rosenberg dkk. pada tahun 1977. Terapi ABPA yang direkomendasikan adalah prednison dan/atau agen antifungal azol seperti itrakonazol. Di samping itu, terapi ABPA dengan antibodi monoklonal memliki potensi namun masih perlu diteliti lebih lanjut. Diagnosis yang terlambat dan terapi yang tertunda dapat menyebabkan komplikasi seperti bronkiektasis dan fibrosis. Kajian pustaka ini membahas ABPA pada pasien asma, mulai dari etiopatogenesis hingga penatalaksanaannya.

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INTRODUCTION

Asthma is the most common disorder due to chronic airway inflammation. It was estimated that more than 300 million people have asthma globally, and 5 to 10% suffer from severe asthma.[19] Risk factors of asthma such as heredity and various environmental conditions trigger asthma; however, definite identification is still challenging.[1012] Asthmatic symptoms occur due to airway hyperresponsiveness that causes airway obstruction and phenotypically can be divided into two; allergic and intrinsic asthma. The most common asthma triggers are allergens such as house dust mites, pollen, and mold spores.[13,14,15] In terms of its etiopathogenesis, allergic asthma is characterized by a hypersensitive response involving the activation and increase of Th2 cells and allergen-specific IgE.[1,2,16,17]

Fungal-related allergic asthma has a broad spectrum depending on the host condition and the fungus that causes it. One of the spectra often encountered is allergic bronchopulmonary aspergillosis (ABPA), mainly caused by *Aspergillus fumigatus*.[17,20] From 11-70% of asthmatic patients who are sensitized by fungi, 16-38% are confirmed to be sensitized by *A. fumigatus*.[15,18,19,21,26] ABPA is characterized by a progressive allergic lung disease resulting from a hypersensitive reaction to antigens from Aspergillus spp. In contrast, allergic bronchopulmonary mycosis (ABPM) is used when the antigen comes from other fungi.[2,3,18,19,22] The first case of ABPA was reported in 1952; then, there has been an increasing number of cases everywhere over the years.[2,27] Globally, it is reported that more than 4 million people are affected by ABPA, predominantly adults.[22,28,29] Moreover, diagnosing and beginning therapy for asthma patients with ABPA is challenging because there is no universal consensus on the diagnostic criteria, and they are still being modified due to a lack of a standard. This review focused on ABPA in asthma patients, from etiopathogenesis to managing the disease.

METHODS

A comprehensive data-based literature search was conducted from PubMed, Web of Science, Scopus, and Google Scholar on topic-related articles using the keywords “asthma”, “allergic bronchopulmonary aspergillosis”, “etiology”, “pathogenesis”, “diagnosis”, and “treatment”. No time limits were applied. Articles of all types such as research, guidelines, systematic reviews and meta-analyses, and other narrative reviews are included. All authors are responsible for conducting a literature review and compiling the results into this review.

DISCUSSION

Etiopathogenesis

Characteristics of fungi

Aspergillus is a saprophytic fungus that is thermophilic and thermotolerant to grow at a temperature of 37 to 75°C. The conidia of *Aspergillus* wind-dispersed easily in the atmosphere so that they can be found in the environment outdoors; soil, plant, decaying vegetation, and indoor air.[30] *Aspergillus fumigatus* is the most common cause of ABPA, but other *Aspergillus* fungi such as *A. niger, A. flavus, A. nidulans,* and *A. terrus* can also be the cause.[15,20,23,30,33] Although not yet standardized, isolated fungal on cultures media such as sabouraud dextrose agar (SDA), potato dextrose agar (PDA), or malt extract agar (MEA) media can be carried out to identify species based on a variety of colors colony *Aspergillus* spp.[28,30,34,39]

This saprophytic fungus has a bilayer cell wall dominated by polysaccharides synthesized by transmembrane synthase, transglycosidase, and glycosyl hydrolase. The cell wall is also composed of proteins generally associated with polysaccharides, forming glycoproteins. The central core comprises an α-1.3-glucan polymer, β-1.3-glucan, galactomannan, galactosaminogalactan, and chitin. The outer cell wall consists of rodlet layers followed by melanin. Macroscopically, A. fumigatus exhibits a velvety, woolly, or powdery surface texture with colors ranging from blue-green to gray, often with a narrow white border. (FIGURE 1A).[40] Microscopic morphological structure of *Aspergillus* is constructed from hyaline septate hyphae and conidiophore consisting of conidia chains, phialides, metulae, and vesicles derived from foot cells (FIGURE 1B). However, in A. *fumigatus* there is no metulae.[30,33,41,42]

Factors contributing to the failure of Aspergillus clearance in asthmatic patients

Aspergillus is an opportunistic pathogen and rarely causes disease if inhaled by an immunocompetent individual. Therefore, it is known that ABPA commonly occurs in patients with previous pulmonary disease as a predisposing factor, including a history of asthma or cystic fibrosis. In asthmatic patients, inhalation of conidia *Aspergillus* spp. can not be eliminated as well as in healthy people.[2,17]The defect in conidia elimination is caused by goblet cell hyperplasia, damage to the ciliary structure, and function that interferes with mucociliary clearance. These hyperplastic goblet cells secrete excessive mucus but fail to eliminate it due to damaged cilia.[17,43] Besides host immune status and structural defect, genetic factors also increase the risk of developing conidia in the host, leading to asthma aspergillosis (TABLE 1).[2,1719,23,44-49]

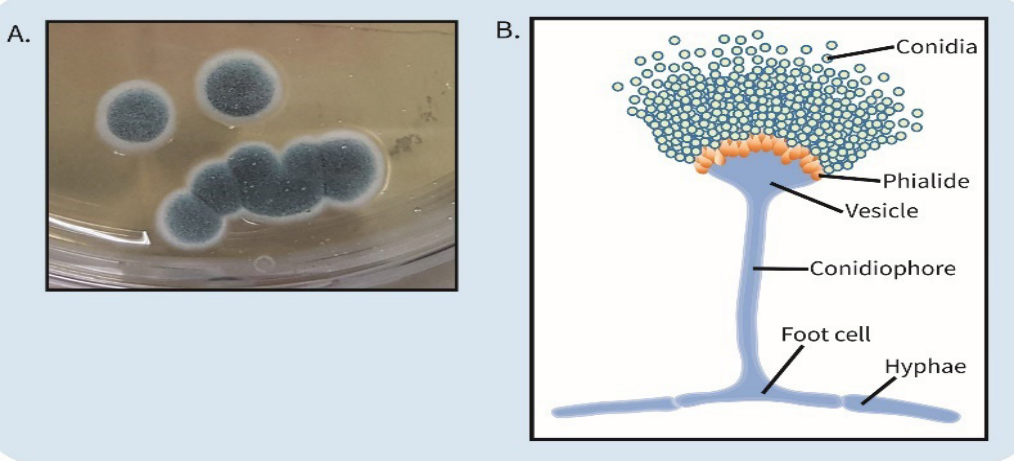

FIGURE 1. Morphology of *A. fumigatus*. A. Colony in SDA media.[40] B. Microscopical illustration.

TABLE 1. Host genetic factors related to the pathogenesis of aspergillosis asthma.[2,17,49,18,19,23,44–48]

PRRs	Cytokines
• TLR-9 gene polymorphism • TLR-3 gene polymorphism	• IL-10-1082GA promoter polymorphism • IL-4 alpha receptor polymorphism • IL-13 polymorphism • IL-15 polymorphism • TNF-α polymorphism • TGF- β polymorphism
Others	
• EEA-1 gene mutations • CHIT-1 gene mutations • Mannose-binding lectin gene mutations • Surfactant protein A2 gene polymorphism • CARD9 gene polymorphism • ZNF77 gene polymorphism	• HLA-DR2 • HLA-DR5 • HLA-DQ

CHIT: chitotriosidase; EEA: early endosome antigen; TLR: toll-like receptor; CARD: caspase recruitment domain-containing protein; IL: interleukin; TNF: tumor necrosis factor; TGF: transforming growth factor; HLA: human leukocyte antigen; PRRs: pattern recognition receptors.

Immune evasion mechanisms of Aspergillus

Asthmatic patients inhale the conidia of *Aspergillus* spp., leading to exacerbations. The conidia of *Aspergillus* are very small, around 3-5 μm, which allows them to reach the lower respiratory tract very easily. The cell wall of *A. fumigatus*, the most common cause of ABPA, plays an essential role in the activation host immune system.[17,50,52] However, the exact pathogenesis of asthma aspergillosis is still in debate.

The cell wall of the conidia and hyphae of *A. fumigatus* has different components. The conidia have a rodlet layer and melanin at the outer. Meanwhile, the hyphal cell wall has an extracellular polysaccharide galactosaminogalactan, a component essential for fungal adherence and virulence. These differences in components affect the activation mechanism of the host immune system.[19,44,50,52]

Conidia have a rodlet and melanin layer on the outside initially, which causes pathogen-associated molecular patterns (PAMPs) not to be recognized by pattern recognition receptors (PRRs) of host cells; thus, the activation of the immune system is inhibited. The rodlet layers outer have been shown to inhibit the activation of dendritic cells, alveolar macrophages, and T cells. Therefore, the rodlet layer is crucial for preventing the activation of the immune system. Later the rodlet layer will be degraded at the beginning of germination so that the PRRs of host cells will recognize the conidia: dectin-1, toll-like receptor (TLR)-2, TLR-4, C-type lectin receptors (CTLs), complement receptor 3 (CR3). On the contrary, hyphae of *A. fumigatus* do not have an outer protective layer, so PRRs can directly recognize PAMPs to activate the host immune system.[19,42,51,53,54]

The inhaled conidia can be completely cleared by professional phagocytic cells (macrophages) and non-professional phagocytes (bronchial epithelial cells/ alveoli) in individuals without prior pulmonary impairment. However, professional phagocytic cell function is impaired in individuals with chronic lung diseases such as asthma; thus, conidia can colonize bronchial/ alveolar epithelial cells. Clearance of conidia begins with adhesion to phagocytic cells, which then enter to form vesicle sacs (phagosomes) and combine with lysosomal enzymes to form phagolysosomes; then, some will be lysed and eliminated. Meanwhile, the non-eliminated conidia will swell, germinate, and grow hyphae.[19,31,54,56]

The PRRs function to recognize PAMPs in dendritic cells will induce the release of C-C motif chemokine ligand 17 (CCL17), which will regulate the differentiation of T cells into Th2 cells and activate regulatory T cells, which will suppress Th1 cell and macrophage response.[19,31,54,57] In addition, the protease enzyme (Alp-1/ Asp f13 (alkaline serine protease), which is secreted by hyphae at the beginning of its growth, can also induce epithelial cells to release pro-inflammatory cytokines: interleukin (IL)-33, IL-25, dan thymic stromal lymphopoietin (TSLP).[19,42,44,58] The secretion of these cytokines cause inflammation and ultimately cause damage to epithelial cells. On the contrary, these cytokines can also activate type 2 lymphoid cells (ILC2) and regulate the differentiation of T cells into Th2 cells, reducing the inflammatory response. Type helper 2 cells will then produce IL-4, IL-5, and IL-13. Activation of ILC2 will cause the release of type 2 cytokines, such as IL-5, IL-13, IL-9, and amphiregulin, in large quantities. Later, cytokines produced by ILC2 and Th2 cells will regulate the differentiation of B cells into plasma cells, produce IgE, and stimulate eosinophils. Moreover, the IgE will sensitize the mast cells and basophils, triggering hypersensitivity reaction type I. Furthermore, plasma cells will produce IgG-mediated immune complexes associated with types III hypersensitivity reactions (FIGURE 2).[19,57]

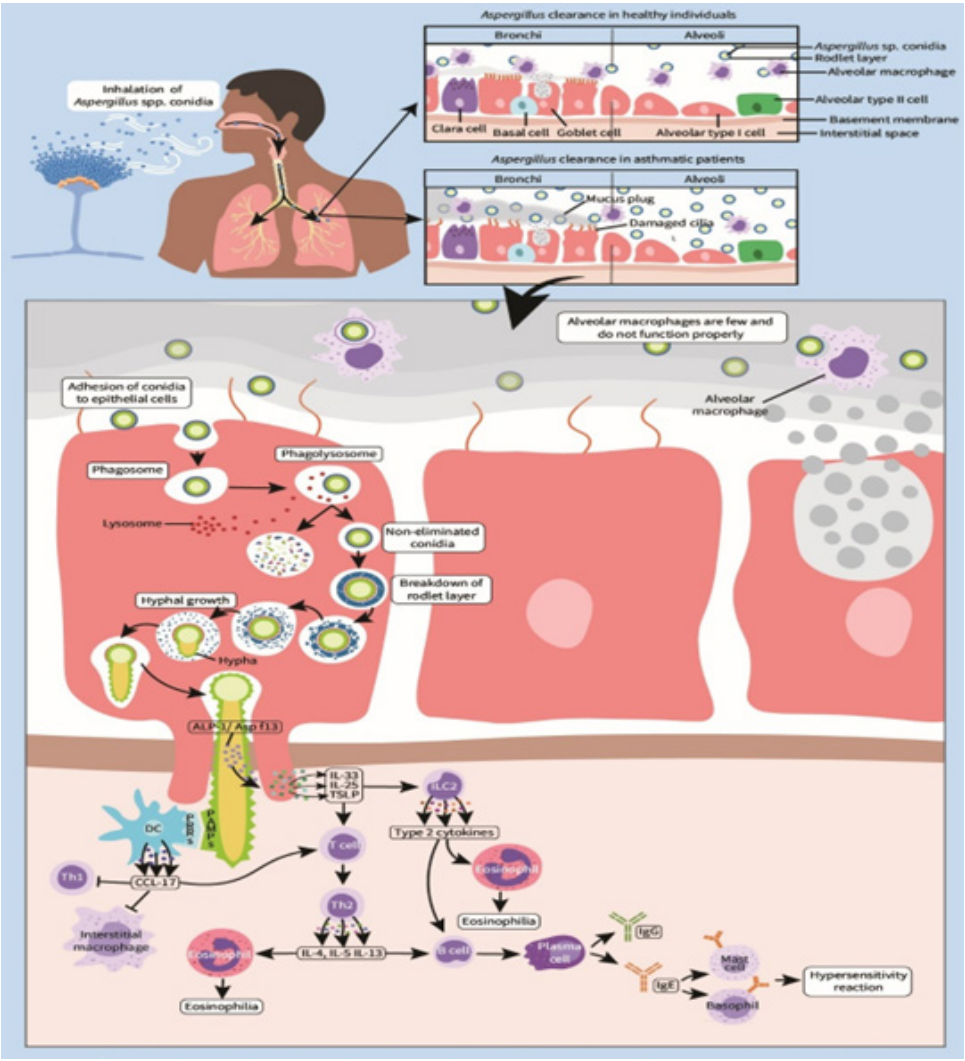

FIGURE 2. Asthma aspergillosis immunopathogenesis. Inhaled conidia in healthy individuals are mostly entirely cleared by phagocytic cells, but in asthmatic patients, conidia can persist and develop within bronchial/alveolar epithelial cells.

In asthmatic patients, a series of host immune responses fail to eliminate the fungus and cause an acute and persistent inflammatory reaction. The inflammatory reaction causes molecular to tissue damage that will increase mucus production and chronic inflammation and trigger airway hyperresponsiveness. If this continues uninterrupted, it can lead to bronchiectasis and pulmonary fibrosis.[19,44]

Diagnosis

Clinical features

Although most ABPA patients are asymptomatic, it can trigger exacerbations and worsen asthma symptoms in asthmatic patients.[18] Productive cough with dark green or brownish mucus, or even hemoptysis, is commonly observed in asthma patients. Other common features are dyspnea, chest pain, wheezing, clubbing fingers, and coarse crackles.[18,44,59] A more advanced ABPA may trigger a more severe manifestation, such as low-grade fever, headache, anorexia, and weight loss, that can hinder the patient's daily activities.[60] These manifestations may lead to complications such as bronchiectasis and fibrosis in the longer term.[18,44,60,61] The conventional progression staging of ABPA disease may be divided from acute (stage 1), remission (stage 2), exacerbation (stage 3), corticosteroid-dependent asthma (stage 4), to fibrosis (stage 5).[62] Meanwhile, the International Society for Human and Animal Mycology proposed a new staging, which consists of asymptomatic (stage 0), acute (stage 1), exacerbation (stage 2), remission (stage 4), treatment-dependent ABPA or glucocorticoid-dependent asthma (stage 5), and complicated ABPA (stage 6).[28]

Laboratory investigations

There has been no agreed laboratory test as the gold standard of ABPA, so a combination of tests is needed to establish the diagnosis. The primary laboratory test used for screening is the serum-specific IgE level of A. fumigatus due to its high sensitivity.[63] *Aspergillus fumigatus* IgE levels of more than 0.35 kUA/l are supposed to be associated with the incidence of ABPA in asthmatic patients.[44] The sensitivity of this method is up to 100%, and the specificity is 70% (TABLE 2).[63] Contrary to the previous test, total serum IgE levels are preferred in post-treatment follow-up ABPA patients because they tend to decrease promptly as therapy progresses but must be done several times to determine the various baseline between individuals. Moreover, there is a minimum total IgE level of 1000 IU/mL to prevent overdiagnosis due to its high sensitivity but very low specificity (TABLE 2).[18,44,63]

A positive *Aspergillus* skin test can help diagnose ABPA patients, but the sensitivity and specificity are not satisfactory[63] (TABLE 2), so it needs to be supported by an increase in total serum IgE levels for diagnosis. If both of these tests lead to a diagnosis of ABPA, additional tests, such as the precipitin test for *A. fumigatus*, may be performed to rule out other diagnoses because of their high specificity.[64] Unfortunately, this test has low sensitivity and variable cut-off, making it unreliable in the initial diagnosis (TABLE 2).[63] In addition, there is a test of specific IgG levels against *A. fumigatus* with good sensitivity and specificity for the diagnosis of ABPA, but it is not recommended for monitoring therapy (TABLE 2).[65] Various commercial IgG A. fumigatus assays may yield varying sensitivity results in the diagnosis of chronic pulmonary aspergillosis. However, this study utilized only one commercial test for ABPA.[65,66] Furthermore, an increased number of eosinophils in the peripheral blood is also one of the examination criteria for diagnosing ABPA. Nevertheless, the test is unreliable because it can lead to misdiagnosis due to very low sensitivity (TABLE 2).[28,44,64]

TABLE 2. Sensitivity and specificity of laboratory investigation in diagnosis ABPA.[63,66]

Methods	Sensitivity (%)	Specificity (%)
Specific IgE against *A. fumigatus*	100	70
Total serum IgE level	92-97	24-40
Skin test against *A. fumigatus*	88-95	80-87
A. fumigatus precipitins	33-43	97-98
Specific IgG against *A. fumigatus*	75-90	97-99
Peripheral blood eosinophils	30-36	93

Other tests, such as sputum culture, can identify species other than *A.* fumigatus that cause ABPA and determine the antifungal susceptibility test. Pulmonary function tests can also determine the severity of ABPA and the patient's progress in therapy. However, these two types of tests are rarely used by clinicians for both diagnosis and follow-up therapy.[18,44,64]

Radiologic findings

The modality used in ABPA imaging is a CT scan with infiltrates on the upper lobes as the most common finding.[18] In addition, the feature of bronchiectasis (especially central bronchiectasis with bronchus walls thickening), mucus-filled bronchus, high-attenuating mucus (HAM), centrilobular nodules consolidation, tree-in-bud opacities, and mosaic attenuation may be found.[19,44] Subsequently, the imaging results are classified as transient or fixed. Nonetheless, ABPA patients often do not show any abnormalities in imaging results. On the contrary, another modality, MRI, is not recommended for diagnosis.[18,19,44,63,64]

Diagnostic criteria for ABPA

Asthma-related ABPA can be diagnosed through clinical features, laboratory examinations, and imaging. The first ABPA diagnostic criteria were made by Rosenberg *et al.*[67] in 1977, consisting of seven major and three minor items. It is also the most widely used criterion in diagnosing ABPA. In 1988, Greenberger and Patterson suggested an additional item, elevated *A. fumigatus*-specific IgE and IgG levels in the serum.[68] In 2002, Greenberger suggested minimal essential criteria that consisted of 5 items.[69] Greenberger then simplified this criterion in 2013 into truly minimal diagnostic criteria consisting of four items.[70] Eventually, in 2013, the International Society for Human and Animal Mycology (ISHAM) suggested diagnostic criteria for asthma or cystic fibrosis patients as a predisposing factor. The criteria consisted of two major and three minor items. Both major items and at least two minor items should be present.[28]

Agarwal *et al.*[71] proposed seven types of simplified criteria for comparison with the modified ISHAM-AWG criteria. Of the seven criteria, criteria 5, with the combination of IgE result (total and *A. fumigatus*-specific) and an increase in *A. fumigatus*-specific IgG or bronchiectasis, can be alternative criteria for diagnosing ABPA. In addition, criteria three based on a specific IgE examination can be another alternative, especially in places with limited resources, because it comprises only three components. Criteria 4 with based skin tests can also be used to confirm ABPA due to its high specificity in settings without access to *A. fumigatus* immunoassays.[71]

Asano *et al.*[72] proposed ten new criteria that can be used for diagnosing ABPM and ABPA from the modified Rosenberg and ISHAM criteria. If the patient meets six or more of these

criteria, they can be diagnosed with ABPM. This study result shows more excellent sensitivity than the previous criteria with fairly good specificity. However, this criterion has limitations due to the variable cut-off values of some laboratory tests, and the patient sample includes only Japanese cases.[72] Therefore, these new criteria still require international multicenter investigation to be validated. All criteria for ABPA diagnostics that have been used and proposed are shown in TABLE 3.

TABLE 3. ABPA Diagnostic Criteria.[61,65,67-71]

References	• Major	• Minor
Rosenberg *et al.*[61,65]	• History of asthma • Peripheral eosinophilia • Positive skin hypersensitivity test against *Aspergillus* antigen • Elevated total IgE level in serum • Fixed or transient opacity in the lung • Central bronchiectasis	• Presence of *A. fumigatus* found in the sputum • History of brownish mucus • Arthus-type reactivity against *Aspergillus* antigen
ISHAM[71]	• Positive skin test against type 1 *Aspergillus* or increased IgE level against *A. fumigatus* • Increased total IgE level (>1000 IU/ mL)	• Presence of antibody or IgG serum against *A. fumigatus* • Thorax imaging support the presence of ABPA • Total eosinophil count over 500 cells/µL in patients who never used steroid therapy
Minimal Essential Criteria[67]	• History of asthma • Positive skin test against *A. fumigatus* • Total IgE level in serum higher than 1000 ng/mL • Elevated *A. fumigatus* specific IgE or IgG level • Central bronchiectasis without distal bronchiectasis	
Truly Minimal Criteria[68]	• Same as Minimal Essential Criteria but without elevated *A. fumigatus* specific IgE or IgG level	
Agarwal *et al.*[69] (from modified ISHAM-AWG)	Any of the criteria listed below: • *A. fumigatus* specific IgE serum > 0.5 kUA/L, total IgE serum > 500 IU/mL and either *A. fumigatus* specific IgG serum > 27 mgA/L or bronchiectasis (criteria 1) • *A. fumigatus* specific IgE serum > 0.5 kUA/L, total IgE serum > 500 IU/mL and either bronchiectasis or peripheral eosinophilia (>500 cells/mL) (criteria 2) • *A. fumigatus* specific IgE serum > 0.5 kUA/L, total IgE serum > 500 IU/mL and bronchiectasis (criteria 3) • Positive skin test against type 1 *Aspergillus*, total IgE serum > 500 IU/mL, and bronchiectasis (criteria 4) • *A. fumigatus* specific IgE serum > 0.5 kUA/L, total IgE serum > 500 IU/mL and either *A. fumigatus* specific IgG serum > 27 mgA/L or bronchiectasis (criteria 5) • *A. fumigatus* specific IgE serum > 0.5 kUA/L, total IgE serum > 500 IU/mL and either bronchiectasis or peripheral eosinophilia (> 500 cells/mL) (criteria 6) • *A. fumigatus* specific IgE serum > 0.5 kUA/L, total IgE serum > 500 IU/mL and either *A. fumigatus* specific IgG serum > 27 mgA/L or peripheral eosinophilia (> 500 cells/mL) (criteria 7)	
Asano *et al.*[70]	• History of asthma or symptoms of asthma • Peripheral eosinophilia (higher than 500 cells/mm^3) • Increased total IgE in serum (higher than 417 IU/mL) • Positive skin hypersensitivity or specific IgE against filamentous fungi • Presence of precipitins or specific IgG against filamentous fungi • Presence of filamentous fungal in sputum or bronchial lavage fluid • Fungal hyphae in bronchial mucus plug • Central bronchiectasis • Mucus plug in central bronchi • High attenuation mucus in bronchi	

In addition, there are comparison diagnosis criteria by Saxena *et al.*[73] between existing criteria such as Patterson and ISHAM with three other modified criteria but with the same patients from Agarwal *et al.*[71]respectively, on an MDT evaluation. Results: We analyzed data from 543 asthmatic subjects (58.8% women; mean age, 36.8 years The results of this study showed that the sensitivity and specificity of the diagnostic criteria of ISHAM were slightly better than Patterson's. Moreover, of the three modified ISHAM criteria, the best one is the presence of asthma, *A. fumigatus* specific IgE >0.35 KUA/L, total serum IgE level >500 IU/mL; and meets 2 of the following: *A. fumigatus* specific IgG >27 mgA/L, bronchiectasis on chest CT, and eosinophil count >500 cells/mL.[73]

Despite the mentioned criteria above, there is still no standardized universal consensus. Rosenberg's criteria have a high specificity yet low sensitivity. The one proposed by ISHAM has better sensitivity and is still quite specific but too complicated to use. In recent years, there have been new criteria proposed by Agarwal *et al.*[71] with patients from a single tertiary care referral center in India and Asano *et al.*[72] from two health institutes in Japan. Thus, the two newer criteria still require multicenter studies to be validated to be used in varied populations.

In the ISHAM's diagnosis criteria, the cut-off total serum IgE levels >1000 IU/mL still has no clear reason. In addition, there is also no receiver-operating characteristic/ ROC analysis regarding IgE levels with ABPA, SAFS, and Aspergillus-sensitized asthma. Aspergillus IgG may also not be specific for ABPA because high levels are also found in other forms of aspergillosis, such as chronic pulmonary aspergillosis/ CPA. Finally, a peripheral eosinophil count of >500 cells/μL is very common in many other diseases and the specificity of this criterion is questionable. Therefore, further research is needed to validate this diagnostic criterion.

The newly proposed simplified criteria by Asano *et al.*,[72] state that the diagnostic accuracy of the newer criteria should be considered acceptable if the efficiency exceeds 95% and the false-negative rate is less than 5%. Although several criteria qualify for acceptance, there are some criteria (criteria 3, 4, and 7) that shows false-negative rate was >5%. The study is also a single-center study conducted at a tertiary care hospital, with large proportion of patients had bronchiectasis. It is also mentioned that the diagnostic performance of the criteria might be different in the milder asthmatics seen in the community or those with serological ABPA. Thus, the criteria still need prospective multicenter investigation for validation.

Treatment

Treatment of ABPA aims to treat inflammation in the lung, prevent asthma exacerbations, overcome acute symptoms of ABPA, and delay complications. Glucocorticoids, such as prednisone, and antifungals like itraconazole, are utilized in conservative therapy (TABLE 4).[43,44] The administration of oral glucocorticoid remains the first line of ABPA in all stages.[18,19,44,69] The glucocorticoid that is widely used as an anti-inflammatory is prednisone or prednisolone. Clinicians must be careful if there is an indication of an increase in serum IgE ≥ 100% of the initial value because this indicates an exacerbation. Other routine evaluations are required for ABPA patients, such as CXR and eosinophil count, based on the patient's ABPA stage at diagnosis.[18,19,44,69] In addition to reducing the patient's symptoms, mucolytic drugs can be given.[43] Other novel treatments, such as monoclonal antibodies, on the other hand, are still under further study.[74]

TABLE 4. Recommendation therapy for ABPA

Stage	Therapy recommendation	Notes
Stage 1: Acute	*First line* Prednisone 0.5-0.75mg/kg/d for 6 wk; then TO 5-10 mg every 2 wk	*Glucocorticoid therapy follows up*: • Evaluate total serum IgE after 6-8 wk of therapy (the expected result is a decrease more than 35%); CXR and eosinophil count evaluation every 3 m.o. • To decide the normal range of total serum IgE, evaluations can be done every 8 wk for a yr • In discontinuing corticosteroid therapy, evaluate the remission status of the patient every 6-8 wk • *Antifungal administration*: • Susceptibility test (confirm that the patient is not resistant) • Evaluate liver enzyme, triglyceride, potassium, drugs level in blood
Stage 2: Remission	*Second line* Itraconazole 200-400 mg p.o. 2x1 for 16 wk	
Stage 3: Exacerbation	*Combination therapy* Prednisone 0.5-0.75mg/kg/d for 6 wk; then TO 5-10mg every 2 wk and Itraconazole 200-400mg PO 2x1; for 16 wk	
Stage 4 Corticosteroid-dependent asthma	*First line* Prednisone 10-40mg AD, for a few yr *Second line* Itraconazole 200-400mg PO 2x1; for 16 wk	
Stage 5 : Fibrosis	Prednisone 10-40mg AD	• Must be combined with cor pulmonale and hypoxemia therapy

• TO: tapper off; p.o.:per oral; CXR: chest X-ray; AD: on alternate days

Inhalation and intravenous routes are alternative routes of glucocorticoid administration in ABPA patients. For inhaled glucocorticoid, it is known to improve asthma symptoms and increase total serum IgE when used in combination with a long-acting beta agonist. However, the combination of inhaled glucocorticoids and itraconazole is associated with increased risk of adrenal insufficiency. Pulse steroid therapy or intravenous glucocorticoid used in ABPA is methylprednisolone (10-20mg/kg/d), given for three consecutive days every four weeks and reported in most cases that intravenous glucocorticoid therapy is showing improvement to ABPA symptoms with minimal side effects.[19,75]

Antifungal therapy in ABPA treatment has multiple roles as an alternative therapy (second line) and combination therapy. Itraconazole is widely used antifungal, with an orally recommended dosage of 200-400 mg/d divided into two doses for 16 wk. Nevertheless, it is necessary to periodically evaluate liver enzymes, triglycerides, blood potassium, and blood drug levels in patients receiving these antifungals until their administration is

discontinued. Administration of other azole alternatives with voriconazole, 400-600 mg/d, divided into two doses for 16 wk, or posaconazole, in patients with treatment failure or intolerance of itraconazole shows clinical improvement. The side effects of itraconazole and posaconazole are better tolerated than voriconazole. However, these alternative newer azoles still need further evidence from randomized controlled trials to be considered the first-line antifungal therapy for ABPA.[18,19,44,76] Although antifungal therapy is more effective and has fewer side effects than glucocorticoid therapy, a susceptibility test against various species of *Aspergillus* is still necessary.

Despite the mentioned therapies, there is also a novel treatment: monoclonal antibodies such as omalizumab, mepolizumab, tezepelumab, and dupilumab.[74] Some of these antibodies are known to have fewer side effects than conventional therapy and have been proven effective in reducing clinical manifestations and the number of exacerbations. Nonetheless, most of these monoclonal antibodies still require further studies.[43,74]

CONCLUSION

Despite aspergillosis and asthma having long been acknowledged, the diagnosis and treatment approaches are still a challenge for clinicians. Several ABPA overlapping diagnostic criteria without universal consensus are one of the causes. The laboratory test mainly used in screening is the serum-specific IgE level of *A. fumigatus* because of its high sensitivity. Hence, physicians should be vigilant in screening asthmatic patients for this infection to prevent long-term complications such as bronchiectasis and fibrosis.

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Potential biomarkers of IFN-γ, IL-2 and CXCL9 for diagnosis of Q fever disease

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ABSTRACT

The pathogen responsible for Q fever disease, *Coxiella burnetii*, is a zoonosis classified as a pathogen due to its airborne transmission. The *C. burnetii* infection could be both acute or chronic in humans. The main and most common entry of the pathogens to the body is through the breathing of polluted aerosols containing a resistant substance similar to *C. burnetii* spores. This small cell variant (SCV) or spore-like morphotype is extremely stress-resistant, therefore inadequate treatment causes serious effects even death. Due to the diversity of clinical manifestations of Q fever and the presence of less specific and sensitive diagnoses for other diseases, multiple platforms for exploring Q fever biomarkers are required. Apart from serological studies to determine a biomarker for Q fever, it will be prudent to concentrate on the more appropriate cell-mediated immune response. This article discusses *C. burnetii* causing *Q fever* disease and how the host develops humoral and cellular immunity, particularly IFN-γ, IL-2 and CXCL9, as potential biomarkers for the diagnosis of *Q fever* disease.

ABSTRACT

Coxiella burnetii penyebab penyakit Q fever merupakan zoonosis yang termasuk sebagai pathogen yang penularannya lewat udara. Infeksi *C. burnetii* dapat besifat akut atau kronis. Jalur utama dan umum masuknya patogen ke dalam tubuh adalah pernafasan aerosol yang terkontaminasi bahan resisten mirip spora *C. burnetii*. Varian sel kecil (*small cel variant*/SCV) atau morfotipe mirip spora ini sangat resisten sehingga pengobatan yang tidak tepat menyebabkan efek serius bahkan kematian. Manifestasi klinis Q fever yang bervariasi dan keberadaan diagnosis penyakit yang sudah ada masih kurang spesifik dan sensitif maka dibutuhkan berbagai platform untuk mengeksplorasi biomarker Q fever. Selain studi serologi sebagai biomarker Q fever, akan sangat bermanfaat juga berfokus pada respon imun yang dimediasi sel yang lebih relevan. Makalah ini memaparkan tentang *C. burnetii* sebagai agen penyebab Q fever dan bagaimana pejamu mengembangkan imunitas humoral dan seluler khususnya IFN-γ, IL-2 dan CXCL9, sebagai biomarker potensial untuk diagnosis penyakit Q fever.

Keywords:
biomarker;
Coxiella burnetiid;
immune response;
Q fever

INTRODUCTION

Q fever is caused by the zoonotic agent *Coxiella burnetii,* a bacterium that spreads through aerosol transmission.[1] These pathogens are isolated or clustered cells or spores that adhere to particulate matter or dust. As a result, *C. burnetii* can adhere to dust particles, which plays a significant role in wind dispersal.[2,3] Inhalation becomes the primary route of infection via contaminated aerosols containing an environmentally resistant material that resembles *C. burnetii* spores.[4] This spore-like morphotype is also known as the small cell variant (SCV), exhibiting exceptional resistance to environmental stresses (heat, drying, UV rays).[5] *Coxiella burnetii* is classified as a type B agent by the Centers for Disease

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Control and Prevention (CDC) due to its resistance to environmental factors, low infection doses, human transmission via aerosol, and previous use as a bioweapon.[5,6]

Acute infection of *C. burnetii* in humans shows nonspecific clinical symptoms until pneumonia or hepatitis develops. The other clinical manifestation, characterized by persistent infection of the heart valve, prosthesis, or vascular aneurysm, was reported in 1 to 5% of the individuals with chronic infection of Q fever.[7] High mortality will occur if not treated properly.[8-10] Impaired health status in more than 30% of Q fever patients despite following the prescribed antibiotic regimen reported in one 24-month cohort study.[10,11] Because chronic Q fever infection can be fatal, early diagnosis of different stages of this disease is essential for the prevention of this condition.[12] Clinical application and diagnosis have included immunoglobulin G (IgG) phase 1 of *C. burnetii* phase I, C-reactive protein, 18F-FDG-PET/computerized tomography scan, and PCR antibodies.[13]

Several countries also reported outbreaks of Q fever, including the United States, Spain, Australia, Japan, and Israel exemplifying how widespread *C. burnetii* infection is worldwide.[14] However, with the world's largest outbreak of Q fever in the Netherlands,[7] it is necessary to identify and test biomarkers that can better discriminate against the different stages of Q fever infection.[13]

In another bacterial infection, *Mycobacterium tuberculosis*, measuring particular IL-2 production in addition to IFN-γ distinguishes between active and latent infection in tuberculosis. In this instance, active infection is characterized by high specific IFN- and low IL-2 production, while both high IFN- and high IL-2 production imply latent tuberculosis.[15,16] During active tuberculosis treatment, a change from T cells secreting only IFN- and IFN- /IL-2 to T cells secreting IFN- /IL-2 and only IL-2 has been documented.[17]

Measurement of specific antibodies is still the standard detection method for Q fever.[18] The detection approach through the measurement of T-lymphocyte immunity can be an alternative and provides advantages based on cellular immunity as a defense against these intracellular pathogens, including macrophage activation mediated by interferon-gamma (IFN-γ).[19] This paper describes *C. burnetii* causing Q fever diseases, how the immune system reacts to *C. burnetii* and identifies the immune system's response as potential biomarkers for enhancing diagnostic tools against *C. burnetii* infection.

DISCUSSION

Coxiella burnetii

Coxiella burnetii includes small, gram-negative bacteria, ranging from 0.2-0.4 μm in width and 0.4-1 μm in length, forming a *coccobacillus*. Originally classified in the order *Rickettsiales* because of their presence in ticks and intracellular morphology.[20] It is stated that *C. burnetii* can last up to 10 mo at temperature 15 to 20°C, and if kept in the cold storage, can last more than one month in meat and at room temperature in skim milk could be stored for more than 40 mo.[3] Studies continue using experimental animals to determine virulence factors[21,22] and genetic mechanisms in LPS.[23] Phase I (nine-mile phase I/NM I) that cause Q fever in humans has complete and smooth lipopolysaccharide, very virulent and pathologically challenges laboratory animals. A virulent called Phase II, or nine-mile phase II (NM II), does not have the O-side chain of LPS, is rough, and shows growth impairment in immunocompetent animals.[22,24]

Pathogenesis

In contrast to other intracellular obligate pathogens, *Coxiella's* stability in environmental stresses includes

enhancement of temperature, ultraviolet light, and osmotic pressure.[25] It has a biphasic development cycle with characteristics of small cell variant (SCV) forms, and the form that is more metabolically active and replicative than these organisms is the large cell variant forms (LCVs).[26] The main mode of infection in humans is inhalation of contaminated aerosols. *Coxiella* can replicate in the most inhospitable compartments of host cells, i.e., phagolysosomes, indicating its extracellular stability.[25] In the intracellular cycle of *C. burnetii*, after interacting with macrophages, *C. burnetii* is absorbed passively by binding to αvβ3 integrin with an actin-dependent phagocytosis process.[27] Newborn *Coxiella*-containing vacuole (CCV) will combine with the autophagosome after absorption, then mature. In addition to joining the cellular lysosomes, the mature CCV also lowers the pH to 4.5. It is stated that the transition from SCV to a metabolically active morphotype, namely, LCVs occurs after inhalation.[25,28]

Protein synthesis by *Coxiella* is required to start the initial interactions of autophagosome to promote the cessation of the VP that has matured. Type IV secretion system (T4SS) in *Coxiella* is the same as in *Legionella*, known as Dot/Icm T4SS system. The Dot/Icm of T4SS plays an important role in forming the *Legionella* vacuole replication, and T4SS is used for gram-negative pathogen advantage by translocating proteins that can modulate certain mechanisms in host bodies.[27,28] An acute Q fever pathological condition may progress to chronic Q fever, different tissues show granulomatous lesions in different varieties including, but not limited to, the lung, liver, and spleen are common in *C. burnetii infection.* In contrast, granuloma formation in the chronic condition of Q fever is declared reduced.[29]

Clinical symptom and detection

Conxiella burnetii is a highly contagious intracellular obligate γ-proteobacterium. Flu-like pneumonia often becomes an acute clinical manifestation in humans.[30] Other symptoms include dry cough, malaise, myalgia, fever, and chills, usually appears within 2-3 weeks of *C. burnetii* exposure.[31] Most cases typically resolve on their own; following primary infection, between 1% and 5% of patients develop a chronic condition of Q fever, the manifestation of which can occur several years after the initial infection.[7,32] The development of chronic condition Q fever is affected by risk factors including heart valve disease, aortic aneurysms, immune disorders, pregnancy, and vascular grafts.[33,34]

If left untreated, chronic Q fever will cause significant morbidity and mortality, up to 60% and requires long-term treatment with antibiotics such as hydroxychloroquine and doxycycline.[35] Diagnosis of chronic condition Q fever is still quite a challenging task. The difficulty of diagnosis is due to several things, including culturing *C. burnetii*; which takes time, requires a laboratory with security level 3, and less sensitive.[36] Currently, with samples from blood or tissue, the polymerase chain reaction (PCR) is still a potential tool for serology and detection of DNA is used to confirm the chronic condition of Q fever. Detection of *C. burnetii* DNA in the sample from tissue or blood by PCR without acute infection was expressed as chronic Q fever although the sensitivity of this technique is low.[33]

The antigenic variation of *C. burnetii* forms the basis of serological diagnosis.[37] The first detected in the time of acute infection is antibodies related to phase II antigen, then the antibodies against phase I antigen.[37] High levels of long-lasting antibodies to phase I and phase II antigens are regarded as an indicator of the chronic condition of Q fever, which is interpreted as the result of persistent antigen stimulation. The limit determination for IgG virulence (phase I) of serological from in-house immunofluorescence assay (IFA) is

≥1: 800; this value has been accepted internationally to chronic diagnosis condition of Q fever. However, this test yields a high number of false positives.[33]

Conxiella burnetii infection has significantly impacted the health of humans and domesticated livestock and the sustainability of human food.[1,5] This condition showed in the epidemic occurrence of acute cases of Q fever over four years (2007-2011) in the Netherlands, reported over 4,000 acute Q fever cases occurred.[1,7,34] Test consistency is critical with cases reported throughout the Netherlands as of 2007, during the large Q fever outbreak. Moreover, the result test interpretation also impacts on improving patient care.[33] Because there is still much uncertainty in the algorithms used by diagnostic tools for the chronic condition of Q fever.[33] It is very important to carry out further research in the development of biomarkers that can be used for early detection of Q fever.[13]

Host immune response against *C. burnetii* infection

Innate immune response

Neutrophils, monocytes, and macrophages

In a study conducted by Elliott *et al.*[38] neutrophils are initially recruited to the lungs during the early infection stage due to cytokine production from infected macrophages. That role can be seen from the decrease in neutrophils using the lowering antibody RB6-8C5, resulting in a more severe infection in mice.[38] The elimination of *C. burnetii* is associated with the formation of reactive oxygen species (ROS), followed by reactive nitrogen species (RNS) in collaboration with other immune cells via the production of cytokines.[39] Neutrophils also enhance the ability of macrophages in killing phagocytic bacteria. Both of these play a significant part in the elimination of this bacterium.[39] However, one study showed that the ability to inhibit protein transfer from this bacterium is needed in ROS production into CCV.[25]

It is interesting that *C. burnetii* targets not only monocytes but also macrophages.[24,40] A subversion mechanism from the microbicidal properties of these cells makes *C. burnetii* persistently reside and carry out intracellular trafficking. The drastic changes of the actin cytoskeleton, activation of the protein tyrosine kinase pathway, and cytokine production occur after this bacteria interaction through αvb3 integrins with macrophages and monocytes.[39] This functional polarization occurs as a result of monocyte/macrophage interaction with *C. burnetii*.[24,40] Currently, the resting phase of the monocyte, which this bacteria exist but does not multiplicate, results in the induction of M1 polarization. After being triggered in response to infectious agents or IFN-γ, these M1-polarized cells contribute to the host's microbicidal function.[24,40] The *C. burnetii* replication occurs in macrophages and induces M2 polarization-related gene expression. This leads us to the conclusion that *C. burnetii* controls its intracellular life by polarization M1 or M2-like phenotype of macrophages.[24,40]

Professional antigen presentation cells and TLRs and NOD-like receptors (NLRs)

Through a study, virulent types of this bacterium can replicate and infect human dendritic cells without inducing a response to inflammatory cytokines.[25] Differs from a virulent *C. burnetii* resulting in the induction of dendritic cells. This is related to the virulence properties of the *Coxiella's* complete virulent lipopolysaccharide (LPS) related to the ability of the molecule to protect the outer membrane.[25] In avirulent organisms, *C. burnetii* or phase II *C. burnetii* is easily eliminated through the complement system's membrane attack complex (MAC). In addition to inhibiting the interaction of *Coxiella*

with the CR3 receptor (αMβ2 integrin) from macrophages,[41] the *Coxiella* toll-like receptor (TLR) ligand is also masked by phase I LPS, so can't recognize this bacteria.[42] Chemically identical parts and molecules from LPS lipid in phase I and phase II apart not only fail to bind to TLR-4, but is also different to TLR-4 signalling by another LPS.[42]

In response to *C. burnetii* infection in humans, a study by Ammerdorffer *et al.*[42] demonstrated that stimulation of cytokines required receptor recognition occurs via heterodimers with NOD-like receptors as well as TLR1/TLR2.[42] The absorption process of *C. burnetii* bacteria during infection indicates that the ability of TLR2 receptors to form a heterodimer with either TLR1 or TLR6 greatly assists the function of TLR2 receptors compared to TLR4.[42] It also appears that the signalling of lipid A from *C. burnetii* via TLR2 is important in producing inflammatory cytokines.[42]

Adaptive immune response

Humoral response

The paradigm that protection against intracellular pathogens is solely cell-mediated has been challenged through research trials. In one experiment,[43] wild-type mice were protected from the challenge by passive transfer of antibodies to *C. burnetii*, and another study demonstrated that the antibodies act by neutralizing *C. burnetii*.[44] In acute infection, a potential role for B cells during the primary immune response is demonstrated. One study discovered that mice lacking B cells had more severe histopathological damage to their liver and spleen than mice with intact B cells.[43]

In laboratory experiments with *C. burnetii* phase I vaccination in cell-deficient mice showed no protection. However, the protection remained under other conditions even though the mice had $CD4^+$ T cell deficiency compared to wild-type mice. Under these conditions, we can see that IgM production, which can take place independently of T cells, mediates the protection. In contrast, IgG-mediated protection requires the role of $CD4^+$ T cells. As a result, humoral immunity appears to play a role; in the immunological response to this bacterial infection.[45]

According to Fournier *et al.* in Shannon, *et al.*[46] on acute Q fever, antibodies to phase II antigen acquire in less than 3 until 4 weeks of disease progression.[46] Acute Q fever is detected by combining anti-phase II antibodies with a low dose with anti-phase I antibodies directed primarily against LPS. If a patient has an anti-phase I titer greater than 800, they are diagnosed with chronic Q fever.[47] Given that nearly all effective vaccines are antibody-dependent, a better understanding of antibody-mediated immunity to *C. burnetii* will aid in vaccine development.[46]

Chemokines and cytokines

During the early phases of infection with intracellular pathogens, IFN-γ is an essential cytokine. In both phagocytic and non-phagocytic cells that are infected, IFN-γ interacts synergistically with bacterial products to trigger a range of bacteriostatic or bactericidal effector pathways.[48] The real presence of IFN-γ in eradicating intracellular infections was confirmed by antibody-mediated neutralization of IFN-γ *in vivo* and subsequent confirmation using mice with the IFN-γ knockout (KO) gene. The severity of infection with *C. burnetii* was elevated in these mutant mice.[49] TNF-α is also involved in the cytokines response to *C. burnetii* infection.[49] When IFN-γ and TNF-α are combined, macrophages and/or other target cells become more capable of controlling growth and/or killing certain intracellular organisms.[14,48] Although the contribution of the cytokine IL-10 in chronic infection with *C. burnetii* is not clear, data suggest an increase in IL-10 in patients with chronic infection.[29]

No less important mediators in the resistance of intracellular pathogens are chemokines. Early chemokine induction is not only necessary for recruiting professional antigen-presenting cells (APC), but it is also necessary for maintaining the primary role of specialist phagocytic cells and other target cells.[48] Through the defensin pathway, several chemokines that induce IFN-γ like (MIG or CXCL9), IFN-γ induced by IP-10 or CXCL10, a protein with a size of 10 kDa and IFN-γ induced chemoattractant T cells (CXCL11) can directly neutralize microbes.[48] Throughout BALB/c infections associated with *C. burnetii* during acute infection, they show high production of the specific antigen CXCL10. However, elevated CXCL10 is more likely to be a marker of inflammation than a specific marker of acute Q fever.[13]

Lymphocyte T CD4^{+} and CD8^{+}

The importance of $CD8^{+}$ and $CD4^{+}$ T cells in this bacterial infection was demonstrated clearly in a study,[50] in which both SCID and nude mice lacked T cells exhibiting severe infection that could be fatal. When $CD8^{+}$ T cells were combined with $CD4^{+}$ T cells or observed alternately in this study, they demonstrated a greater protective ability than $CD4^{+}$ T cells. This finding may be explained by lymphocyte T-$CD8^{+}$ ability to produce IFN-γ, a cytokine that $CD4^{+}$ Th1 T cells also produce.[45] Inactivated bacteria in phase I have been shown to elicit robust Th1 protection and response, although inactivated bacteria in phase II elicit a weak Th1 response.[51] This identical death rate documented in *C. burnetii* infections of the IFN-$\gamma^{-/-}$ mouse model demonstrates the critical role of IFN-γ in *C. burnetii* protection.[51]

Potential biomarkers

Humoral immune response

It is important to carry out further studies to identify the humoral response to *C. burnetii* with a powerful tool. A comprehensive study of the antigen profile using protein microarrays has been reported that new seroreactive antigens have been identified.[52] Through the capacity to determine specific antigens against specific IgM antibodies and subsequent IgG antibodies,[52] it is possible to determine better when a person is exposed to the pathogen, determine the progression of infection, and the therapeutic response. In the diagnosis of infectious diseases, detection of IgM needs to be done early.[52] A study conducted by Vigil *et al*.[52] used protein microarrays to profile the antibody repertoire generated in response to infection. It is important to detect IgM immune response in infectious disease early diagnosis. That IgM production will continue by the development of IgG antibodies, the ability to determine when an individual was exposed to a pathogen, and potentially the progression of infection and therapeutic response by selecting antigen-specific IgM antibodies and subsequent IgG antibodies. This study identified a significant reactive protein capable of discriminating between acute and chronic conditions of Q fever, namely the CBuK 1974 protein. CBuK 1974 is a 63-amino acid small protein discovered in the genome of *C. burnetii* strains isolated from people with endocarditis.[52]

It is stated in the literature study conducted by Kowalczewska *et al*.[53] that some proteins have been found by proteomic analysis as potential marker candidates and have been cross-validated. CBU 0952 (acute disease antigen A), CBU 0236 (elongation factor Tu/tuf-2), and CBU 0092 (tol-pal system protein/Ybgf) are proteins that have the potential to be a significant markers in acute Q fever conditions.[53] The protein rpoA (CBU_0263) and the universal stress family (CBU_1916) are potential markers for chronic Q fever.[53]

INF-γ and IL-2

Even though anti-phase I IgG has only a minor protective role against *C. burnetii*, it is still used to diagnose and monitor Q fever's chronic condition. Anti-phase I IgG is also used to detect infections and complications. It is a manifestation of the humoral response to specific B cells and CRP and IL-6-mediated inflammatory products.[13] However, with the dominance of the IFN-γ-mediated T-helper 1 response as a protective immune response against *C. burnetii*, the existing markers have not accurately reflected disease progression.[50]

It is critical to investigate whether IFN-γ is a cytokine that plays a critical role in determining whether chronic Q fever progresses from a previously cured infection. Measurement of other cytokines that also confirm IFN-γ is important to evaluate.[19] Type I interferon, IL-12, IL-18, and IL-23 were inhibited in the presence of IFN-γ.[54] When M2 macrophages are polarized in vitro, we observe increasing production of IL-10[29] and an increase in the regulation production of receptor antagonists IL-1 (IL-1Ra) and IL-6 as a decrease in the production of TNF-α.[19] Proliferation and development of lymphocytes in the memory response are also critical, as is the role of the IL-2 cytokine in the induction process.[55] To protect the host cell from *C. burnetii*, the combined effort of IFN-γ producing T cells and natural killer cells stimulates the microbicidal activity of the macrophage.[40]

Schoffelen *et al*.[19] demonstrated that an IFN-γ greater than eleven compared to IL-2 had a specificity of 96% and a sensitivity of 79% for diagnosing chronic Q fever, respectively. These study results demonstrate that the effector response and memory of $CD4^+$ T cells play a role; the high ratio IFN-γ compared to IL-2 is indicative of the dominance of T-cell effectors and T-cell memory effectors as a result of the prolonged stimulation of the infecting organism. Two distinct populations of T-cell memory effectors and T-cell memory centers are important in our understanding of the functioning of memory T lymphocytes. T cell effectors and T cell memory effectors will produce IFN-γ, while T cell memory centers will produce IL-2 (Figure 1).[56] As a result, subsequent studies examining the ratio IFN-γ to IL-2 found a significant reduction in chronic Q fever patients who had recovered from treatment.[57]

Another study by Schoffelen *et.al*[58] in 2013 regarding the specific detection of IFN-γ for previous *C. burnetii* infection through Bayesian analysis, with sensitivity and (87.0% and 90.2%, respectively,) comparable to the results of serology and ST. The concordance between IFN-γ detection and a combination of serology and ST measurements was moderate (84% concordance; = 0.542).[58]

It is noteworthy that Schoffelen *et al.*[19] found that the IFN-γ/IL-2 value among test subjects with chronic Q fever did not meet the criteria established by Raoult D. or the Dutch consensus.[33,59] These subjects would have phase I IgG without a definitive clinical, PCR, or imaging diagnosis of chronic *C. burnetii* infection. This low ratio of IFN-γ to IL-2 could indicate the absence of infection with *C. burnetii* or infection on a small scale. The sensitivity of PCR and imaging techniques, on the other hand, affects this condition. Thus, in such difficult circumstances, an elevated IFN-γ to IL-2 ratio may be a useful marker for diagnosing chronic Q fever.[19] The wide difference in IFN-γ to IL-2 ratios among study participants necessitates further research to formulate solid guidelines for using these biological markers. However, this study establishes a potentially important impact on the IFN-γ to IL-2 output profile as a biomarker of chronic Q fever.[19]

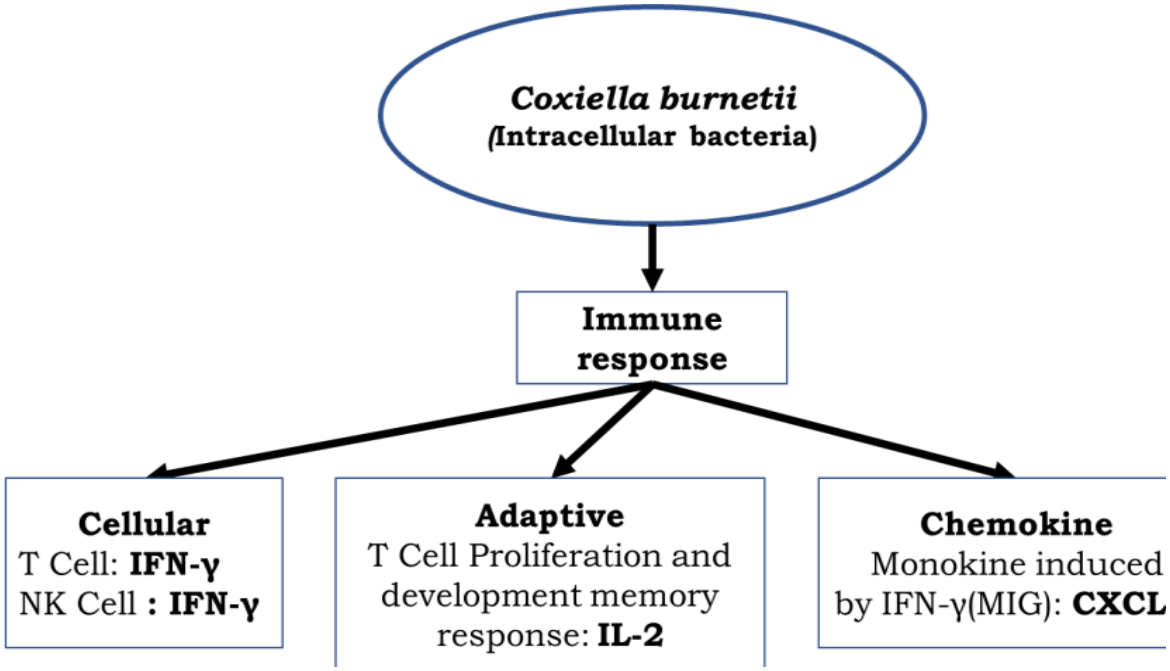

FIGURE 1. Schematic overview of potential biomarkers from the immune response toward *C. burnetii* that cause Q fever disease.

Chemokine CXCL-9

Recognizing the critical role of biomarkers in the early detection of Q fever, Jansen *et al.*[13] discovered the potential of the chemokine CXCL9 (FIGURE 1) as a biomarker through transcriptome analysis of peripheral mononuclear cells (PBMC) from chronic Q fever sufferers induced to *C. burnetii*. Transcriptome analysis of serum from sick people with Q fever and a validated comparison group revealed the presence of four chemokines stimulated by IFN-γ in high concentrations in responding to inactive *C. burnettii* after heating.[13] The fact that CXCL9 and CXCL11 serum levels have increased in individuals with persistent Q fever than in patients who have had Q fever demonstrates that CXCL9 and CXCL11 can aid in diagnosing chronic Q fever.[13] Then, more conclusive results demonstrated that the chronic condition of Q fever patients had a greater increase in CXCL9 than patients who had been exposed to Q fever.[13]

The chemokine found in the study of Jansen *et al.*[13] is a small chemotactic protein with characteristic features, including four cysteines with the amino acid variable 'X' dividing it.[60] The four CXCL chemokines are CXCL10, CXCL11, CXCL8, and CXCL9. IFN-γ induces all four chemokines, but only CXCL10, CXCL11, and CXCL8 can be stimulated by type 1 interferon as well as by TNF-α. CXCL9 can be expressed by macrophages, endothelial cells, fibroblasts, and peripheral blood mononuclear cells (PBMCs). Although the chemokines CXCL9, CXCL10, and CXCL11 share CXCR3 receptor ligands on the same cells, their affinity for CXCR3 varies, as does their expression, which is stimulus and time-dependent, indicating critical production as well as functional differences.[60,61] The presence of the CXCR3 chemokine also varies and has been used as a surrogate marker for active and latent tuberculosis conditions, treatment monitoring, and, of course, this is related to the immune system response of *C. burnetii*, another intracellular bacterium.[13] According to Van den Steen *et al.*[60] in Jansen *et al.*[13], different active concentrations of MMP-8 (metalloproteinase-8) or MMP-9 affect

the level as well as the functionality of CXCL9 and CXCL10.[13]

Given that elevated CXCR3 ligand levels are not increased specifically through the chronic condition of Q fever, careful consideration is warranted in further interpretation.[62] Likewise, the increase in the concentrations of CXCL9 and CXCL10 in heart failure patients, which, if observed, were still lower when compared to when patients were in chronic Q fever conditions.[62,63] Some lack in the study of Jansen *et al.*[13] the specificity of CXCL9 in chronic infection by *C. burnetii* in comparison with any other pathogen that also elicits a T helper-1 response, which of course also induces an increase in the serum concentration of the CXCR3 ligand.[13] In addition, transcriptome analysis is very helpful, but this test without validation from other tests makes it less than optimal. Similarly, the number of samples analyzed will significantly impact the representativeness of the condition of patients with persistent Q fever.[13]

CONCLUSION

The varied clinical manifestations of Q fever and a diagnosis that is often based solely on systematic consideration have been a barrier to the assessment and diagnosis of Q fever. Therefore, the availability of other relevant biologic markers may be helpful. The diagnosis of Q fever is less specific and sensitive in different stages of the disease through serological approaches and PCR techniques, makes it necessary to have multiple platforms to explore biomarkers of Q fever. Combining more than one potential biomarker could be done to obtain a more precise diagnosis. Cell-mediated immune responses, particularly IFN-γ, IL-2, and CXCL9, have shown potential biomarkers for diagnosing Q fever disease, but validation with a large number of samples is needed.

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Serpentine supravenous hyperpigmentation (SSH) in nasopharyngeal carcinoma patient on docetaxel and carboplatin chemotherapy: a case report

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ABSTRACT

Serpentine supravenous hyperpigmentation (SSH) is a rare but unique side effect of intravenous anticancer. It manifests as linear hyperpigmentation eruption on the skin that radiates along the superficial vein accompanied by mild pain and/or itch. This SSH does not cause systemic alterations, however, most patients complained about its cosmetic effects. The diagnosis of SSH can be made clinically, although histopathological examinations can aid in excluding differential diagnoses. We reported a case of SSH found in a nasopharyngeal cancer patient during docetaxel and carboplatin chemotherapy. It is a potentially alarming interface dermatitis that is not lot reported in the literature. It was reported, the patient tolerated the second and third cycles well with less severe side effects when premedicated with 250 mL NaCl 0.9% bolus intravenously before and after chemotherapy sessions, 10 mg cetirizine every 24 hr orally, and desoximetasone cream 0.25% every 12 hr topically added.

ABSTRAK

Hiperpigmentasi supravenosa serpentine (HSS) adalah efek samping antikanker intravena yang jarang terjadi namun unik. Manifestasi HSS berupa erupsi hiperpigmentasi linier pada kulit yang menyebar sepanjang vena superfisial disertai nyeri ringan dan/atau gatal. Efek samping ini tidak menyebabkan perubahan sistemik, namun sebagian besar pasien mengeluhkan efek kosmetiknya. Diagnosis SSH dapat ditegakkan secara klinis, meskipun pemeriksaan histopatologi dapat membantu menyingkirkan diagnosis banding. Kami melaporkan kasus SSH yang ditemukan pada pasien kanker nasofaring selama kemoterapi docetaksel dan carboplatin. Ini adalah dermatitis antarmuka yang berpotensi mengkhawatirkan dan tidak banyak dilaporkan dalam pustaka. Dilaporkan, pasien mentoleransi siklus kedua dan ketiga dengan baik dengan efek samping yang tidak terlalu parah bila diberikan premedikasi dengan 250 mL NaCl 0,9% bolus intravena sebelum dan sesudah sesi kemoterapi, 10 mg cetirizine setiap 24 jam secara oral, dan krim desoximetasone 0,25% setiap 12 jam ditambahkan secara topikal.

Keywords:
docetaxel;
carboplatin;
nasopharyngeal carcinoma;
serpentine supravenous hyperpigmentation;
side effects

INTRODUCTION

Serpentine supravenous hyperpigmentation (SSH), also known as serpentine supravenous dermatitis (SSD) or persistent supravenous hyperpigmentation (PSSH) is a term used to describe skin morphology of linear hyperpigmentation eruption which is along the superficial veins. This rare phenomenon is related to the use of intravenous anticancer therapy. The SSH was first described by Hrushesky in 1976 to describe peculiar cutaneous-manifesting side effects after intravenous 5-fluorouracil (5-FU) administration.

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Even though SSH does not cause systemic alterations, the majority of the patients complained about its cosmetic effect.[1]

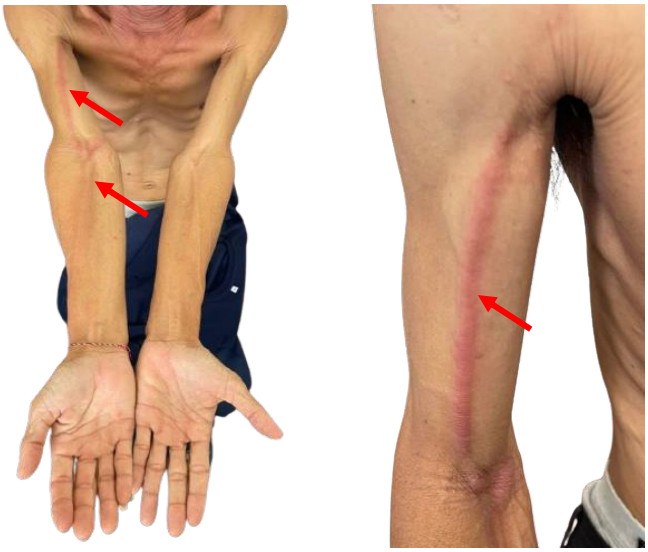
Nasopharyngeal carcinoma is a rare malignant case that originates from the nasopharyngeal epithelium. The incidence of nasopharyngeal carcinoma varies based on its geographical distribution. In Indonesia, nasopharyngeal carcinoma came in fourth as the most common malignancy, treated with either radiotherapy and/or chemotherapy.[2,3] However, the use of anticancer puts a variety of side effects at risk. These side effects are dependent on the type of anticancer used. On the other hand, administration, and dosage of the medications also correlate to the side effects. The side effects may manifest in every organ system, for instance, the cardiovascular, respiratory, gastrointestinal, nervous, and immune systems. Some side effects may also manifest in the skin with distinctive clinical manifestations. Serpentine supravenous hyperpigmentation is considered one of the unique and distinctive side effects manifestations in the skin.[4]

The side effects during chemotherapy must be taken into consideration when deciding the management options for cancer patients, from dosing to further treatment planning. Furthermore, this side effect can dominantly affect the life quality of the patients. Hence, physicians need to know, distinguish, and diagnose the commonly found reactions, so that prevention and also comprehensive early management can be addressed for these specific patients.

In this case report, we reported one case of docetaxel and carboplatin-induced SSH. This report was made to increase the knowledge about one of the side effects of chemotherapy agents manifesting in the skin, along with establishing a diagnosis, an etiology investigation, and management planning for the patient.

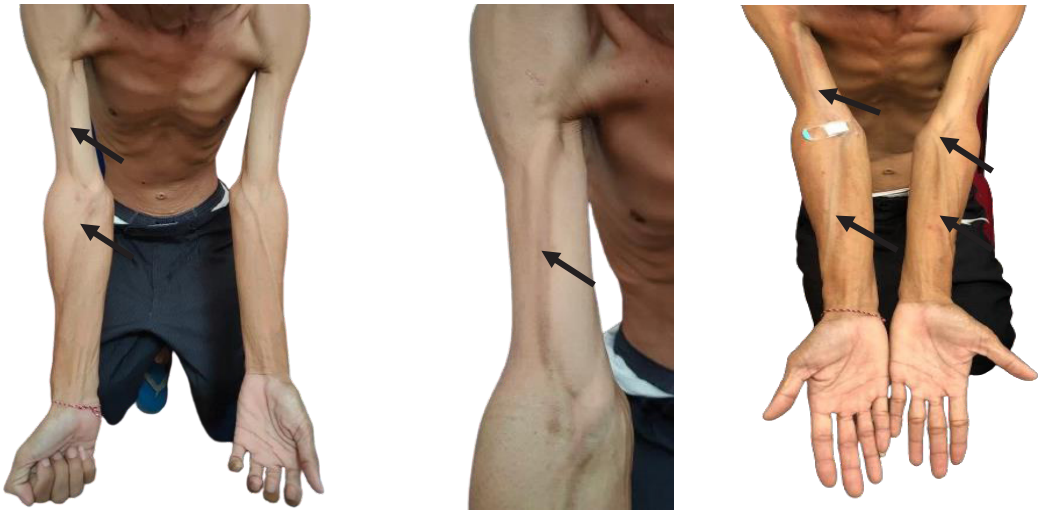
CASE

A 48 y.o. male from the Ear, Nose, and Throat (ENT) Outpatient Care was consulted to the Department of Dermatology and Venereology (DV), Sanglah General Hospital with 5 d history of linear, erythematous, and pruritic eruption on his right lower arm without systemic symptoms, 2 d after receiving chemotherapy infusion at this location. His past medical history was remarkable for a recent diagnosis of nasopharyngeal carcinoma. He had his first cycle of chemotherapy with 100 mg of docetaxel in 500 mL NaCl 0,9% infused over 6 hr followed by 400 mg of carboplatin in 500 mL NaCl 0,9% infused over 2 hr, with premedication of 10 mg of dexamethasone intravenously, 10 mg of diphenhydramine intravenously, along with 100 mL of NaCl 0.9% 20 drops per min. After the chemotherapy session was conducted, another 100 mL of NaCl 0.9% (20 drops per min) along with 8 mg of ondansetron 2 x daily, 20 mg of omeprazole 2 x daily, 500 mg of paracetamol 3 x daily, and vitamin B complex once daily were prescribed. He was known to have no drug allergies. He had no other medical conditions and took no regular medications, vitamins, or supplements. His social and family histories were non-contributory. On dermatologic examination, he had multiple erythematous macules to patch, a well-defined margin, annular to linear shaped by the vein tracks, with the size of 0.5 cm x 0.5 cm to 1 cm x 22 cm, linear in configuration, locally distributed on his right upper and lower arm (FIGURE 1A and 1B). The patient was suggested for a biopsy examination, but the patient refused. Based on the clinical pattern, a diagnosis of SSD was made. The patient was discharged home with a prescription for a 5 d course of 10 mg of cetirizine 10 mg intraorally, desoximetasone 0.25% cream applied to the affected areas 2 x daily for 7 d.



A B

FIGURE 1. Multiple erythematous macules to patch (red arrow) characterized by well-defined margin, annular to linear shaped by the vein tracks (A and B).



C D E

FIGURE 2. Multiple hyperpigmented patch (black arrow). The first visit to the DV outpatient care (C and D) after receiving 1 wk treatment from the DV outpatient care. The third visit to the DV outpatient care after receiving second and third session of chemotherapy (E).

A week later, his lesion had significantly improved when he arrived for DV outpatient therapy (FIGURE 2A and 2B). He was scheduled for a second chemotherapy session with the same regimen the next week. For the next chemotherapy session, he was suggested to receive premedication of 250 mL of NaCl 0.9% bolus intravenously before and after the administration of the chemotherapy agent and communicate with the ENT department. He tolerated

his second and third cycles with milder adverse events. The linear, erythematous-hyperpigmented, and slightly pruritic eruption appeared on his left and right arm and lower arm after receiving the second and third cycle chemotherapy infusions (FIGURE 2C). The eruption slowly disappeared after a few days.

DISCUSSION

Serpentine supravenous hyperpigmentation rarely happens to patients on anticancer therapy. There have been several reports of SSH cases globally, but the exact incidence is unknown. Some anticancers susceptible to inducing SSH include fluorouracil, taxanes, vinca alkaloids (vincristine and vinorelbine), proteasome inhibitors, methotrexate, dacarbazine, actinomycin, docetaxel, doxorubicin, daunorubicin, cyclophosphamide, cisplatin, fotemustine, nitrogen mustard, nitrosoureas, and carboplatin. The majority studies reported that most of the SSH occurred during chemotherapy sessions using multiple treatment regimens at the same time.[5-10]

The exact pathophysiology of SSH is yet to be known, thus additional studies are still needed to understand more about this phenomenon. Currently, two hypotheses try to explain this phenomenon. The first one is the direct cytotoxic effect of anti-cancer medications on endothelial cells causing increased permeability. Thus, the medications extravasate to the nearest epidermal cells. Hence, the extravasated medications will cause toxic effect on the nearest melanocytes and keratinocytes, ultimately causing hyperpigmentation throughout the blood vessels.[6] The second hypothesis is the accumulation of anti-cancer medications in the skin around the blood vessels can cause local and generalized hypersensitivity reactions followed by hyperpigmentation.[9,11]

Serpentine supravenous hyperpigmentation clinically manifests as hyperpigmented macule lesions, and/or linear- or serpentiform-shaped erythema across the cutaneous veins. In some cases, these lesions can manifest as purpuric papules with hyperpigmentation or erythema. It is distributed locally to the skin blood vessels which provide access for anti-cancer drugs. The lesion will first show in the proximal area where the intravenous medication administration is located, then it slowly extends to distal areas. In some cases, SSH can be preceded by some lesions in the other parts of the body, for example, the back of the neck and the thorax.[12] SSH lesions may or may not be preceded with erythematous lesions. The lesions usually show in 24 hours up to 15 days after exposure to the causative agent(s).[6,11,12] Regardless, one study has reported that there was a unique SSH patient who slowly began to manifest 1-6 mo after the exposure. This opens up the possibility of the mechanism of delayed reaction causing SSH.[5,11] Most of the patients complained of itch on the lesions. Systemic symptoms are never found on SSH.[8]

Most cases of SSH do not need additional diagnostics because of their pathognomonic clinical manifestation. The only additional examination which can distinguish SSH from other conditions (even without the presence of the pathognomonic sign) is histopathology examination.[7,9] On histopathology examination, the lesion typically shows basement layer degeneration, pigment incontinence, melanophages, focal band-like infiltrates, and perivascular mononuclear infiltrates.[9] Some histopathological examinations will also show acanthosis, hyperkeratosis, and necrotic keratinocytes.[12] Some differential diagnosis that can be considered include thrombophlebitis, cutis marmorata, erythema ab igne, livedo reticularis, and linear lichen planus which manifests across the

superficial cutaneous veins.[13]

Several precautions that can be taken by patients who will undergo anti-cancer therapy to help reduce the risk of SSH. Applying moisturizing cream to the arms and legs regularly can help repair the skin barrier. In addition, avoiding irritants such as alcohol and tight clothing, extreme temperatures (too cold or too hot) and friction on the skin are also recommended.[8] Several studies have also shown that exposure to sunlight can increase the risk of developing SSH. Therefore, patients should use anti-UV creams and avoid excessive sun exposure.[4,8,12] In acute cases, several studies recommend the administration of moderate potency topical steroids plus oral glucocorticoids. One study used oral prednisone at a dose of 20 mg per day (for 7 d) along with betamethasone valerate 0.1% cream (14 d) with favorable outcomes.[12] Another study used oral dexamethasone at a dose of 20 mg for 3 days.[14] Fernandes *et al.*[6] used methylprednisolone once daily for 7 d on two SSH patients with different outcomes. Several studies support the administration of corticosteroids before and after therapy to suppress the inflammatory response.[6] Hossain *et al.*[12] gave dexamethasone 2 mg orally for 3 d to patients who had just undergone a second chemotherapy after experiencing SSH on their first chemotherapy. In this patient, no further side effects were found.[12] To avoid further reactions, the intravenous infusion of the drug should be given at a slower rate. In addition, administration of the drug should be initiated and terminated with a bolus of normal saline (250 mL initially, 500 mL at the end) to remove residual toxic metabolites from the vasculature. Bolus administration should be considered in patients who can not tolerate fluid overload.[6] For longer therapy sessions (>1 hr), central venous access may be a better option than peripheral access. Cold compresses can be applied to the infusion site to cause vasoconstriction in the veins and degradation of drug metabolites. In addition, cold compresses can also be used during acute reactions to reduce pain and inflammation.[6,12] To treat pruritic complaints, antihistamines, for instance, cetirizine or loratadine can be used.[14] The interruption of chemotherapy or modification of the standard dose of the chemotherapeutic drug is not indicated.

Resolution of the lesions generally happens spontaneously, after the patient stops the exposure to anti-cancer drugs.[13] The duration of resolution varies from person to person, ranging from weeks to years. However, these post-inflammatory hyperpigmented lesions persist in a minority of patients.[6,12,14]

CONCLUSION

We reported a case of SSH found in nasopharyngeal cancer patient on docetaxel and carboplatin chemotherapy. Diagnosis of SSH is made based on the clinical findings of linear, erythematous, and pruritic eruption at the location used to receive chemotherapy infusion. The patient is given a premedication of 250 mL of NaCl 0.9% bolus intravenously before and after the chemotherapy session, 10 mg of cetirizine every 24 hr orally, and desoximetasone 0.25% cream every 12 hr topically. The patient tolerates his second and third cycles well with milder adverse events.

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Management of non-malignant hyperinsulinemic hypoglycaemia in resource limited setting: a case report

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ABSTRACT

A 37 y.o. Asian male presented with frequent hypoglycaemia in both fasting and post-prandial state. He had elevated blood insulin levels during the hypoglycaemic episodes with normal pancreatic morphology and no extra-pancreatic tumor. Through systematic symptom assessment and the use of simple laboratory examination, non-insulinoma pancreatogenous hypoglycaemia syndrome presented as the most likely diagnosis. Conclusive aetiological diagnosis could not be reached due to the limited availability of diagnostic modalities. Nevertheless, modest symptom control and frequency reduction of hypoglycaemia were achieved with empirical dietary modification and α-glucosidase inhibitor treatment.

ABSTRAK

Laki – laki ras Asia berusia 37 tahun mengalami hipoglikemia berulang yang terjadi saat puasa maupun setelah makan. Pasien mengalami peningkatan kadar insulin pada saat episode hipoglikemi dengan morfologi pankreas normal serta tanpa tumor ekstra-pankreas. Pendekatan diagnosis berdasarkan analisa sistematik gejala dan pemeriksaan laboratorium sederhana menghasilkan diagnosa kerja *non-insulinoma pancreatogenous hypoglycaemia syndrome*. Diagnosis etiologis yang konklusif tidak dapat dicapai karena keterbatasan modalitas diagnostik. Akan tetapi, perbaikan gejala dan penurunan episode hipoglikemi dapat dicapai melalui terapi empiris modifikasi diet dan obat penghambat α-glukosidase.

Keywords:
hyperinsulinemic hypoglycaemia; non-insulinoma pancreatogenous hypoglycaemia syndrome; medical management

INTRODUCTION

Adult-onset hyperinsulinemic hypoglycaemia (HH) is uncommon and mostly caused by insulinoma.[1] Non-malignant aetiologies are rare, requiring advanced and costly diagnostic modalities to confirm, which might not be accessible in resource-limited countries.[2] These barriers might result in low awareness among health practitioners, causing underdiagnosis/misdiagnosis and ultimately, inappropriate treatment. In contrast, the treatment approach for this clinical entity can be simple, effective, and affordable, even without conclusive aetiological diagnosis.[3,4] We presented a 37 y.o. Asian male with non-malignant hyperinsulinemic hypoglycaemia, well-managed with acarbose and diet modification, although no conclusive aetiological diagnosis was reached.

CASE

A 37 y.o. Asian male presented with frequent hypoglycaemia for the past 3 yr, manifesting as sudden general weakness accompanied by dizziness, sweating, tremor, and palpitation. He

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experienced these symptoms almost daily. These symptoms usually occur with a blood sugar level < 70 mg/dL and improve with starchy food, such as rice and potato consumption. Events of hypoglycaemia occurred in prolonged fasting, after consumption of liquid sugar (most frequent), and after strenuous physical activity. He also experienced significant weight gain of roughly 20 kg in 3 yr due to frequent snacking. He denied other symptoms and was not on any medication. He does not has history of gastric surgery and no history of alcohol consumption. He does not has significant familial history. His physical examination was unremarkable. His abdominal CT scan with contrast was unremarkable, with normal pancreatic dimension. His cranial CT scan with contrast and chest X-ray were also within normal limits. His initial lab results were within normal limits (TABLE 1). However, his plasma insulin level was elevated during hypoglycaemic episodes (fasting, after strenuous activity) (TABLE 2). He was clinically diagnosed with non-insulinoma pancreatogenous hypoglycaemia syndrome (NIPHS) and empirically treated with acarbose 25 mg t.i.d. He was advised to have frequent, smaller meals low in carbohydrates, which resulted in modest symptom control and less frequent hypoglycaemic episodes. He still experiences weekly symptoms, especially after a huge meal intake and strenuous physical activity. The notable treatment-related side effect in this patient was nausea.

TABLE 1. Initial laboratory results

Parameter	Value	Reference	Parameter	Value	Reference
Fasting blood glucose	77 mg/dL	70-90	Corresponding insulin level (fasting)	10.5 µIU/mL	2.6-24.9
2-hr post-prandial (2-hr PP) blood glucose	127 md/dL	< 140	Corresponding insulin level (2-hr PP)	89.3 µIU/mL	16-166
Anti-insulin autoantibody	Negative	Negative			
Anti-dsDNA	8.2 U/mL	< 25			
ANA IF	Negative				
Cortisol (morning)	8.9 µg/dL	3.7-19.4			

TABLE 2. Insulin level during hypoglycaemic event

Parameter	Value	Reference
Fasting blood glucose (8 hr fasted)	60 mg/dL	70-90
Corresponding insulin level (8 hr fasted)	149 µIU/mL	2.6-24.9

DISCUSSION

Hypoglycaemia in seemingly well patients without diabetic medication is uncommon. In such patients, it is helpful to classify hypoglycaemic manifestation as hyperinsulinemic or non-hyperinsulinemic and fasting or post-prandial.[2,5] It is imperative to evaluate insulin levels during an episode of hypoglycaemia to avoid misclassification. In the case of our patient, his insulin levels were normal during normoglycemia but were markedly elevated when evaluated during a hypoglycaemic state. We recorded a hyperinsulinemic state during fasting hypoglycaemia but not during post-prandial evaluation. However, this patient reported frequent hypoglycaemic episodes both during prolonged fasting (> 8 hr) and post-prandial state at home.

The most common aetiology for adult-onset HH is an insulin-secreting tumor, including insulinoma and extra-pancreatic neoplasia.[6] Both were excluded in our patient due to normal cranial, thoracal, and abdominal radiological findings. Non-malignant aetiologies of HH are NIPHS and insulin autoimmune syndrome (IAS)/ Hirata's disease. Generally considered a rare disease elsewhere, the incidence of IAS is significantly higher in the Asian population.[2] Hypoglycaemic manifestation of IAS occurs in post-prandial and fasting states and can not be clinically distinguished from other aetiologies of HH.[7] Confirmatory diagnosis of IAS requires the detection of IgG insulin autoantibody, which was negative in our patient. The exclusion of IAS left us with NIPHS as the most likely diagnosis.

Hyperinsulinemia in NIPHS is caused by hypertrophy and sometimes hyperplasia of pancreatic islet cells, which doesn't alter the macroscopic dimension of the pancreas.[8] The etiology of NIPHS is currently unknown and still under investigation. Hypoglycaemic manifestation of NIPHS mainly occurs post-prandially and occasionally in a fasting state.[9] Confirmatory diagnosis requires a selective arterial calcium stimulation test (SACT) or histopathologic examination of pancreatic tissue.[10] A conclusive diagnosis could not be reached since biopsy was not performed on our patient, and SACT wasn't available in our hospital. NIPHS is challenging to diagnose due to its rarity, non-specific symptoms, and radiological findings.[11] Suggestive patient history, which might lead to suspicion of NIPHS, is usually a history of gastric bypass surgery, which was not present in our patient.[12-14] SACT is the primary diagnostic test, usually performed after other exhaustive radiological examinations have been performed with no significant pathological finding.[15-17] Unfortunately, SACT is an advanced examination requiring skilled interventional radiologists, uncommon in limited-resource hospitals. Nevertheless, effective and safe treatment to prevent episodes of hypoglycaemia was essential.

The general treatment approach for HH is to prevent sudden spikes in blood glucose levels. Patients should be advised to have frequent, smaller meals consisting of low carbohydrates to avoid fasting, the sudden elevation of blood glucose, and, subsequently, insulin level.[18] Slowly digestible carbohydrates such as corn starch can be good carbohydrate sources for these patients, as it is absorbed slowly in the digestive tract, resulting in a steadier blood glucose level. Acarbose (25-100 mg t.i.d, with each main meal), an α-glucosidase inhibitor, can be prescribed to delay carbohydrate absorption, which helps stabilizes post-prandial blood glucose level.[3,19] These approaches can be safely implemented both in IAS and NIPHS.[3,4] Other pharmacological therapy for HH includes diazoxide, a somatostatin analog, and Ca-channel blockers, such

as verapamil, amlodipine, nifedipine, and diltiazem. Diazoxide is the primary pharmacological treatment for HH as it directly inhibits insulin secretion by β-cells which unfortunately wasn't available in our region.[20] Somatostatin analog, such as octreotide, is a less compelling option than diazoxide and is usually reserved in cases unresponsive to diazoxide. It requires parenteral administration and should be used in higher doses to achieve the desirable effect.[21] Calcium-channel blockers may impede calcium-channel at β-cells, inhibiting insulin secretion.[22] Albeit not curative, these approaches can effectively reduce the incidence of hypoglycaemia due to endogenous hyperinsulinemia and should be considered when a conclusive aetiological diagnosis can not be reached.

CONCLUSION

The most common cause of non-malignant hyperinsulinemic hypoglycaemia includes NIPHS and IAS, which are difficult to differentiate in a resource-limited setting conclusively. Frequent, smaller, and low carbohydrate meals and consumption of acarbose with each main meal present safe, effective, and affordable management in such patients, even without a conclusive aetiological diagnosis.

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