

# Secondary Prevention Post Stroke in Primary Care in an Asian Setting: A Retrospective Cohort Study

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## Research Article

**Keywords:** Stroke, Primary care, neurology, Secondary Prevention

**Posted Date:** December 2nd, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-111876/v1>

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**Version of Record:** A version of this preprint was published on March 25th, 2021. See the published version at <https://doi.org/10.1186/s12875-021-01412-0>.

# Abstract

**Background:** Stroke is one of the top contributors to burden of disability-adjusted life-years worldwide. Family physicians have key role in optimal secondary prevention post-stroke helping patients make appropriate lifestyle changes and take medication in accordance with the recommended clinical practice guidelines.

**Methods:** Our aim was to characterize the profile of patients who were being managed in primary care setting for secondary prevention of stroke. Our specific objectives were: (i) to examine the level of overall and individual risk factors control after an index-stroke event in patients visiting the primary care setting and (ii) to describe the factors associated with the achievement of overall risk factors control in post-stroke patients.

**Study Design:** Retrospective cohort study.

We conducted a study looking retrospectively at records from our electronic chronic disease database. Our study included post-stroke patients who visited public primary care setting in Singapore between 1st January 2012 to 31st December 2016. Based on AHA Stroke guidelines, we operationalized our outcome of secondary prevention post-stroke as overall control, which comprised of patients having blood pressure, lipids, and glucose reading (for those who had diabetes) all well-controlled. We conducted descriptive analysis and performed multivariable logistic analysis to determine factors associated with overall control.

**Results:** There were 24,240 patients in our study. Overall control was better in post-stroke patients without diabetes (49.2%) as compared to those with diabetes (28.1%).

Among post-stroke patients without diabetes, factors significantly associated with overall control were sex [OR: 1.23, 95% CI: 1.10, 1.39], ethnicity [OR: 0.72, 95% CI: 0.58, 0.90], BMI [OR: 0.72, 95% CI: 0.62, 0.84] and atrial fibrillation [OR: 1.47, 95% CI: 1.21, 1.78].

Among post-stroke patients with diabetes, factors significantly associated with overall control were sex [OR: 1.28, 95% CI: 1.12, 1.46], ethnicity, BMI, atrial fibrillation [OR: 1.24; 95% CI: 1.02, 1.51], chronic kidney disease and smoking status.

**Conclusion:** We reported a sub-optimal level of overall control. Among post-stroke patients, those with diabetes had a higher proportion of sub-optimal control as compared to those without diabetes. Irrespective of diabetic status, being female, having high BMI, and of Malay ethnicity were associated with poor overall control compared to Chinese.

## Background

Stroke is one of the top contributors to the burden of disability-adjusted life-years (DALYs) worldwide with close to 100 million DALYs attributed to it.[1] It is the third largest contributor to the burden of DALYs

within Singapore, along with other cardiovascular ailments.[2, 3] After a stroke event, patients have an increased risk of recurrence with poorer health outcomes. The prevalence of recurrent stroke varies from 5.4% to 18.1% [4-7] and one review summarizing the prevalence of recurrent stroke across Asian settings reported stroke recurrence to be up to 25.4%.[8] Recurrence is associated with adverse outcomes like increased all-cause mortality and increased risk of disability, making it important to prevent such an event from occurring.[7, 9] As more patients are surviving the acute event, the need for optimal secondary prevention measures becomes greater. Family physicians (FP) have an important role in engaging the patients after stroke and helping implement measures in accordance with the recommended clinical practice guidelines.[10]

While some studies have tried to quantify adherence to secondary prevention guidelines in the general population [11-14], not many have focused on examining the achievement of targets for secondary prevention post-stroke. Bohn et al. studied the adherence to guidelines for secondary prevention of stroke or myocardial infarction in post-stroke patients with type 2 diabetes in Germany and Austria.[15] They reported the proportion of post-stroke patients with diabetes achieving the targets for secondary prevention being 61.1%, 42.2% and 89.9% for HbA1c, LDL and blood pressure respectively. However, they did not report the proportion of individuals achieving overall control across all three parameters or risk factors, and it is this assessment of overall control which gives a more meaningful picture of secondary prevention. Another recent study from Wales reported the level of HbA1c in patients with diabetes before and one year after a stroke event, with improvement in glycemic control post-stroke.[16] A US-based study reported trends in vascular risk factor control and treatment in post-stroke patients over the years. They reported point prevalence of control of risk factors post-stroke being 68.6%, 64.4% and 87% for blood pressure, HbA1c and cholesterol levels in men respectively.[10] Similarly, a Spanish study reported suboptimal control of risk factors in post-stroke patients, and this control was lower when compared with patients with coronary artery disease (CAD).[17] None of these studies were done in the Asia Pacific region, and their results are not generalizable to the Asian context due to differences in patient characteristics and healthcare systems. This study was designed to address this gap.

Our study aimed to highlight the areas where secondary prevention of stroke could be improved for patients who were on follow-up in the primary care setting following a stroke. Our specific objectives were: (i) to examine the level of overall and individual risk factors control after index-stroke in patients visiting primary care setting and (ii) to describe the factors associated with the achievement of overall control in post-stroke patients.

## **Methods**

### ***Participants and setting***

This was a retrospective cohort study reviewing data from the institutional electronic healthcare record database. Our study included post-stroke patients visiting public primary care setting in Singapore, also known as polyclinics, for follow-up with a family physician. Participants were aged 21 years and above

with a past diagnosis of stroke (ICD-9 Classification of Diseases, Ninth Revision) and who received care in any of the National Healthcare Group Polyclinics (NHGP) between 1<sup>st</sup> January 2012 to 31<sup>st</sup> December 2016.

### ***Outcome Variables***

For participants meeting the above eligibility criteria, we retrospectively extracted information on both exposure and outcome variables over the stated observation period. The outcome variables were extracted at the end of one year of follow-up from the baseline visit for each participant within the observation period.

Our outcome variables of secondary prevention at one-year post stroke were based on the American Heart Association (AHA) Stroke 2014 guidelines. [18]. We operationalized overall control as patients having a systolic blood pressure <140 mmHg, and a diastolic blood pressure < 90 mmHg, LDL-cholesterol < 2.6 mmol/L and, if the patients also had diabetes mellitus, a haemoglobin A1c (HbA1c) < 7.0%. For all patients meeting the study eligibility criteria, we extracted the systolic and diastolic blood pressure, LDL cholesterol level and HbA1c as continuous variables.

Based on the above cut-offs, these parameters were converted into binary categorical variables of 'yes' or 'no'. Similarly, the recommended cut-offs for the parameters were converted to binary variables indicating whether secondary prevention targets were achieved or not.

### ***Exposure Variables***

For all patients meeting the study eligibility criteria, we extracted socio-demographic and clinical variables. Sociodemographic data included age (continuous variable in years), sex (categorical variable with two categories of 'male' and 'female'), ethnicity (four categories of 'Chinese', 'Malay', 'Indian' and 'Others'). Clinical data included body mass index (BMI) (continuous variable converted to categorical variable based on the Asian cut-off with three categories of 'low', 'moderate' and 'high' risk) and smoking status (categorical variable with two categories of 'yes' or 'no'). In addition, we also captured the presence of the following comorbid clinical conditions (as binary categorical variables of 'yes' or 'no'): hyperlipidemia, hypertension, diabetes, chronic kidney disease (CKD), ischemic heart disease (IHD), and atrial fibrillation (AF). Clinical relevance and information availability in the institutional electronic healthcare record database guided the selection of above mentioned cardiometabolic comorbid conditions.

### ***Analysis***

We used de-identified data to conduct our analysis. We used descriptive analysis to summarize our categorical variables using proportions and frequencies. Complete case analysis was used to study the implementation of treatment goals for secondary prevention in patients with a prior history of stroke. To describe the factors associated with the achievement of overall control in post-stroke patients, we

performed multivariable logistic regression analysis since our outcome variable was binary (overall control achieved versus not), and we controlled for the following pre-decided medically relevant variables: age, sex, ethnicity, BMI, smoking status, presence of IHD, presence of AF and presence of CKD. All the analysis was performed using STATA version 14.2, and the statistical significance level was set at 5%.

*Sample size:* As per our original intention, we selected all available patients from Institutional electronic healthcare record database between the period of time from 1<sup>st</sup> January 2012 to 31<sup>st</sup> December 2016, with a past diagnosis of stroke (ICD-9 Classification of Diseases, Ninth Revision) and who received care in any of the National Healthcare Group Polyclinics (NHGP). The sample extracted was 24,240 patients. Choosing the level of confidence of 95%, 0.05 precision level, expected prevalence or proportion of stroke patients meeting overall control target (as per clinical practice guidelines) being set at 50%, our sample size meets the minimum requirement.[19, 20] This calculation estimated the minimum sample size required to observe a significant effect.

## Results

We have summarized the basic socio-demographic information of all the included participants in **Table 1**. With regards to age, 58.9% of the participants were 65 years old and above while 38.7% were between age of 45 to 64 years old. With regards to sex, 57.7% of the participants were male and 42.3 % were female. With regards to race, majority were Chinese (80.1%), followed by Malay (10.8%), Indian (6.5%) and others (2.6%). About 41.4% had a body mass index (BMI) between 23.0 to 27.4 kg/m<sup>2</sup>. Hyperlipidemia was the most common chronic condition (98.1%) followed by hypertension (92.9%), diabetes (54.9%), CKD (chronic kidney disease) (54.1%), IHD (Ischemic heart disease) (20.1%) and AF (Atrial fibrillation) (12.3%).

**Table 2** shows the proportion of patients with optimally controlled hypertension, diabetes, and hyperlipidemia as 71.4%, 52.9%, and 66.6% respectively. Among patients who had complete data, overall control was better in stroke patients without diabetes (49.2%) as compared to stroke patients with diabetes (28.1%).

**Table 3** shows the multivariable regression analysis results for post-stroke patients without diabetes, demonstrating the association of socio-demographic and clinical variables with overall control. Among those stroke patients without diabetes, sex, ethnicity, BMI and AF were significantly associated with overall control. With regards to sex, being male increased the odds of overall control by 1.23 (CI 1.10, 1.38) times as compared to females. With regards to ethnicity, Malay had 0.72 (CI: 0.58, 0.90) times the odds of achieving overall control as compared to Chinese ethnicity. Compared to the low-risk category of BMI, those in the high-risk category had 0.72 (CI: 0.62, 0.84) times the odds of overall control. Having AF increased the odds of overall control by 1.46 (CI: 1.21, 1.78) times when compared to those without AF.

**Table 4** shows the multivariable regression analysis results for post-stroke patients with diabetes, demonstrating the association of socio-demographic and clinical variables with overall control. Among

those post-stroke patients with diabetes, sex, ethnicity, BMI, AF, chronic kidney disease and smoking status were significant covariates. With regards to sex, being male increased the odds of overall control by 1.28 (CI: 1.12, 1.46) times as compared to females. With regards to ethnicity, Malay and Indian had 0.81 (CI: 0.65, 0.99) and 0.70 (CI: 0.55, 0.88) times the odds of having overall control as compared to Chinese ethnicity respectively. Compared to the low-risk category of BMI, those in the moderate and high-risk category had 0.84 (CI: 0.72, 0.98) and 0.71 (CI: 0.59, 0.84) times the odds of overall control. Having AF increased the odds of overall control by 1.24 (CI: 1.02, 1.52) times when compared to those without AF. Having CKD decreased the odds of overall control by 0.63 (CI: 0.54, 0.72) times when compared to those without CKD. Being a smoker decreased the odds of control by 0.68 (CI: 0.54, 0.88) times as compared to a non-smoker.

**Table 1.** Baseline characteristics of participants (N=24,240)

Variable		N <sup>a</sup> (%)
Age (in years)	Less than 45	572 (2.4%)
	45 to 64	9,393 (38.7%)
	65 and above	14,275 (58.9%)
Sex	Female	10,258 (42.3%)
	Male	13,982 (57.7%)
Race	Chinese	19,438 (80.1%)
	Malay	2,613 (10.8%)
	Indian	1,570 (6.5%)
	Others	619 (2.6%)
Body Mass Index (kg/m <sup>2</sup> )	Less than 23	4,476 (31.0%)
	23 to 27.4	5,968 (41.4%)
	More than 27.4	3,983 (27.6%)
Smoking status	Yes	1,624 (6.7%)
Comorbid conditions(yes)	Hyperlipidemia	23,779 (98.1%)
	Hypertension	22,525 (92.9%)
	Diabetes	13,307 (54.9%)
	Chronic kidney disease*	13,104 (54.1%)
	Ischemic heart disease	4,877 (20.1%)
	Atrial fibrillation	2,990 (12.3%)
<p>*: those patients with documented eGFR (estimated glomerular filtration rate) value less than 60 ml/min/1.73m<sup>2</sup></p> <p>Data from patients seen at polyclinics from 1<sup>st</sup> Jan 2012 to 31st Dec 2016 with a diagnosis of stroke coded. Total sample size (N=24,240)</p> <p><sup>a</sup>: All numbers may not add up to total because of missing data.</p>		

**Table 2.** Risk factor control profile within the first post-stroke year.

Risk Factor (individual or composite)	Numerator	Denominator	Proportion (%)
Overall Control (Non-Diabetic) <sup>b</sup>	3,195	6,501	49.2%
Overall Control (Diabetic) <sup>c</sup>	1,964	6,999	28.1%
Blood Pressure Control <sup>d</sup>	13,934	19,529	71.4%
Lipids (LDL) Control <sup>e</sup>	9,804	14,714	66.6%
Glycaemic Control <sup>f</sup>	4,782	9,046	52.9%
<p>Based on the American Heart Association/ American Stroke Association (AHA/ASA) Stroke 2014 guidelines[18], we operationalized our outcome variable of secondary prevention post-stroke as overall control which comprised of patients having a systolic blood pressure &lt;140 mmHg and a diastolic blood pressure &lt; 90 mmHg, LDL-cholesterol &lt; 2.6 mmol/L, and haemoglobin A1c (HbA1c) &lt; 7% (for diabetes mellitus) for those post-stroke patients with diabetes.</p> <p><sup>b</sup>: For the post-stroke patients without diabetes, only 6,501 had readings available for both blood pressure and LDL.</p> <p><sup>c</sup>: For the post-stroke patients with diabetes, only 6,999 patients had readings available for all three (HbA1c, blood pressure and LDL).</p> <p><sup>d</sup>: Out of the 22,525 patients with hypertension, only 19,529 had blood pressure readings available.</p> <p><sup>e</sup>: Out of the 23,779 patients with hyperlipidaemia, only 14,714 had LDL readings available.</p> <p><sup>f</sup>: Out of the 13,307 patients with diabetes, only 9,046 had HbA1c readings available. Glycaemic control is determined by the level of HbA1c.</p>			

**Table 3.** Results for post-stroke patients without diabetes, association of socio-demographic and clinical variables with overall control.

Overall Control	OR (95% CI)	P-value	aOR (95% CI)	P-value
<b>Age (in years)</b>				
Less than 45	Ref		Ref	
45 to 64	0.80 (0.59, 1.09)	0.162	0.88 (0.63, 1.24)	0.759
65 and above	0.87 (0.64, 1.18)		0.88 (0.62, 1.25)	
<b>Sex</b>				
Female	Ref		Ref	
Male	1.20 (1.09, 1.32)	<0.001	1.23 (1.10, 1.38)	<b>0.001</b>
<b>Race</b>				
Chinese	Ref		Ref	
Malay	0.74 (0.61, 0.89)		0.72 (0.58, 0.90)	
Indian	0.89 (0.68, 1.15)	0.012	0.93 (0.69, 1.25)	<b>0.040</b>
Others	0.93 (0.67, 1.29)		0.95 (0.66, 1.38)	
<b>Body Mass Index (kg/m<sup>2</sup>)</b>				
Less than 23	Ref		Ref	
23 to 27.4	0.92 (0.81, 1.04)	<0.001	0.92 (0.81, 1.04)	<b>&lt;0.001</b>
More than 27.4	0.70 (0.60, 0.81)		0.72 (0.62, 0.84)	
<b>Smoking status</b>				
No	Ref		Ref	
Yes	0.91 (0.76, 1.09)	0.287	0.91 (0.75, 1.11)	0.365
<b>Ischemic heart disease</b>				
No	Ref		Ref	
Yes	1.04 (0.90, 1.19)	0.621	0.92 (0.78, 1.08)	0.290
<b>Atrial Fibrillation</b>				
No	Ref		Ref	
Yes	1.53 (1.30, 1.81)	<0.001	1.46 (1.21, 1.78)	<b>&lt;0.001</b>
<b>Chronic Kidney Disease*</b>				
No	Ref		Ref	
Yes	0.96 (0.87, 1.06)	0.451	0.91 (0.81, 1.03)	0.131

Abbreviations: OR: odds ratio; aOR: adjusted odds ratio; CI: confidence interval; Ref: reference value

**Table 4.** Results for post-stroke patients with diabetes, association of socio-demographic and clinical variables with overall control.

Overall Control	OR (95% CI)	P-value	aOR (95% CI)	P-value
<b>Age (in years)</b>				
Less than 45	Ref		Ref	
45 to 64	0.84 (0.55, 1.28)	0.001	0.75 (0.46, 1.22)	0.068
65 and above	1.02 (0.67, 1.56)		0.87 (0.53, 1.42)	
<b>Sex</b>				
Female	Ref		Ref	
Male	1.24 (1.11, 1.37)	<0.001	1.28 (1.12, 1.46)	<0.001
<b>Race</b>				
Chinese	Ref		Ref	
Malay	0.70 (0.59, 0.83)		0.81 (0.65, 0.99)	
Indian	0.67 (0.54, 0.82)	<0.001	0.70 (0.55, 0.88)	0.009
Others	0.81 (0.56, 1.17)		0.87 (0.56, 1.35)	
<b>Body Mass Index (Kg/m<sup>2</sup>)</b>				
Less than 23	Ref		Ref	
23 to 27.4	0.83 (0.71, 0.96)	<0.001	0.84 (0.72, 0.98)	<0.001
More than 27.4	0.65 (0.55, 0.77)		0.71 (0.59, 0.84)	
<b>Smoking status</b>				
No	Ref		Ref	
Yes	0.73 (0.59, 0.91)	0.005	0.68 (0.54, 0.88)	0.003
<b>Ischemic heart disease</b>				
No	Ref		Ref	
Yes	0.93 (0.83, 1.06)	0.282	0.90 (0.77, 1.05)	0.177
<b>Atrial Fibrillation</b>				
No	Ref		Ref	
Yes	1.20 (1.02, 1.41)	0.024	1.24 (1.02, 1.52)	0.028
<b>Chronic Kidney Disease</b>				
No	Ref		Ref	
Yes	0.69 (0.61, 0.77)	<0.001	0.63 (0.54, 0.72)	<0.001

## Discussion

In this study, we estimated the proportion of post-stroke patients whose secondary prevention was optimal over one year post-stroke and described the characteristics of these post-stroke patients. Optimal secondary prevention was recorded in 28.1% of post-stroke patients with diabetes and 49.2% of those without diabetes. In post-stroke patients with diabetes, we found that sex, ethnicity, BMI, smoking, AF, and CKD were significantly associated with the achievement of overall control. While in post-stroke patients without diabetes, we found sex, ethnicity, BMI and AF were significantly associated with the achievement of overall control. Irrespective of the diabetes status, being female, having high BMI, and of Malay ethnicity were associated with poor overall control.

### Comparisons with other studies

Published studies mainly explored the control of individual risk factors rather than the overall control achieved. Comparing our results with single risk factor studies, we found examples of some study populations doing better and others worse. For example, comparing the proportion of post-stroke patients having an optimal level of blood pressure, two studies reported a higher level of control at 89.9% [15], and 86% [21], as compared to our estimate of 71.4%. Those studies reporting a lower level of blood pressure control as compared to our estimate reported values ranging from 23.8% to 62.4%. [10, 17, 22-24] Comparing the level of lipid control across different studies, all the reviewed studies reported a lower level of lipid control as compared to our estimate of 66.6%, with reported estimates ranging from 13.9% to 49.0%. [15, 17, 21, 23, 24]. We found 52.9% of post-stroke patients with diabetes achieving the target level of glycaemic control. Compared to existing literature, two studies reported a higher proportion of glycaemic control [10,15], and another two reported a lower proportion of glycaemic control. [17, 21]

Our study filled an important gap in current literature as a limited number of current studies address the level of secondary prevention attained. Among the studies reviewed, only two attempted to report a composite or combined estimate of control of risk factors achieved, with one reporting 3.3% of ischemic stroke patients achieving control of all risk factors.[17] Another study reported the proportion of post-stroke patients achieving control of both blood pressure and lipids to be about 19.4%.[24] While our study reported more encouraging levels of secondary prevention achieved, as compared to the above two studies, there remains considerable room for improvement. The reasons for not achieving the optimal level of control of risk factors could be multiple. These could be at the patient level, the physician level, the health system level, or a combination of these. A study reported that in spite of 90% of post-stroke patients being on specific drug regimens, only about one-fourth of them achieved the recommended risk factor control. [17] This highlights the complexity of addressing secondary control post-stroke. For example, the patient's adherence to medication, compliance to lifestyle factors or healthcare system factors such as difficulty in accessing services or financial barriers, can all play a part. Another study based in Canada reported poor control of risk factors after either a coronary artery disease (CAD) or

cerebrovascular disease (CVD).[24] Those with CVD had worse control of risk factors as compared to those with CAD, with the former group having 46.0%, 40.5% and 19.4% of post-CVD patients meeting target levels of blood pressure, LDL and both respectively. In spite of good adherence to secondary prevention guidelines (medication rates ranging from 76.5% to 91.3%), it did not translate to the achievement of risk factor control suggesting the importance of other elements like patient factors.

We reported lower overall control in post-stroke patients with diabetes as compared to post-stroke patients without diabetes. There are literature that support poorer control of other risk factors in post-stroke patients who have diabetes. Patients with diabetes were associated with lower odds (OR=0.16; 95% CI: 0.14, 0.19) of achieving the target blood pressure level compared to patients with a previous cerebrovascular event.[24] Another study reported lower levels of control of lipids in post-stroke patients with diabetes.[15] Another possible explanation could be the difficulty managing co-occurrence of multiple chronic conditions experienced by both healthcare providers and patients.

Our finding was in agreement with other studies that showed, a significant association between sex and achievement of target levels of risk factors.[15, 24] One such study reported the largest difference across males and females in the achievement of the target level of serum LDL levels, with 46.1% of men and 38.3% of women achieving the target.[15] It is important to further study these sub-groups of post-stroke patients to intervene in an evidence-based manner and promote optimal secondary prevention since diabetes itself is an independent predictor of recurrent stroke with about 9.1% of stroke cases being attributable to it.[25-27]

### ***Strengths and Limitations of this study***

Our study has several strengths including the large sample size from 10 polyclinics over a period of five years. We captured major risk factors associated with recurrent stroke with a large database. Compared to observational study design which includes self-reported data, our study has the advantage of including a relatively objective source of data from electronic health records. This was one of the few studies to provide estimates of the overall control of risk factors post-stroke in an Asian setting, and we have added new knowledge to the existing literature on the prevalence of control of individual risk factors.

The study also has several limitations. The database could not provide the causation of stroke (ischemic versus haemorrhagic) experienced by each patient, which may influence treatment recommendations by clinicians. Moreover, the database did not have information on other relevant variables such as the functional status of the stroke patients, education level, employment status and available psychosocial support. Another limitation was related to missing data, for which we opted to conduct complete case analysis. Another shortcoming was that we could only assess the proportion of patients meeting or not meeting the treatment goals but could not elicit the reasons why. Qualitative research exploring the experiences of post-stroke patients and their caregivers engaging in secondary prevention related behaviours will be needed.

## Conclusion

Optimizing secondary prevention post-stroke should be made a priority given the burden of stroke to our healthcare system. Among post-stroke patients, those with diabetes had a higher proportion of sub-optimal control as compared to those without diabetes. Irrespective of the diabetes status, being female, having high BMI, and of Malay ethnicity were associated with poor overall control and interventions targeting these sub-groups might be helpful. Highlighted gaps represent a significant missed opportunity for the prevention of adverse events in individuals at high risk for a recurrent stroke. Future research efforts should focus on exploring the association of overall control with outcomes such as stroke recurrence or other cardiovascular events.

## Declarations

### Ethics approval and consent to participate

The study was approved by the National Healthcare Group Domain Specific Review Board (NHG DSRB Ref: 2017/01165).

The study was a retrospective record review after extracting de-identified data from the Chronic Disease Management System, so there were no identifiers collected that would enable the investigators to identify the participant, hence informed consent was not required by ethics committee (National Healthcare Group Domain Specific Review Board).

All methods were performed in accordance with the relevant guidelines and regulations (Declaration of Helsinki).

### Consent for publication

Not applicable

### Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

### Competing interests

The authors declare that they have no competing interests

**Funding:** This study was funded by the following grant awarded to Dr Vivek Bansal: NHG-LKC Clinician Scientist Preparatory Program (CSPP). GRANT / AWARD NUMBER: CSPP – 18004. The funders had no role except for providing research training program for the awardee. The funders were not involved with the data collection, analysis and writing of this manuscript.

This research was supported by the Singapore Ministry of Health's National Medical Research Council under the Centre Grant Programme (ref no. CGAug16C019).

### **Authors' contributions**

VB was involved in conceptualization and design of the study, analysis and interpretation of data, original draft preparation and incorporating revisions in manuscript based on critical inputs from other co-authors.

LES was involved in conceptualization and design of the study, interpretation of data, and providing critical inputs to revision of Manuscript.

HS was involved in conceptualization and design of the study, interpretation of data, drafting of the manuscript and providing critical inputs to revision of manuscript.

All authors read, reviewed and approved the manuscript.

### **Acknowledgements**

We thank Dr Tang Wern Ee, Dr Shilpa Tyagi, Ms Teo Sok Huang and Mr Lim Hai Thian for their assistance and support for this project.

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