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“The man who has no imagination has no wings.”

Muhammad Ali (1942 –)
American athlete

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Angiotensin 2 Type 1 Receptor Blockade with Neprilysin Inhibition for Chronic Heart Failure: A New Paradigm?

Arthur Mark Richards, ¹MD, PhD, FRACP

A new treatment for heart failure (HF) combining angiotensin 2 type 1 receptor blockade with inhibition of the widespread membrane-bound enzyme, neprilysin (NEP), seems likely to take treatment of chronic heart failure (CHF) a major step forward. Neurohormonal pathways are central to the evolution and progression of HF irrespective of the exact initial triggering cardiac injury or overload, and our current evidence-based treatments for this condition depend upon antagonism of the renin-angiotensin-aldosterone (RAAS) and sympathetic nervous (SNS) systems through prescription of angiotensin converting enzyme inhibitors (ACEIs), angiotensin 2 type 1 receptor blockers (ARBs), mineralocorticoid antagonists (MRAs) and blockers of beta-adrenoceptors (beta blockers). Properly deployed, these therapies reduce 1-year mortality to less than half than that suffered by patients with HF in the pre-1980s era.^{1,2} However, despite this clear impact upon the outcome of this deadly syndrome, 5-year mortality in CHF remains greater than 50%. This poor outlook plus the continuing high prevalence of HF mandates an ongoing search for more effective treatments.³

The therapeutic advances outlined above were established by the turn of the 21st century and until now, trials of new neurohormonal interventions over the last 15 years have been largely disappointing. Examples include therapeutic trials, all based on impeccable rationales and supported by encouraging preclinical data and positive phase 1 and 2 clinical data of endothelin 1 antagonists, arginine vasopressin blockers and direct renin inhibitors.⁴⁻⁶ When subjected to the test of full phase 3 randomised controlled clinical trials, none of these approaches has proven to reduce cardiovascular or all-cause mortality in HF, although some secondary end-points such as readmission with recurrent, acute decompensated HF may show a beneficial trend and useful niche applications have developed such as the use of endothelin antagonists in pulmonary hypertension and of AVP blockers in accelerating correction of hyponatraemia complicating HF. In the 1990s, an effort was made to add enhancement of beneficial endogenous adaptive responses

to HF (exemplified by activation of the natriuretic peptide system) together with the established efficacy of blockade of the “culprit” RAAS by combining ACEI with neutral endopeptidase (NEP, EC 3.4.24.11) inhibition. This enzyme plays a role in the degradation of the natriuretic peptides (NPs) which have an array of biological actions beneficial in HF including natriuresis, diuresis, vasodilation, suppression of the RAAS and SNS and antihypertrophic and antifibrotic effects. Despite some promising findings such as reduction in rates of readmission for HF, mortality was not lowered by the “ACEI-NEPI” approach and the combination caused an unacceptable rate of angioedema leading to cessation of clinical trials of these agents.⁷

However, the combination of NEP inhibition with angiotensin receptor blockade appears to offer a true beneficial advance without an increased burden of side effects. LCZ 696 consists of the NEP inhibitor sacubitril and the ARB valsartan. After initial success in surrogate end-point trials in hypertension and HF with preserved ejection fraction, this approach has now passed its sternest test to date in a phase 3 trial (“PARADIGM-HF”) in CHF with reduced ejection fraction.⁸ In centres in the United States, United Kingdom, Canada and Sweden, 8442 patients were randomised to receive either LCZ 200 mg twice daily or enalapril 10 mg twice a day in addition to other guideline-based therapy. Patients were selected for relatively high risk of recurrent acute HF and mortality by inclusion criteria comprising New York Heart Association (NYHA) class II to IV HF, a left ventricular ejection fraction less than 40% (later shifted to 35%) and plasma B type cardiac peptide levels above set thresholds (BNP or NT-proBNP at least 150 or 600 pg/mL respectively or 100 and 400 pg/mL respectively if admission to hospital for HF had been required within 12 months prior to randomisation). Exclusion criteria included symptomatic hypotension, systolic blood pressure below 100 mmHg at screening or 95 mmHg at randomisation, eGFR below 30 mL/min per 1.73m², elevated serum potassium and a history of angioedema or unacceptable side effects on either ACEI or ARBs.

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The trial was halted early, after median follow-up of 27 months, because of emerging overwhelming benefit from LCZ696 therapy. The primary end-point, death or rehospitalisation with HF occurred in 914 (21.8%) of patients receiving LCZ696 versus 1117 (26.5%) in those given enalapril (hazard ratio 0.80 with 95% CI, 0.73 to 0.87; $P < 0.001$). All-cause mortality considered as a stand-alone end-point was also reduced (hazard ratio 0.86 [0.76 to 0.93] $P < 0.001$) as was death from cardiovascular causes (HR 0.80 [0.71 to 0.89], $P < 0.001$). LCZ also reduced rate of readmission for HF by 21% and improved HF symptoms ($P < 0.001$). With respect to safety, treatment with LCZ696 was associated with more symptomatic hypotension with systolic pressures below 90 mmHG (2.7 versus 1.4%, $P < 0.001$) but, importantly, less renal impairment (serum creatinine elevated to 2.5 mg/dL or more, 3.3 versus 4.5%; $P = 0.007$) and less hyperkalemia (serum potassium over 6.0 mmol/L, 4.3 versus 5.6%; $P = 0.007$).

When compared with historical placebo rates of clinical events recorded in a previous landmark placebo-controlled trial of enalapril, in PARADIGM-HF the relative risk reduction for the composite end-point of death or readmission with HF for LCZ696 compared with placebo was 43% (95% CI, 34% to 50%; $P < 0.0001$), for cardiovascular death 34% (21% to 44%; $P < 0.0001$), HF hospitalisation 49% (39% to 58%; $P < 0.0001$) and for all-cause mortality 28% (95% CI, 15% to 39%; $P < 0.0001$). Analyses based on previous placebo-controlled trials of candesartan gave putative risk reductions relative to placebo of 39% (95% CI, 27% to 48%; $P < 0.0001$) for the composite outcome of cardiovascular death or HF hospitalisation, 32% (95% CI, 16% to 45%; $P < 0.0001$) for cardiovascular death, 46% (33% to 56%; $P < 0.0001$) for HF hospitalisation, and 26% (95% CI, 11% to 39%; $P < 0.0001$) for all-cause mortality.⁹

This is the first time a new pharmaceutical approach has demonstrated such clear cut, across-the-board benefit to HF in the last 15 years. The power of this trial supports Food and Drug Administration (FDA) approval for its use in CHF. Some caveats apply. Duration of experience and exposure to this drug class (now dubbed “ARNIs”) to date is relatively brief. Whether or not adverse effects emerge over time remains to be seen. NEP catalyses metabolism of a wide range of substrates in addition to the natriuretic peptides including, but not limited to, enkephalins, tachykinins, endothelin 1, angiotensin 2, bradykinin, and amyloid beta.^{10,11} The central role of the latter in cerebral amyloid accumulation and dementing processes has been highlighted recently and the effects of ARNIs upon cognitive function will be subject to close ongoing scrutiny.¹² However, given

the clear net benefit for multiple key outcomes over a 2-year period in PARADIGM HF, it seems highly likely that in the near future this new treatment will become recommended as the first-line therapy in CHF.

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Electrocardiographic Criteria for Left Ventricular Hypertrophy in Asians Differs from Criteria Derived from Western Populations—Community-based Data from an Asian Population

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Abstract

Introduction: Electrocardiographic (ECG) criteria for left ventricular hypertrophy (LVH), such as the Cornell and Sokolow-Lyon voltage criteria were derived from Western populations. However, their utility and accuracy for diagnosing echocardiographic LVH in Asian populations is unclear. The objective of this study was to assess the accuracy of ECG criteria for LVH in Asians and to determine if alternative gender-specific ECG cut-offs may improve its diagnostic accuracy. **Materials and Methods:** ECG and echocardiographic assessments were performed on 668 community-dwelling Asian adults (50.9% women; 57 ± 10 years) in Singapore. The accuracy of ECG voltage criteria was compared to echocardiographic LVH criteria based on the American Society of Echocardiography guidelines, and Asian ethnicity and gender-specific partition values. **Results:** Echocardiographic LVH was present in 93 (13.6%) adults. Cornell criteria had low sensitivity (5.5%) and high specificity (98.9%) for diagnosing LVH. Modified gender specific cut-offs (18 mm in women, 22 mm in men) improved sensitivity (8.8% to 17.5%, 0% to 14.7%, respectively) whilst preserving specificity (98.2% to 94.2%, 100% to 95.8%). Similarly, Sokolow-Lyon criteria had poor sensitivity (7.7%) and high specificity (96.1%) for diagnosing LVH. Lowering the cut-off value from 35 mm to 31 mm improved the sensitivity in women from 3.5% to 14% while preserving specificity at 94.2%. A cut-off of 36 mm was optimal in men (sensitivity of 14.7%, specificity of 95.5%). **Conclusion:** Current ECG criteria for LVH derived in Western cohorts have limited sensitivity in Asian populations. Our data suggests that ethnicity- and gender-specific ECG criteria may be needed.

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Key words: Cornell, Ethnicity, Sokolow-Lyon, Voltage

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Introduction

Traditionally, the diagnosis and assessment of left ventricular hypertrophy (LVH) was made with the use of electrocardiography (ECG) and echocardiography, and more recently, with cardiac magnetic resonance (CMR) imaging.¹ ECG remains widely used due to its convenience, low cost, widespread availability and high reproducibility. The importance of accurately detecting LVH has increased in recent years, with the recognition that LVH can be reversed with therapy, and that this can prevent or delay adverse clinical outcomes.^{2,3} Since the development of ECG criteria for the diagnosis of LVH a hundred years

ago,⁴ numerous ECG voltage criteria for LVH have been proposed. Despite its high specificity, ECG voltage criteria for LVH has however, yielded poor sensitivity.⁵

Commonly used ECG voltage criteria for LVH include the gender-specific Cornell voltage criteria⁶ and gender non-specific Sokolow-Lyon voltage criteria.⁷ Many studies have been performed to evaluate these ECG voltage criteria^{1,8} and some have suggested the use of ethnicity-^{9,10} and gender-specific^{6,8} ECG criteria can improve its sensitivity and maintain its high specificity in diagnosing LVH. These studies were however, based on Western populations, and there is a lack of similar studies in Asian populations.

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Taking into account the smaller body habitus of Asians, the applicability of such ECG criteria in Asian populations remains uncertain.

The purpose of this study was to assess the accuracy of these ECG voltage criteria for the diagnosis of echocardiographic LVH in a community-based cohort of Asian adults. We chose to evaluate the Cornell voltage criteria and Sokolow-Lyon voltage criteria due to their ubiquity and easy applicability. We further aimed to investigate if alternative gender-specific ECG cut-offs may improve the diagnostic accuracy of LVH in Asians.

Methods

Study Population

A total of 668 Asian adults (50.9% women; 57 ± 10 years) from the Singapore Longitudinal Ageing Study (SLAS) were included in this present study.¹¹ The SLAS involves whole population sampling of all residents (Singapore citizens and permanent residents) in contiguous precincts within 5 districts in the Southeast region of Singapore who were identified from a door-to-door census and invited to participate. Our study population therefore consisted of asymptomatic community-based adults who self-reported their ethnicity to be Chinese, Malay or Indian. All participants underwent standard 12-lead resting ECG and transthoracic echocardiography at a reference echocardiography laboratory as part of the control arm for a concurrent Singapore Heart Failure Outcomes and Phenotypes (SHOP) study.¹² Patients with a history of valvular heart disease, previous myocardial infarction, ventricular pre-excitation and left or right bundle branch block were excluded. The study was approved by the institutional ethics review committee. Written informed consent was obtained from all subjects.

Electrocardiography

Standard 12-lead ECGs were recorded at 25 mm/s and 10 mm/mV standardisation. All measurements were interpreted by experienced investigators blind to clinical information and echocardiographic measurements. ECG parameters of the 2 commonly used ECG voltage criteria for LVH, Cornell voltage criteria⁶ ($SV_3 + RaVL \geq 28$ mm for men; ≥ 20 mm for women) and Sokolow-Lyon voltage criteria⁷ ($SV_1 + RV_5$ or $V_6 \geq 35$ mm for both genders) were collected (Fig. 1).

Echocardiography

All subjects underwent standard M-mode and 2-dimensional (2D) and Doppler echocardiography at rest, performed by an experienced research sonographer using Vivid 7 Dimension and E9 ultrasound systems (General



Fig. 1. Two ECG depictions about Cornell voltage and Sokolow-Lyon voltage. ECG in A is that of a 62-year-old woman with LVH: $RaVL + SV_3 = 24.5$ mm (more than 22 mm); $SV_1 + RV_5$ or $V_6 = 44$ mm (more than 35 mm). ECG in B is that of a 64-year-old man with no LVH: $RaVL + SV_3 = 7.5$ mm (less than 28 mm); $SV_1 + RV_5$ or $V_6 = 24$ mm (less than 35 mm).

Electric Healthcare, Milwaukee, WI, USA) equipped with wideband transducers. In accordance with American Society for Echocardiography (ASE) recommendations,¹³ left ventricular end-diastolic diameter (LVEDD), diastolic posterior wall thickness (PWTd) and diastolic septal wall thickness (SWTd) were imaged from a parasternal long-axis window at the level of the mitral chords using 2D-targeted M-mode echocardiography. Left ventricular mass (LVM) was calculated based on the ASE-cube formula by Devereux et al.¹³

$$LVM (g) = 0.8 \times [1.04 \times \{(LVEDD + PWTd + SWTd)^3 - (LVEDD)^3\}] + 0.6$$

LVM was indexed for body surface area (BSA) to obtain left ventricular mass index (LVMI). In accordance with ASE recommendations,¹³ LVH was defined by LVMI >95 g/m² in women and >115 g/m² in men in our primary study.

Further recognising that the (ASE) criteria for LVH has not been validated in Asians, we also applied Asian-specific cut-offs derived in our own echocardiography laboratory¹⁴ in secondary analyses, where LVH was defined by LVMI >96 g/m² in women and >106 g/m² in men.

Statistical Analysis

Data were presented as the mean $\pm$ SD for normally distributed parameters. Groups were compared using chi-square test for discrete variables and one-way analysis of variance (ANOVA) for continuous variables. Multiple linear regression models were used to analyse the relationship between LVMI, Sokolow-Lyon voltage, Cornell voltage by gender adjusted for age, body mass index (BMI) and hypertension status. Sensitivity and specificity for both Sokolow-Lyon voltage criteria and Cornell voltage criteria

were tested using different partition values for men and women both separately and as a combined cohort. Receiver operating characteristic (ROC) curves were used to compare the performances of both ECG voltage criteria for LVH. This was first tested using the ASE criteria for LVH as the gold standard, followed by the Asian-specific criteria for LVH¹⁴ in secondary analyses. For all tests, a 2-tailed *P* value <0.05 was required for statistical significance. All analyses were performed using IBM SPSS Statistics software, version 21.0 (IBM Corporation, Armonk, NY, USA).

Results

Clinical, demographic, ECG and echocardiographic variables according to gender are presented in Table 1.

Except for BMI, LVEF, the prevalence of diabetes mellitus and Asian echo-defined LVH, all other variables were significantly different between women and men. Of note, men were slightly older and had a greater prevalence of hypertension. Using the Cornell ECG criteria, the prevalence of LVH was notably 0% in men and 2.9% in women, and using the Sokolow-Lyon criteria, the prevalence of LVH was 7.4% in men and 2.1% in women. Compared to women, men had higher LVM and LVMI. Women had greater prevalence of echocardiographic LVH than men.

Relationship of LVMI, Cornell Voltage and Sokolow-Lyon Voltage

Scatter plots of Cornell voltage (Figs. 2A and 2B) and Sokolow-Lyon voltage (Figs. 2C and 2D) showed a positive correlation with LVMI in men and women respectively (Cornell voltage: $r = 0.320$ and $r = 0.315$; Sokolow-Lyon: $r = 0.227$ and $r = 0.194$, for men and women respectively and $P < 0.001$ for all). These relationships remained statistically significant after adjusting for age, BMI, hypertension status, Cornell voltage ($r = 0.312$ and $r = 0.209$ for men and women respectively; $P < 0.001$ for both) and Sokolow-Lyon voltage ($r = 0.226$ and $r = 0.155$ men and women respectively; $P < 0.001$ for both).

Performance Comparison of Different Cut-off Values of ECG Criteria against ASE LVMI Cut-off for Echocardiographic LVH

Overall performance of Cornell and Sokolow-Lyon voltage cut-off values for LVH was assessed in terms of sensitivity and specificity (Table 2, Table 3 and Fig. 3).

To select the optimal cut-off criteria for our Asian population, we aimed for 95% specificity of the combined modified criteria¹⁵ with preservation of overall accuracy in both sex-stratified and combined analyses.

Table 1. Clinical, Demographic, ECG and Echocardiographic Variables of Community-based Subjects by Gender

	Men (n = 328)	Women (n = 340)	P Value
Age (years)	58 ± 11	56 ± 9	0.015
Height (cm)	1.68 ± 0.06	1.55 ± 0.06	<0.001
Weight (kg)	69.89 ± 11.96	59.88 ± 10.78	<0.001
BMI (kg/m ²)	25.10 ± 3.87	24.97 ± 4.37	0.665
BSA (m ²)	1.79 ± 0.17	1.60 ± 0.15	<0.001
Smokers (%)	16.5	0.4	<0.001
Hypertension (%)	17.7	12.9	0.003
Diabetes mellitus (%)	4.5	3.6	0.322
LVM (g)	161.58 ± 38.74	123.69 ± 30.49	<0.001
LVMI (g/m ²)	90.04 ± 20.01	77.20 ± 17.05	<0.001
LVEF (%)	63.46 ± 4.01	65.21 ± 4.01	0.432
Echocardiographic LVH (n, %)	34 (10.4)	57 (17)	0.013
Asian echocardiographic-defined LVH (n, %)	66 (20.2)	49 (14.6)	0.059
Sokolow-Lyon voltage (mm)	24.48 ± 6.96	22.16 ± 5.89	<0.001
ECG LVH by Sokolow-Lyon (%)	7.4	2.1	0.001
Cornell voltage (mm)	13.57 ± 5.31	10.91 ± 4.94	<0.001
ECG LVH by Cornell (%)	0	2.9	0.002

BMI: Body mass index; BSA: Body surface area; ECG: Electrocardiographic; LVEF: Left ventricular ejection fraction; LVH: Left ventricular hypertrophy; LVM: Left ventricular mass; LVMI: Left ventricular mass index

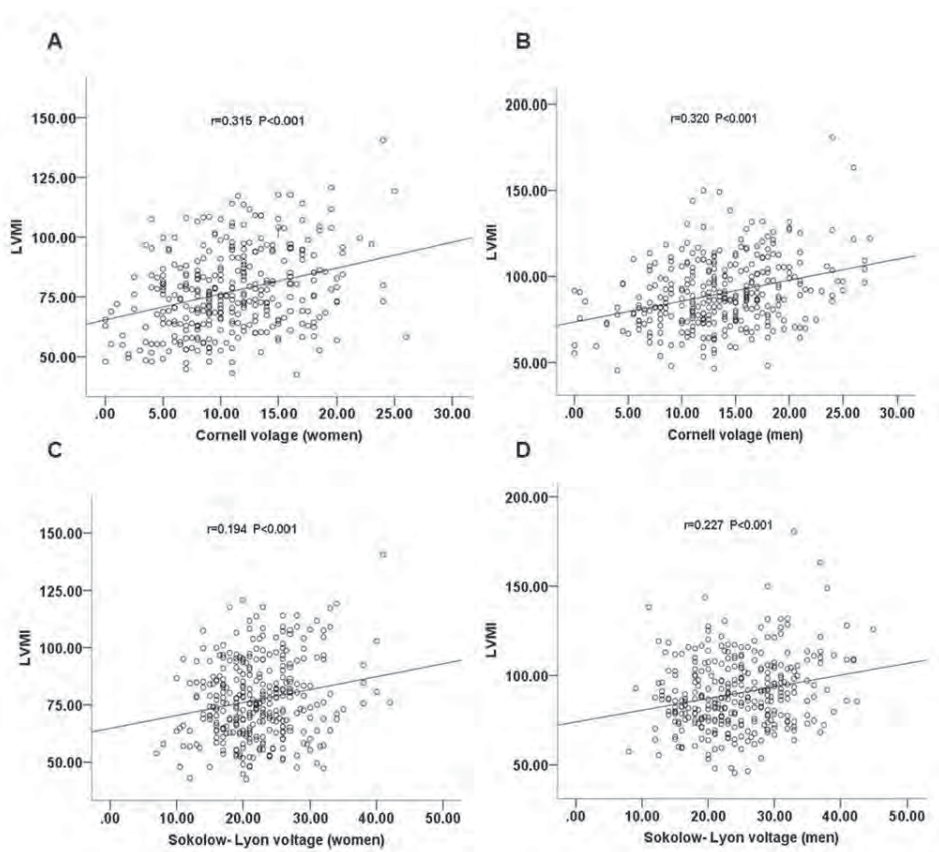


Fig. 2. Scatter diagrams showing the correlation between the left ventricular mass index (LVMI) and Cornell voltage or Sokolow-Lyon voltage in women (A and C) and men (B and D), respectively.

Table 2. Cornell Voltage Criteria Performance with Different Cut-off Values for Diagnosing ASE Echocardiographic LVH

	RaVL + SV3	Sensitivity	Specificity	Accuracy	PPV	NPV
	Cut-off	%	%	%	%	%
Women	14	40.4	79.1	72.5	28.4	86.6
	16	22.8	86.7	75.8	26	84.6
	18	17.5	94.2	81.2	38.5	84.8
	20	8.8	98.2	83	50	84
	22	5.3	98.9	83	50	83.6
	24	1.8	99.6	83	50	83.2
	26	0	100	83	0	83
	28	0	100	83	0	83
Men	14	70.6	60.1	61.2	17	94.6
	16	52.9	72.7	70	18.4	93
	18	35.3	85.0	79.8	21.4	93.9
	20	17.6	91.5	83.8	19.4	90.5
	22	14.7	95.8	87.5	29.4	90.6
	24	8.8	98.0	88.7	33.3	90.3
	26	2.9	99.0	89	25	89.9
	28	0	100	89.6	0	89.6
Cornell criteria		5.5	98.9	84.6	45.5	86.8
Modified Cornell criteria	Women >18 mm					
	Men >22 mm	16.5	95.0	84.3	34.9	87.7

ASE: American Society of Echocardiography; LVH: Left ventricular hypertrophy; NPV: Negative predictive value; PPV: Positive predictive value

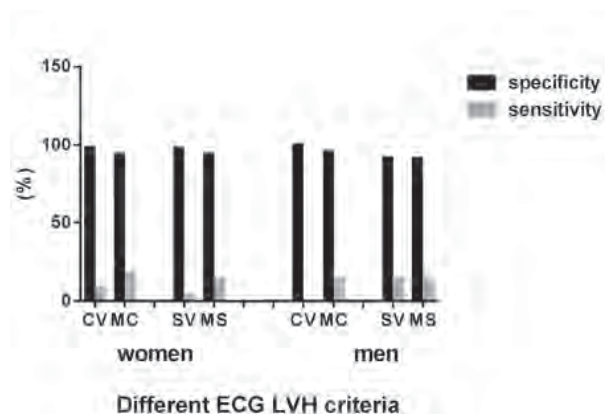


Fig. 3. A bar diagram comparing the sensitivity and specificity of the original Cornell voltage criteria (CV) and Sokolow-Lyon voltage criteria (SV) with that of the modified Cornell voltage criteria (MC) and Sokolow-Lyon voltage criteria (MS). The modified cut-off values preserved specificity while increasing sensitivity.

Overall, Cornell voltage criteria showed a low sensitivity (5.5%) and high specificity (98.9%) for the diagnosis of LVH among Asians, particularly in Asian men (Table 2). In Asian women, lowering the cut-off from 20 mm to 18 mm doubled the sensitivity (8.8% to 17.5%), but preserved specificity (98.2% to 94.2%). In Asian men, lowering the cut-off from 28 mm to 22 mm improved sensitivity (0% to 14.7%) and maintained specificity (100% to 95.8%).

Hence, the modified Cornell criteria (SV3 + RaVL >22 mm in men and >18 mm in women) had 16.5% sensitivity, 95% specificity, 34.9% positive predictive value (PPV) and 87.7% negative predictive value (NPV) for the diagnosis of LVH.

Similarly, Sokolow-Lyon voltage criteria had poor sensitivity (7.7%) and high specificity (96.1%) for the diagnosis of LVH among Asians (Table 3). In Asian women, lowering the cut-off from 35 mm to 31 mm improved the sensitivity by 4 times from 3.5% to 14% while preserving a high specificity of 94.2%. In Asian men, increasing the cut-off to 36 mm maintained the sensitivity at 14.7% but improved the specificity from 92% to 95.2%. The modified Sokolow-Lyon criteria (S in V1+R in V5 or V6 >36 mm in men and >31 mm in women) doubled the sensitivity from its original 7.7% to 14.2%, yet retained a specificity of 95%, a PPV of 28.6% and a NPV of 87.1% for the diagnosis of LVH.

The PPV estimates provided in this study should be considered with caution: while PPV is often used to estimate the probability of true LVH among test-positive cases, such estimates can be misleading in non-hospitalised populations such as ours, where the true prevalence is low and the test (ECG in this case) has a high false positive rate.

The ROC area under curve (AUC) was 0.644 for the traditional Cornell voltage criteria and 0.586 for the

Table 3. Sokolow-Lyon Voltage Criteria Performance with Different Cut-off Values for Diagnosing ASE Echocardiographic LVH

	SV1 + RV5 or V6	Sensitivity	Specificity	Accuracy	PPV	NPV
	Cut-off	%	%	%	%	%
Women	25	45.6	75.2	70.1	27.7	86.9
	27	28.1	86.5	76.4	37.2	85.3
	29	19.3	90.9	78.5	30.6	84.4
	31	14.0	94.2	80.4	33.3	84
	33	5.3	97.1	81.3	27.3	83.1
	35	3.5	97.8	81.6	25	83
	37	3.5	98.2	81.9	28.6	83
Men	25	52.9	59.9	59.1	13.4	91.5
	27	44.4	67.1	65	14.4	91.5
	29	35.3	76.5	72.1	15	90.9
	31	23.5	84.8	78.3	15.4	90.4
	33	14.7	91.3	83.2	16.6	90.1
	35	14.7	92.0	83.9	17.9	90.2
	36	14.7	95.5	87	27.8	90.5
Sokolow-Lyon criteria	37	8.8	96.9	87.6	25	90
	35	7.7	96.1	83.8	24.1	86.6
Modified Sokolow-Lyon criteria	Women >31 mm	14.2	95.0	83.3	28.6	87.1
	Men >36 mm					

ASE: American Society of Echocardiography; LVH: Left ventricular hypertrophy; NPV: Negative predictive value; PPV: Positive predictive value

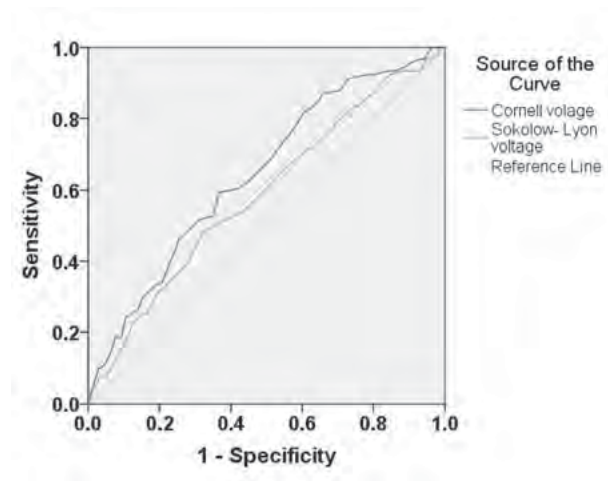


Fig. 4. Receiver operating characteristic (ROC) curve for 2 electrocardiography (ECG) criteria based on the American Society of Echocardiography (ASE) echocardiographic left ventricular hypertrophy (LVH). Cornell voltage criteria area under curve (AUC) = 0.644; Sokolow-Lyon voltage criteria AUC = 0.586.

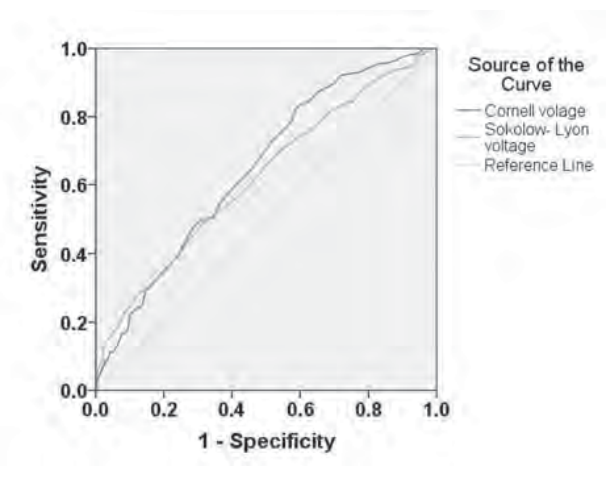


Fig. 5. Receiver operating characteristic (ROC) curve for 2 electrocardiography (ECG) criteria based on Asian-specific echocardiographic left ventricular hypertrophy (LVH). Cornell voltage criteria area under curve (AUC) = 0.649; Sokolow-Lyon voltage criteria AUC = 0.621.

traditional Sokolow-Lyon voltage criteria. However, pair-wise tests comparing the AUC for both criterion did not show any significant difference ($P > 0.05$) (Fig. 4).

Secondary Analyses Using Asian-specific Echocardiographic Cut-offs for LVH

By applying the lower Asian echocardiographic LVMI cut-off for LVH as the gold standard, a further 32 men who did not meet the ASE LVMI cut-off were reclassified as having LVH, while 8 women were reclassified as not having LVH based on the higher Asian-specific echocardiographic LVMI cut-offs for women. With this new threshold, the standard Cornell criteria performed just as poorly for sensitivity (3.5% vs 5.5%) and had similar specificity (98.9% vs 98.9%) as when ASE defined cut-offs were used.

The standard Sokolow-Lyon criteria had higher sensitivity

(13% vs 7.7%) and specificity (97.4% vs 96.1%) for LVH using Asian echocardiographic criteria as gold standard compared to using ASE criteria as gold standard.

We similarly derived modified cut-offs for both ECG criteria by maintaining specificity at 95% which were 18 mm for women and 22 mm for men for the Cornell criteria (which is identical to when the ASE LVH cut-off was used) and 30 mm for women and 35 mm for men with the Sokolow-Lyon criteria. Our modified criteria were associated with improved sensitivity with both criteria (Cornell criteria 13.1% vs 3.5%; Sokolow-Lyon criteria 19.1% vs 13%) (Table 4).

The ROC AUC was 0.649 for the traditional Cornell voltage criteria and 0.621 for the traditional Sokolow-Lyon voltage criteria. However, pair-wise tests comparing the area under the ROC curve for both criterion did not show any significant difference ($P > 0.05$) (Fig. 5).

Table 4. Cornell and Sokolow-Lyon Criteria Performance for Diagnosing Asian-specific Echocardiographic LVH

ECG Criteria	Cut-off (mm)	Sensitivity (%)	Specificity (%)	Accuracy (%)	PPV (%)	NPV (%)
Cornell criteria	20 & 28	3.5	98.9	82.3	40.0	83.8
Modified Cornell criteria	18 & 22	13.1	95.0	80.7	34.9	83.8
Sokolow-Lyon criteria	35	13.0	97.4	82.6	51.7	84.0
Modified Sokolow-Lyon criteria	30 & 35	19.1	95.0	81.3	43.1	84.6

ECG: Electrocardiographic; LVH: Left ventricular hypertrophy; NPV: Negative predictive value; PPV: Positive predictive value

Discussion

The main finding in our study was that existing ECG criteria for LVH derived from Western cohorts had good specificity but unacceptably low sensitivity in a community-based Asian cohort. We also found that by modifying these criteria, it may be possible to improve their performance.

Traditional Cut-offs Perform Poorly in Non-Western Populations

The most commonly used ECG LVH criteria were originally derived from Western populations but are applied universally to all ethnic groups. In 1949, Sokolow and Lyon⁷ compared the ECGs of 147 patients with cardiovascular disease and 151 healthy patients, to define the ECG changes associated with LVH. While many observations were reported, the ECG criterion that remains most commonly in clinical practice is the sum of the S wave in lead V1 plus the larger of the R wave in V5 or V6 greater than 35 mm. They found that the sensitivity of this criterion was 32% and specificity was 100. Notably, LVH was not confirmed with imaging techniques in this early seminal study. More recently, Casale et al⁶ derived the Cornell voltage criteria from 414 “learning series” patients and 129 “test series” patients and the majority of whom had cardiovascular disease. They found that the R wave in lead aVL and the S wave in lead V3 correlated best with M-mode echocardiographic LVMI, and reported a sensitivity of 41% and specificity 90% in the learning series, and similar sensitivity of 41% but higher specificity of 98% in the test series. These 2 ECG criteria remain in general use largely due to their simplicity and accuracy as reported in the original series.

In contrast, the present study showed that the widely used Cornell and Sokolow-Lyon ECG voltage criteria for LVH had poor sensitivities in our Asian population. This is especially so for the Cornell voltage criteria in Asian men, where sensitivity in our cohort was 0%. The low sensitivities of Cornell voltage criteria and Sokolow-Lyon voltage criteria from our study (5.5% and 7.7%, respectively) is in sharp contrast to that found in the original reports of these criteria^{6,7} (41% and 32%, respectively). A possible explanation for this is the higher LVMI cut-off of >132 g/m² for men and >109 g/m² for women was used to define LVH in the former study.⁶ Other studies performed on largely Western populations that also found higher sensitivities and specificities for these criteria than in our cohort had used similar cut-off values.^{5,8} The higher LVMI cut-off would have reduced the number of patients who would have been classified as having LVH and these patients would also have had higher LVM on average. Hence, they may also have had higher voltages on their ECGs making it more likely that they would meet the ECG voltage criteria cut-off values and

resulting in increased sensitivity of these criteria. In addition, the accuracy of ECG-LVH criteria have been reported to be substantially improved by combining repolarisation abnormalities with high ECG voltage criteria.¹⁶ However, our data from a community-based healthy population has a very low incidence of repolarisation abnormalities (only 10 cases of T-wave inversions), so this was not included in the current analyses.

Two recent studies in East Asian populations also lend weight to this notion. In a study by Xie et al¹⁷ of hypertensive Chinese patients, Cornell product and voltage criteria were good predictors of LVH (sensitivity of 28% and 30.1% in men and women respectively; specificity of 95.9% and 91.4% in men and women respectively) based on LVMI cut-off values of >125 g/m² men and >110 g/m² women which was similar to those in Western cohorts.⁶ Of note, these cut-off values for echocardiographic LVH were higher than the ASE¹³ recommended cut-offs (>115 mg/m² men; >95 mg/m² women) used in this present study. The selected population of only hypertensive subjects as compared to our study population of healthy community-based adults may also have increased the number of patients with LVH in their cohort compared to our study.

In contrast, a study of Korean patients by Park et al¹⁸ that diagnosed echocardiographic LVH using the same ASE guidelines as our study, found that the sensitivity of Cornell voltage criteria in Korean men was much lower (and similar to our findings) at 1.4% and 9.7% in women. The ROC AUC for the Cornell voltage criteria against LVMI was 0.648 in men and 0.735 in women, which was similar to the AUC for our cohort at 0.644. Park et al proposed reducing the cut-off values to 20 mm in men and 16 mm in women leading to a markedly improved sensitivity of 19.7% in men and 22.6% in women at a fixed specificity of 95%. This again was similar to our recommendation of 22 mm in men and 20 mm in women. These studies not only highlight the potential limitations of applying conventional ECG cut-off values in the Asian population but also suggest that the apparent performance of these ECG voltage criteria could well be affected by which standard of echocardiographic LVH they are compared against. This is especially since standard ASE criteria for echocardiographic LVH has not been validated in Asian populations. Although different LVMI cutoff values for LVH has been proposed for Southeast Asian populations (106 g/m² for men and 96 g/m² for women),¹⁴ it should be noted that these defined LVH by 95th percentile partition values rather than by the probability of future clinical outcomes in a “normal” population. In the present paper, we have chosen to use these arbitrary cut-off values because they are the only available echocardiographic LVH criteria derived from Southeast Asian populations. These have yet

to be validated against clinical outcomes. It is likely that further validation of these echocardiographic LVH criteria will allow us to more accurately define patients who have LVH and allow us to further refine ECG LVH criteria.

Previous studies have shown that Sokolow-Lyon voltage and Cornell voltage parameter correlated well with LVM or LVMI in Western populations.^{6,19} These were clinic-based cohorts and had higher rates of hypertension than in our community-based cohort. Nonetheless, our data from a community-based cohort also showed that the Sokolow-Lyon voltage and Cornell voltage parameters correlated with LVMI in an Asian cohort. Even when adjusted for age, BMI and hypertension, the correlation persisted. This suggests that the poor performance of conventional voltage criteria in our cohort may be due to the fact that these cut-off values were not suitable for our population rather than a lack of correlation with LVH. It is also likely that cut-off values more tailored to specific ethnic groups may make these voltage criteria more accurate in our Asian population.

Recent studies by Bacharova et al^{20,21} based on the solid angle theory^{22,23} found that LVM is not the only determinant of QRS voltage changes in LVH. Instead, it is a combination of anatomic and electric remodelling that result in the altered ECG voltage. In other words, the creation of the QRS voltage depends on spatial (the size and anatomy of the heart) and non-spatial (the electrical properties of myocardium) factors, which challenges of the traditional conceptual model that the QRS voltage reflects increased LVM. However, these studies were based on computer modelling of underlying myocardial changes and its effect on the ECG. Hence, the clinical implications of these findings are uncertain. In this paper, we chose a more simplistic view of relating ECG voltage criteria to echocardiographic LVH defined by LVM as the latter has been used in the past to define LVH. The aim was to allow us to refine the ECG criteria for diagnosing LVH in Asian patients without the added expense and complexity of echocardiography.

Gender Specific Cut-offs May Be More Appropriate in Asians

In keeping with earlier studies in both Western and Asian cohorts,^{6,8,17,18} men and women in this present study had significantly different LVMI, and Sokolow-Lyon and Cornell voltage parameters. Moreover, the sensitivity and specificity of both criteria varied when assessed by gender. This is particularly true for the Sokolow-Lyon criterion that has conventionally used the same cut-off for both genders. In our women subjects, this resulted in a sensitivity of only 3.5%, but this was increased to 14% by reducing the cut-off to 31 mm, while preserving specificity at 94%. In contrast, the cut-off of 35 mm resulted in acceptable sensitivity and specificity in men in our study (14.7% and 92.0% respectively). These could be further improved

by raising the cut-off to 36 mm in our cohort (14.7% and 95.5% respectively) and we propose that this cut-off is optimal in Asian men.

It is expected that different cut-offs are required in men and women because previous studies, as well as ours, have found that men have significantly higher LVMI, absolute LV mass, LV diastolic and systolic dimensions, height, weight and BSA.^{8,18} Significant sex-specific voltage differences in the S wave in lead V3 were also noted (13.2 mm $\pm$ 8.2 mm males; 7 mm $\pm$ 4.4 mm females; $P < 0.001$).⁶ Widely accepted explanations for this observation include the larger distance between precordial leads and myocardium due to the presence of breast tissue in women²⁴ and smaller ventricular mass in women leading to smaller S wave amplitude in V3 which measures posteriorly directed myocardial electrical activity.⁶ Despite this, Casale et al⁶ showed that S in V3 correlated best with LVMI in both genders and hence it was proposed as part of the Cornell voltage criteria, but importantly, with different cut-off values for men and women.

In a study comparing cardiac MRI diagnosed LVH with ECG voltage criteria for LVH in a group of hypertensive patients and healthy controls, Alfakih et al⁸ demonstrated that the Sokolow-Lyon criteria had non-significantly higher sensitivity (32% vs 23.3%) but lower specificity (90.5% vs 94.2%) in men compared to women. The reverse was true of the Cornell criteria which had lower sensitivity (18.5% vs 25.6%) but higher specificity (99.2% vs 89.3%) in men. The authors proposed new cut-offs that would fix specificity at 95% but that resulted in reduced sensitivity of the Sokolow-Lyon criteria in men (18.5% from 32%), and also reduced sensitivity of the Cornell criteria in women (16.3% from 25.6%). Hence, using gender-specific cut-offs may not necessarily improve the sensitivity of ECG criteria when adequate specificity is targeted. This is in contrast with our study, where the ECG criteria generally had higher specificities but lower sensitivities. As a result, our proposed cut-offs (which stipulated a specificity of 95%) had higher sensitivities than conventional cut-off values. For Cornell voltage criteria, a new cut-off value (RaVL + SV3 >22 mm for men; >18 mm for women) increased sensitivity (0% to 14.7% for men; from 8.8% to 17.5% for women), while maintaining a high specificity (>94%). Likewise, introducing sex-specific cut-offs (SV1 + RV5 or V6 >36 mm for men; >31 mm for women) for Sokolow-Lyon voltage criteria increased its sensitivity in women from 3.5% to 14% and an overall improved sensitivity of 14.2% (as compared to the initial 7.7%) while still maintaining a high specificity (>94%).

Our findings suggest that revised gender-specific cut-off values for both Cornell and Sokolow-Lyon voltage criteria are appropriate.

Limitations

The study subjects in this study were community-dwelling individuals and are very likely to differ significantly from some previous studies that only examined patients with hypertension or cardiovascular disease in a hospital or clinic setting. It is likely that this may affect the sensitivity and specificities of these ECG criteria derived from a different population. Criteria derived in this study may also not be applicable to younger adults. Nonetheless, these are the first available combined ECG and echocardiographic data from a large random sample of older Asian adults from the general community. These findings warrant validation in larger Asian cohorts across the full range of age.

ECG LVH voltage criteria can vary significantly due to lead placement variability, hence reproducibility of this test may be low.²⁵ Furthermore, we used echocardiographic-LVH as the gold standard rather than CMR, and the former may be affected more by inter-operator variability. Nonetheless, we found that there was still significant correlation between the ECG voltage criteria and LVMI. Given the wider availability and lower costs of these tests compared to CMR, it can be argued that our findings may be more easily applicable.

Conclusion

Conventional ECG voltage criteria for LVH developed and validated in Western populations perform poorly in Asian patients. However, tailoring cut-off values to ethnicity and gender improves the ability of ECG voltage to detect LVH in our multi-ethnic Asian cohort. We propose the use of modified Cornell voltage criteria (R in aVL + S in V3 >22 mm men; >18 mm women) and modified Sokolow-Lyon voltage criteria (S in V1 + R in V5 or V6 >35 mm men; >31 mm women) to improve detection of LVH in the Asian population.

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Thyroid Autoimmune Antibodies and Major Depressive Disorder in Women

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Abstract

Introduction: Anti-thyroid antibodies are associated with extra-thyroid diseases such as Graves' ophthalmopathy and Hashimoto's encephalopathy. Some evidence suggests that anti-thyroid antibodies are also associated with depression. Interleukin (IL)-17 appears to play an important role in autoimmune thyroid disease. This study investigated whether specific thyroid autoantibodies and IL-17 distinguished persons with depression from non-depressed controls. **Materials and Methods:** Forty-seven adult females with non-psychotic, current major depressive disorder and 80 healthy female controls participated in this study. Thyroid peroxidase antibodies, thyroglobulin antibodies, thyroid-stimulating hormone (TSH) receptor antibodies, free T3 and T4, TSH and IL-17 were measured from the serum. Measurements were repeated to assess test-retest reliability. Receiver operating characteristic (ROC) curves were used to estimate discriminatory values of the measurements. Differences between groups and associations between the clinical and biochemical assessments were analysed. **Results:** Median TSH receptor antibody concentration was significantly higher in the depressed than control group ($P < 0.001$). Area under the ROC curve was 0.80 (95% CI, 0.73 to 0.88). Higher TSH receptor antibody titres were associated with greater depression severity scores ($r = 0.33$, $P < 0.05$). IL-17 levels were not associated with TSH receptor antibody levels or depression severity scores. Thyroid function and other thyroid autoantibodies were not associated with depression severity. **Conclusion:** TSH receptor antibodies might be a biomarker of immune dysfunction in depression.

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Key words: Cytokines, Depression, Immunology

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Thyroid Autoimmune Antibodies and Major Depressive Disorder in Women

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Introduction

Autoimmune thyroid diseases such as Graves' disease and Hashimoto's thyroiditis are defined by elevated serum levels of anti-thyroid antibodies and thyroid hormone dysfunction.¹ Anti-thyroid antibodies could also affect extra-thyroidal tissues and organs.

In Graves' ophthalmopathy, antibodies target thyroid stimulating hormone (TSH) receptor or thyroid peroxidase (TPO) antigens in orbital tissues to activate cytokine-mediated inflammatory changes.^{2,3} These cytokines stimulate glycosaminoglycan and metalloproteinase

inhibitor production and fibroblast proliferation to cause soft tissue oedema and proptosis.⁴ Patients with Hashimoto's encephalopathy may present with focal neurological symptoms, cognitive deficits, or psychiatric symptoms. Elevated levels of serum anti-thyroid antibodies, particularly antibodies to TPO (TPOAb) and thyroglobulin (TgAb), are hallmarks of this disease.⁵ TPO is a follicular cell enzyme involved in thyroid hormone synthesis, while Tg is a precursor protein used to synthesise thyroid hormones. Although serum levels of anti-thyroid antibodies are elevated in Hashimoto's encephalopathy, thyroid hormone levels are

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often normal. Both TPOAb and TgAb have been detected in the cerebrospinal fluid of patients with Hashimoto's encephalopathy,⁶ which suggests that anti-thyroid antibodies may have direct effects on the central nervous system.

Could depression be a model of extra-thyroidal autoimmune disease? A higher lifetime prevalence of depression, based on self-report, was found in TPOAb positive versus negative individuals (RR 1.4; 95% CI, 1.0 to 2.1).⁷ Another study reported an association between TPOAb positivity and lifetime diagnosis for major depressive disorder (OR 2.7; 95% CI, 1.1 to 6.7)⁸ based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). These studies did not examine patients with current episode major depression. Few studies have investigated other anti-thyroid antibodies in depressed patients. Two studies found no association between TgAb and depression,^{9,10} while 1 study reported higher levels of thyroid-binding inhibitory immunoglobulin in depressed patients compared to healthy controls.⁹ It is not clear whether neutral TSH receptor antibodies (TRAb) are associated with depression.

Thyroid autoantibody's pathophysiological effect in depression may be cytokine mediated. Th17 lymphocytes and its primary cytokine, interleukin (IL)-17, have important roles in autoimmune disease. IL-17 is a critical signaling molecule that induces the release of proinflammatory cytokines and chemokines. Higher proportions of Th17 cells are present in patients with Hashimoto's thyroiditis and Graves' disease^{11,12} and serum IL-17 levels are significantly increased in patients with intractable Graves' disease.¹³ Depression has been frequently associated with elevated levels of proinflammatory cytokines, especially IL-6 and tumour necrosis factor (TNF)- α .¹⁴ However, little is known about whether IL-17 is associated with depression.

The aim of this study was to determine whether depressed patients and healthy volunteers differed in terms of TRAb, TPOAb, TgAb and IL-17 levels. We hypothesised that thyroid autoimmune markers would be greater in patients with current major depressive episode than normal controls and that IL-17 levels would be associated with these thyroid antibody levels. We further explored the relationship between thyroid autoantibodies, IL-17 and depressive symptom severity.

Materials and Methods

Subjects

Psychiatric inpatients and outpatients were recruited from the adult psychiatry service at a general hospital. Healthy volunteers were recruited via printed and online media. The study was approved by the Institutional Review Board and all participants provided written informed consent. Subjects aged 21 to 60 years were eligible to participate.

Major depressive disorder was diagnosed by psychiatrists and trained raters confirmed the diagnosis using the DSM-IV-based Mini International Neuropsychiatric Interview (MINI).¹⁵ Depressed subjects were included if they scored at least 16 on the Montgomery-Åsberg Depression Rating Scale (MADRS).¹⁶ The Quick Inventory of Depressive Symptomatology-Self Report (QIDS-SR)¹⁷ was also used to measure depression severity. Controls were included if they did not have current depression after screening with the MINI and MADRS. Only women were recruited to control for the known gender difference in autoimmune expression.¹⁸ Both control and depressed participants were excluded if they met the diagnostic criteria for bipolar or psychotic disorder (lifetime)—eating, obsessive-compulsive, alcohol or substance use-related disorders (past 1 year). Additional exclusion criteria for all participants included acute infections within the past 1 month; the presence of any allergic, autoimmune, neoplastic, endocrine and other general medical/surgical disorders within the past 3 months; and pregnancy. None of the participants took prescribed or over-the-counter drugs that had known effects on immunological or inflammatory responses.

Serum Measurements

Thyroid autoantibodies (TPOAb, TgAb and TRAb), thyroid hormones (fT3, fT4 and TSH) and cytokine IL-17 were measured from the serum. The blood sample was drawn once from each participant in the day. Samples were then aliquoted into separate sample tubes, stored at -20°C, and assayed in batches. Each blood sample was measured twice in the laboratory to determine test-retest assay reliability.

fT3, fT4 and TSH were measured using the ADVIA Centaur immunoassay system (Siemen Healthcare Diagnostics, Germany). For the fT3 assay, the minimum detectable concentration (analytical sensitivity) was 0.2 pg/mL, reference range was 5.0 pmol/L to 8.9 pmol/L, and manufacturer defined (md) intra-assay CV was <4%. For the fT4 assay, the analytical sensitivity was 0.1 ng/dL, reference range was 9.6 pmol/L to 19.1 pmol/L, and md intra-assay CV was <4%. For the TSH assay, the analytical sensitivity was 0.010 μ IU/mL, reference range was 0.65 mIU/L to 3.7 mIU/L, and md intra-assay CV was <6%.

Thyroid autoantibodies were measured using radioimmunoassays (BRAHMS Diagnostica, Germany). Human receptor assays for the antibodies were used. The analytical sensitivities of TPOAb and TgAb were both 5.5 U/mL, and their measured intra-assay CVs were <5%. The analytical sensitivity of TRAb was 0.3 U/mL and the measured intra-assay CV was <2%.

IL-17 was measured using the Merck-Millipore Milliplex Human Cytokine Panel (Merck KGaA, Darmstadt,

Germany). The assay was performed using a self-antigen specific capture antibody technique in a multiplex immunoassay quantification system. The intra-assay CV was <10%.

Statistical Analysis

All serum specimens were measured twice in the laboratory to assess test-retest reliability of the test procedures. Reliability of the measurements was evaluated by agreement testing (Wilcoxon signed-rank test) and intra-class correlation (Spearman's correlation). Differences between groups and associations between the clinical and biochemical variables were analysed with the appropriate

t-test, Mann-Whitney U test, Pearson's or Spearman's correlation after normality checks. Chi-square test was used for categorical data. Generalised linear regression was used to evaluate potential confounding. Receiver operating characteristic (ROC) curves were used to estimate potential discriminatory values of the laboratory measures.

Results

Forty-seven women with non-psychotic, current episode major depressive disorder and 80 female controls participated in this study. Baseline characteristics are shown in Table 1. Depressed subjects had moderate to severe depression, with a mean MADRS score of 31.3 ($\pm$

Table 1. Baseline Characteristics and Serum Measurements

	Depressed (n = 47)	Controls (n = 80)
Mean age, years (SD)	41.4 (10.4)	33.4 (10.5)
Gender (%)		
Female	47 (100)	80 (100)
Ethnicity (%)		
Chinese	47 (100)	80 (100)
Education level (%)		
Primary or below	5 (11)	0 (0)
Secondary	19 (40)	12 (15)
Tertiary and above	23 (49)	68 (85)
Mean MADRS score (SD)	31.3 (8.2)	0.1 (0.4)
Mean QIDS-SR score (SD)	14.8 (4.6)	-
Mean age of onset for first major depressive episode, years (SD)	35.1 (11.9)	-
Number of major depressive episodes including current episode (%)		
1	18 (38)	-
2	10 (21)	-
≥ 3	19 (41)	-
Number of adequate antidepressant trials (%)		
0	29 (62)	-
1	15 (32)	-
≥ 2	3 (6)	-
Median anti-thyroid antibody titres (range)		
TRAb (U/mL)	0.55* (0.10 – 1.35)	0.15* (0.00 – 4.40)
TPOAb (U/mL)	17.1 (2.4 – 3001.0)	16.4 (0.0 – 2045.3)
TgAb (U/mL)	11.6 (0.0 – 213.1)	13.9 (0.0 – 2031.1)
Median thyroid hormone levels (range)		
fT3 (pmol/L)	4.3 (2.8 – 213.1)	4.3 (3.5 – 5.8)
fT4 (pmol/L)	14.5 (0.8 – 22.3)	14.9 (11.1 – 18.9)
TSH (mol/L)	1.3 (0.3 – 4.7)	1.4 (0.3 – 5.2)
Median cytokine level (range)		
IL-17 (pg/mL)	3.9 (0.0 – 74.4)	6.2 (0.0 – 101.7)

IL-17: Interleukin-17; MADRS: Montgomery-Åsberg Depression Rating Scale; QIDS-SR: Quick Inventory of Depressive Symptomatology-Self Report; TgAb: Anti-thyroglobulin antibody; TPOAb: Anti-thyroid peroxidase antibody; TRAb: Anti-thyroid stimulating hormone receptor antibody; TSH: Thyroid stimulating hormone

*P < 0.001 (Mann-Whitney U test)

11.9 SD) and QIDS-SR score of 14.8 ($\pm$ 4.6 SD). Seventy-nine percent of those depressed were on antidepressant medication (either a selective serotonin reuptake inhibitor, serotonin-norepinephrine reuptake inhibitor or tricyclic antidepressant). Eight depressed and 1 control were smokers. None of the participants had a past history of thyroid disease. Median measures of fT3, fT4 and TSH did not differ between the groups.

All antibody and cytokine measurements in Table 1 had acceptable agreement, as indicated by non-significant differences between repeat laboratory measurements. Correlations between repeat measurements were good. The correlation coefficients for TPOAb, TgAb, TRAb and IL-17 were 1.00, 0.99, 0.78 and 0.94 respectively for cases, and 1.00, 1.00, 0.95 and 0.97 respectively for controls, with all *P* values less than 0.001.

Anti-thyroid Antibodies

The median TRAb level was significantly higher (*P* < 0.001) in those depressed compared with controls. There was 1 TRAb outlier but the difference remained significant (*P* < 0.001) even after exclusion of the outlier. Higher TRAb titres correlated with greater depression scores on the QIDS-SR (*r* = 0.33, *P* < 0.05). The association between TRAb levels and depression remained significant (*P* = 0.033) after adjusting for age and smoking. No difference was found between the depressed and control groups for TPOAb and TgAb.

TRAb levels differentiated the depressed from controls, based on the ROC curve for TRAb. The area under the curve (AUC) was 0.80 (95% CI, 0.73 to 0.88) (Fig. 1), using a cut-off of 0.30 U/mL. At this threshold, TRAb had 87% sensitivity and 73% specificity in discriminating depressed from controls.

Clinically, upper reference limits of >60 U/mL for TPOAb and TgAb, and >10 U/mL for TRAb are used to define positive thyroid autoimmunity in relation to autoimmune thyroid disease. TPOAb positivity was found in 8.5% of depressives and 5% of controls, while TgAb positivity was found in 6.4% of depressives and 7.5% of controls. Depressed individuals did not have significantly greater TPOAb or TgAb positivity compared to controls. None of the subjects were TRAb positive.

Cytokine IL-17

IL-17 levels did not differ between controls and those depressed. IL-17 levels were neither associated with TRAb levels nor depression severity scores. IL-17 was also not associated with age, thyroid hormone levels and other thyroid autoantibody levels.

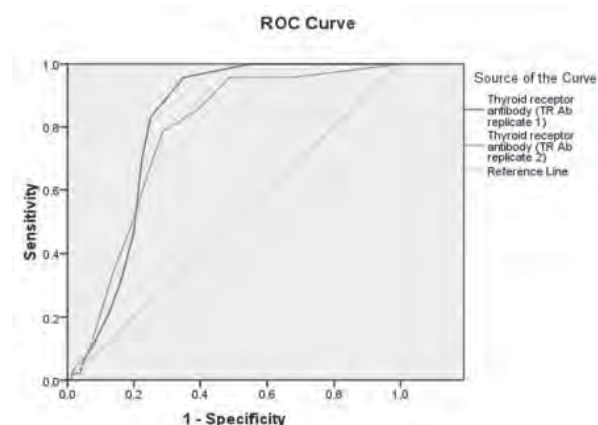


Fig. 1. Receiver operating characteristic (ROC) curve of thyroid receptor antibody (TRAb).

Discussion

Patients with major depressive disorder in a current major depressive episode did not differ from healthy controls in terms of TPOAb and TgAb levels. This is consistent with previous studies that found no difference in TPOAb and TgAb positivity between currently depressed patients and controls.^{9,10,19} However, we found significantly higher TRAb levels in depressed patients than controls.

Approximately 69% of those with systemic autoimmune disease are depressed²⁰ and autoimmune disease is associated with a 45% increased risk of subsequent mood disorder.²¹ Higher titres of autoantibodies are reported in patients with autoimmune disease suffering from depression compared to those without depression and healthy controls.²² In systemic lupus erythematosus (SLE), elevated levels of autoantibodies that are associated with depression include anti-endothelial cell, anti-ganglioside, anti-nuclear, anti-N-methyl-D-aspartate receptor, anti-phospholipid, and anti-P ribosomal autoantibodies.²² To test whether such autoantibodies had a direct pathogenic role in depression, Shoenfeld and colleagues injected purified human anti-P ribosomal autoantibodies into mice intracerebroventricularly. This induced depression-like behaviour in the animal model. The experiment further showed that this depressive behaviour could be blocked by a 4-week treatment with fluoxetine.²³ Immunohistologic staining of anti-P ribosomal autoantibodies revealed stain patterns in the limbic system (cingulate cortex, hippocampus and piriform cortex), a neural network implicated in affect regulation.²³ Although autoimmune antibodies may be involved in depression pathophysiology, studies have yet to show that depression could be induced by thyroid autoantibodies in specific.

The TRAb assayed in our study is not known to be stimulatory or inhibitory, and is thought to be functionally neutral in terms of thyroid hormone production. However, TRAbs may have direct effects on extra-thyroidal tissue and organs, such as the brain. TRAbs were recently shown to bind to human cortical neurons, with localisation to cell bodies and axons.²⁴ Neutral TRAbs activate unique cellular signal cascades that include protein kinase A II, phosphatidylinositol 3K/Akt, MAPK-ERK1/2/p38 α , mammalian target of rapamycin/p70 S6K and nuclear factor- κ B.²⁵ Chronic exposure to these antibodies leads to oxidative stress-induced apoptosis and excess oxidative stress, and generation of reactive oxygen species have been described in depression.^{26,27} Another mechanism through which TRAbs can act is in the stimulation of proinflammatory cytokines. Antibodies that bind to TSH receptors on bone marrow cells have been shown to stimulate IL-6 secretion in the absence of TSH.²⁸

Proinflammatory cytokines are known to cause emotional and cognitive disturbances. Interferon is commonly associated with depression when administered exogenously. Twenty-five percent of patients who started on interferon therapy for hepatitis C go on to develop a major depressive episode.²⁹ Endogenously released cytokines can also cause depressed mood. Healthy volunteers, when given endotoxins to induce proinflammatory cytokine release, experienced both depressed mood and memory impairment. These endotoxins had no effect on physical illness symptoms but caused an increase in cytokines levels that correlated positively with depressive symptoms.³⁰ We measured IL-17 in this study based on previous studies that associated it with autoimmune thyroid disease. IL-17 levels were not significantly raised in our depressed patients. This may be because thyroid autoantibody titres in depression are not as elevated as that in autoimmune thyroid disease, hence there is minimal IL-17 stimulation.

While it is not conclusive that TRAbs are directly involved in the pathogenesis of depression, our findings suggest that neutral TRAbs may be a biomarker of low-grade immunological dysfunction in depression. The AUC for TRAb was 0.8 in this study, which is fairly good for a diagnostic test, considering that there is no reliable laboratory test for depression at present. Other stimulatory and inhibitory TRAbs may be examined in future. Besides the aforementioned autoantibodies that are associated with depression, other cellular markers of altered immune function have been reported in depression, such as leukocytosis, decreased natural killer cell cytotoxicity and reduced lymphocyte proliferation in response to mitogens.³¹ Future studies, with a larger sample size, could look into using the most consistent immunological markers together to characterise depression diagnosis and treatment response.

Conclusion

Our study showed that serum TRAb titres were able to discriminate between depressed cases and controls. IL-17 levels were not associated with TRAb titres or depression severity. TRAbs may be a useful indicator of immune dysfunction in depression.

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Genomics of Hereditary Colorectal Cancer: Lessons Learnt from 25 Years of the Singapore Polyposis Registry

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Abstract

Introduction: The Singapore Polyposis Registry (SPR) was established in 1989 in Singapore General Hospital (SGH). The aims were to provide a central registry service to facilitate identification, surveillance and management of families and individuals at high risk of colorectal cancer. **Materials and Methods:** This is a review of published literature in the department. **Results:** The registry currently has 253 families with several genetic conditions—93 familial adenomatous polyposis (FAP) families, 138 Amsterdam-criteria positive presumed Lynch syndrome (LS) families, 12 families with Peutz-Jeghers syndrome, 2 families with Cowden's syndrome, and 8 families with hereditary mixed polyposis syndrome (HMPS). There are also 169 families with a strong family history of colorectal cancer but no abnormal genes yet identified. In FAP, a diagnostic tool developed has allowed a 94% local APC germline detection rate in FAP families. Knowledge obtained studying the phenotype of FAP patients has allowed better choice of surgery between ileal pouch anal anastomosis (IPAA) against an ileal-rectal anastomosis (IRA). In LS, our review has noted a highly heterogeneous mutational spectrum and novel variants made up 46.7% (28/60) of all variants identified in this cohort. This may suggest that our Southeast Asian ethnic groups have distinct mutational variants from Western populations. Pathogenic mutations were only confined to *MLH1* and *MSH2*, and identified in 28.8% of families. **Conclusion:** The impact of predictive gene testing for hereditary cancer risk in clinical practice has allowed evolution of care. Risk-reducing surgery and aggressive surveillance allows reduction in morbidity and mortality of patients. The SPR will continue to grow and improve outcomes in hereditary colorectal cancer patients and families.

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Key words: Familial adenomatous polyposis, Genetic testing, Lynch syndrome

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Introduction

The significant advancements of genomic information for colorectal cancer (CRC) have been made largely in the last quarter of the century. The loss of the long arm of chromosome 5 in a patient with familial adenomatous polyposis (FAP) was defined in 1986.¹ Significantly, the location was confirmed and refined to 5q22 via linkage studies² and the APC gene was identified as the causative mutation in 1991.³

Up until 1962, FAP was the only known hereditary form of CRC. Henry Lynch's landmark pioneered research work in the now famous Family G, however, identified a different type of hereditary cancer,⁴ which also highlighted the importance of detailed family histories and pedigree

construction. The discovery of mismatch repair (MMR) genes (*MSH2*, *MLH1*, *MSH6*, *PMS2*)⁵ in 1993 enabled identification of mutation carriers and provided certainty that a specific hereditary cancer syndrome other than FAP existed. The roles of microsatellite instability (MSI) and immunohistochemistry (IHC) have also gradually been proven to be of significant aid in the screening and diagnosis of Lynch syndrome (LS).⁶

Hereditary colorectal cancer syndromes are now known to consist of 10% of all colorectal cancers. However, there remains a large proportion (>20%) with a familial predilection for CRC with a syndrome yet to be defined or a genotypic abnormality identified.⁷ The importance of centralising a database, to facilitate identification,

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surveillance and management of families and individuals at high risk of getting CRC has never been more important. The first polyposis registry, the St Mark's Hospital Polyposis Register, was conceived by JP Lockhart-Mummery and C Dukes in 1924.⁸ Since then, many national and regional registries have been set up with considerable impact on the reduction of colorectal cancer in FAP. These include registries in Europe⁹⁻¹¹ as well as Asia,^{12,13} and number more than 50 worldwide. There is increasing evidence that patients with FAP cared for in a registry have lower chance of having CRC at the time of diagnosis, and higher life expectancy than those who are not.^{9,14}

The Singapore Polyposis Registry (SPR) was established in 1989 in Singapore General Hospital.¹⁵ The aims of this initiative were to provide a central registry service to all doctors in Singapore to facilitate identification, surveillance and management of families and individuals at high risk of getting colorectal cancer. FAP families in Singapore were identified so that at-risk individuals may be offered current screening procedures and prophylactic surgery to prevent the almost certainty of developing colorectal cancer. In addition, family members were identified for genetic testing and counselling services provided. From an initial emphasis on FAP and hereditary non-polyposis colorectal cancer (HNPCC), patients with other polyposis types have also been included. The registry has grown in the last 25 years and currently has 253 families with 4 known genetic conditions -93 FAP families, 138 Amsterdam-criteria positive presumed LS families, 12 families with Peutz-Jeghers syndrome, 2 families with Cowden's syndrome, and 8 families with HMPS. There are also 169 other families with a strong family history of colorectal cancer but no abnormal genes yet identified. Key summary of the important findings of these hereditary colorectal cancers will be discussed.

Familial Adenomatous Polyposis (FAP)

FAP has served as an important model for the understanding of carcinogenesis and the functions of the APC gene and protein have been well studied (Figs. 1 and 2). This tumour suppressor gene codes for a large protein that comprises of 2843 amino acid residues. Severity of the disease and extra-colonic manifestations (ECM) appear to correlate with different sites of mutations. The majority of disease associated germline APC mutations causes protein truncation that enables the protein truncation test (PTT) to be adopted as main technique for mutation detection. In our lab, using a combination of cDNA-PTT, multiplex ligation-dependent probe amplification (MLPA) and differential expression techniques, our local APC germline detection rate in FAP families is 94%.^{16,17} This corroborates favourably against worldwide reported frequencies of 50% to 80% detection.¹⁸ In addition, several novel mutations including large genomic deletions were identified and submitted to the Human Gene Mutation Database (HGMD).¹⁹ These novel mutations have loss of either one or both β -catenin binding sites thus inhibiting its tumour suppressor function of regulating the Wnt/ β -catenin-Tcf signaling pathway.

In our recent clinical review of 122 patients from 88 FAP families, 73% underwent restorative proctocolectomy with ileal pouch anal anastomosis (IPAA) and 21% had total colectomies with ileorectal anastomosis (IRA). The most common ECMs were stomach fundic gland polyps and duodenal adenomatous polyps (29%). This was noted early on in the development of the SPR and was reported in 1992.²⁰ Desmoids were found in 14% of the cases and were located either in the abdominal wall or mesentery. Eight percent had extra-colonic cancers of which thyroid cancers were the most common.²¹

Our reviews of the genotypic and phenotypic

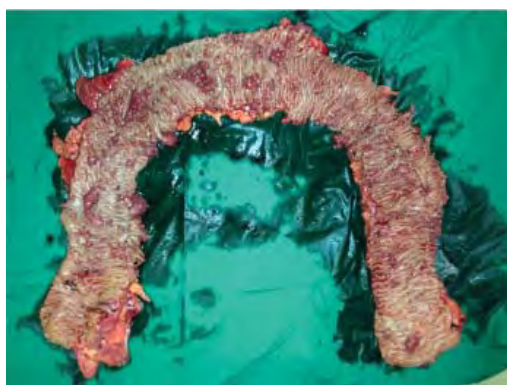


Fig. 1. Endoscopic view of a typical FAP patient.



Fig. 2. The same patient who subsequently underwent prophylactic restorative panproctocolectomy and ileal pouch anal anastomosis.

characterisations indicate that Southeast Asian families have some features similar to, and others distinct from Caucasian families from other regions of the world. Fundic gland polyposis and osteomas appeared to be associated with *APC* mutations throughout the coding region and not confined to codon 767-1573 as reported for Caucasian populations. There were also no patients with hepatoblastomas or brain tumours in our local FAP population suggesting that these ECMs are rare in Southeast Asian populations.^{19,21}

In FAP patients with desmoid tumours, we have noted that the clinical progression can be divided into “stable”, “variable”, “progressive” and “aggressive growth” which have implications on follow-up strategies. The majority of desmoids occur approximately 3 years after initial surgery but almost 30% were found to occur before any surgical stimulus. The most common complications to develop were intestinal obstruction, ureteric obstruction and encasement of superior mesenteric vessels. Unfortunately, the clinical course of desmoids remains unpredictable and no significant genetic marker was identified for prognostication.²²

The 10-year overall survival was 75.6% (95% CI, 67 to 84.2%) and the median age of death was 40 years old. Recurrence and disease-free survival was not significantly different for the type of surgery (IPAA vs IRA) performed ($P = 0.486$). This knowledge from the registry allows appropriate decisions to be made on suitable treatment for these young patients. Prophylactic surgery to minimise the risk of developing colon or rectal cancers is required in FAP families but there are various factors to consider. These considerations will be to provide the most appropriate treatment to reduce the risk of developing CRC and yet at the same time to preserve the patients’ quality of life at a satisfactory level. This is especially important in FAP patients who have to undergo radical surgery and are often young, with minimal or no symptoms. The choice of surgery remains much debated upon. While IPAA was the most common procedure performed in our department, there was a relatively large number of IRAs performed with no difference in disease-free survival. Restorative proctocolectomy was described to eradicate all at-risk colorectal mucosa and yet maintain the anal canal for good functional outcomes. But complications reported include a higher risk of night-time soilage, sexual and urinary dysfunction as well as a need for temporary defunctioning ileostomy. It may be noted from the review that IRA may be a suitable option in a select group. Patients suitable for IRA include an attenuated FAP form of disease, relatively few rectal polyps (usually recommended <20 polyps) and most importantly are compliant and willing to undergo lifelong endoscopic surveillance annually. The use of genetic testing has been proposed for 1 surgical technique over another. Bulow et al identified patients with genetic

errors in codon 0-200 or above 1500 and proposed IRA over IPAA for these patients with mild genetic mutations²³ but this has not been studied in our unit as yet.

While the study of *APC* molecular genetics is relatively mature, it continues to provide insights with general relevance to cancer biology, for example, an indel leading to splicing defect in exon 9 of *APC* has resulted in very severe rather than attenuated FAP in one recent case documented in the SPR.²⁴ In particular, the genetic profiling of our Southeast Asian populations with FAP remains relatively unknown especially in the rural areas of the region. And as travel costs reduce and healthcare access becomes more readily available, it is likely that the SPR will continue to provide more insights into mutational profiles of FAP patients.

Other Polyposis Syndromes

There are other rarer polyposis syndromes include MYH-associated polyposis, Peutz-Jegher syndrome (PJS), Cowden’s syndrome, juvenile polyposis syndrome, hyperplastic polyposis syndrome and hereditary mixed polyposis syndrome (HMPS). Our reported local experience with PJS and HMPS is discussed in more detail here.

PJS is a rare autosomal dominant inherited condition with intestinal hamartomatous polyposis and mucocutaneous melanocytic macules (Fig. 3). Germline mutation of the *STK11* (serine/threonine kinase 11) gene located on chromosome 19p13 has been shown to cause approximately 50% of PJS cases but not all cases have this mutation, raising the possibility of a second gene.²⁵ The SPR reported experience is limited to its first 7 patients and clinical presentations were usually secondary to polyp related complications. These included recurrent episodes of abdominal colic, intestinal obstruction and gastrointestinal bleeding, as well as the need for repeated laparotomies. These patients were also at increased risk of developing both gastrointestinal and extra-intestinal malignancies.²⁶ There were no genotypic or phenotypic differences compared to



Fig. 3. Mucocutaneous melanocytic macules in a Peutz-Jeghers patient.

reported Western literature although our study population is small. Cancer surveillance guideline for these patients has also been suggested based on the experience from the SPR.²⁶

HMPS is characterised by colonic polyps of mixed hyperplastic, adenomatous and juvenile components. HMPS was first described in a large Ashkenazi family, SM96, and the HMPS locus was first mapped to chromosome 6q, then remapped to 15q13 with further clinical data.^{27,28} However, a study of 8 HMPS Chinese families in our local population showed that a *BMPRI1A* gene defect on chromosome 10q23 was the disease-causing mutation in 50% of these families.²⁹ It is thus suggested in our population, that *BMPRI1A* mutation should be first screened for, if patients have large bowel polyps of mixed histology in the absence of upper gastrointestinal abnormalities, and have a family history suggestive of an autosomal dominant inherited condition.

For these polyposis syndromes, the mode of inheritance, clinical manifestations, criteria for diagnosis and screening suggestions are summarised in Table 1.

Hereditary Non-polyposis Colorectal Cancer (HNPCC)/Lynch Syndrome (LS)

LS is the most common autosomal dominantly inherited CRC and defects in the DNA-mismatch repair genes lead to

multiple errors in repetitive DNA sequences (microsatellites) throughout tumours. This genomic instability is the hallmark of LS tumours and is known as microsatellite instability (MSI). MSI itself is not proof of LS as 10% to 20% of sporadic tumours also show some degree of MSI, most commonly due to hypermethylation of *hMLH1* rather than a germline mutation in the MMR genes. The identification of LS individuals is important as it makes it possible to target effective preventative measures and allow surveillance programmes to be implemented in family members.

Clinical criteria has been modified following improved understanding of the disease. The initial Amsterdam I criteria defined in 1991 to facilitate linkage and positional cloning studies³⁰ was highly specific in order to reduce misclassification of families, but suffered from a lower sensitivity. The Amsterdam II criteria in 1999 were widened to include other extra-colonic cancers³¹ (Table 2). At present, the most important clinical criteria used is the updated Bethesda criteria and this version is widely utilised for patients as an indication for selection of families for mutational analysis³² (Table 2). However, because of the varied population-specific gene mutations, clinical phenotypes and clinical outcomes, other countries have developed their own clinical criteria. For Asian populations in particular, gastric cancer is frequently reported in LS families.

Table 1. Summary of Polyposis Syndromes

	Peutz Jegher's Syndrome (PJS)	Hereditary Mixed Polyposis Syndrome (HMPS)
Genetic defect	Mutation in <i>STK 11</i> gene located on chromosome 19p13	Mutation in <i>CRAC1</i> gene located on chromosome 15q13, <i>BMPRI1A</i> on 10q23
Inheritance	Autosomal dominant with variable penetrance	Autosomal dominant with high penetrance
Age of onset	Median of 11 years old	1. First polyp in late 20s 2. Colorectal cancer at 50 to 60 years old
Gastrointestinal manifestations	1. Hammatomatous polyps most frequently involving the small bowel, but can also involve the colorectum and stomach 2. Increased risk of oesophageal, gastric, small intestinal and colonic cancer	3. Colorectal polyps of multiple and mixed morphologies, including serrated lesions, Peutz-Jeghers polyps, juvenile polyps, adenomas and colorectal carcinoma, involving both proximal as well as distal colon 4. Increased risk of colorectal cancer 5. No upper gastrointestinal manifestations
Extra-intestinal manifestations	1. Mucocutaneous hyperpigmentation 2. Increased risk of pancreatic, breast, uterine, ovarian and testicular cancer 3. Hammatomatous polyps in bladder, lungs, nose, uterus and gallbladder	Wilms' tumour and thyroid carcinoma are associated with germline <i>BMPRI1A</i> mutations
Criteria for diagnosis	Histopathologically confirmed hammatomatous polyps and at least 2 of the following clinical criteria: 1. Family history 2. Mucocutaneous hyperpigmentation 3. Small bowel polyposis	No specific criteria. Suspected in individuals and families with a history of multiple polyps of multiple and mixed morphologies, with absence of upper gastrointestinal manifestations
Screening suggestion	Two-yearly surveillance for gastric and small bowel polyposis with upper gastrointestinal endoscopy and small bowel series or capsule endoscopy, beginning at age 10	Two-yearly colonoscopy beginning 5 to 10 years before the age of onset of youngest family member

Table 2. Amsterdam I and II Criteria, and Bethesda Guidelines for Lynch Syndrome

Amsterdam I Criteria - Families Must Fulfill All Criteria	
1.	At least 3 relatives should have histologically verified CRC; 1 of them should be a first-degree relative to the other 2.
2.	At least 2 successive generations should be affected.
3.	In 1 of the relatives, CRC should be diagnosed under 50 years of age.
4.	FAP should be excluded.
Amsterdam II Criteria - Families Must Fulfill All Criteria	
1.	There should be at least 3 relatives with an HNPCC-associated cancer (colorectal, endometrium, stomach, small bowel, ureter or renal pelvis, brain, hepatobiliary tract and skin).
2.	One should be a first-degree relative of the other 2.
3.	At least 3 successive generations should be affected.
4.	At least 1 should be diagnosed before age 50.
5.	FAP should be excluded in the colorectal case(s), if any.
6.	Tumours should be verified by pathological examination.
Bethesda Guidelines for Testing for Colorectal Tumour for Microsatellite Instability	
1.	Colorectal cancer diagnosed in a patient who is less than 50 years of age.
2.	Presence of synchronous or metachronous colorectal or HNPCC-associated tumours.
3.	Colorectal cancer with the MSI-H histology diagnosed in a patient who is less than 60 years of age. (Presence of tumour infiltrating lymphocytes, Crohn disease-like lymphocytic reaction, mucinous/signet-ring differentiation or medullary growth pattern)
4.	Colorectal cancer or HNPCC-associated tumour diagnosed under age 50 years in at least 1 first-degree relative.
5.	Colorectal cancer or HNPCC-associated tumour diagnosed at any age in 2 first-or second-degree relatives.

CRC: Colorectal cancer; FAP: Familial adenomatous polyposis; HNPCC: Hereditary non-polyposis colorectal cancer; MSI-H: Microsatellite instability-high

In LS, there is an estimated 68% to 82% lifetime risk of CRC, early age of onset of related cancers; tumours are predominantly located in the proximal colon (60% to 70%), with approximately 10% to 30% developing synchronous or metachronous cancers. Overall 5-year survival rates in affected family members are better than that seen in sporadic CRC. In our local review of predominantly Chinese patients from 42 families based on Amsterdam I and II criteria, significantly, 69% of tumours in this Amsterdam defined cohort were in contrast left-sided, the majority being sigmoid cancers.³³ This interesting trend in our predominantly Chinese population mirrors a similar review done in China, where a dominant manifestation of left-sided CRCs (60.6%) and lower synchronous tumour incidence (8.5%) was also reported. In this series, only one-third of the kindreds had germline mutations.³⁴ This contrasts with Western reports emphasising the importance of right-sided colon cancer in the diagnosis of LS and has important implications in management of Asian HNPCC patients.

We have recently completed our genetic and epimutational profiling of LS families in Singapore. Amongst the 5 MMR genes screened, pathogenic mutations were only confined to *MLH1* and *MSH2*, and identified in 28.8% of the families.³⁵ The mutational spectrum was highly heterogeneous and novel variants (6 pathogenic, 20 variants of unknown

significance and 2 single nucleotide polymorphisms (SNPs) made up 46.7% (28/60) of all variants identified in this cohort. This again may further reinforce, that our Southeast Asian ethnic groups (Chinese and Malay) have distinct mutational variants from Western populations. Interestingly, 1 recurrent mutation in *MLH1* (c.793C>T) was also observed in 5 patients from 3 unrelated Chinese families, accounting for 21.7% (5/23) of LS cases. This mutation would lead to skipping of exon 10. In Taiwanese Chinese, founder effect of this mutation has been established in 13 LS families.³⁶ While we are not able to confirm the possible founder effect of this mutation in local Chinese LS kindreds, it may potentially be a cost-effective LS screening start point for Singaporean Chinese, before exhaustive scanning of the common MMR genes.

One important finding in our study is the 92.9% sensitivity conferred by IHC that surpassed 64.3% sensitivity by MSI. We also noted that 15.6% of patients with MSS tumours harboured pathogenic mutations and thus may potentially have been excluded from further mutational evaluation for MMR defects.³⁵ Our results may thus provide an initial guide to a diagnostic molecular screening algorithm. The over-reliance of Amsterdam criteria should be taken into consideration and this criteria as well as microsatellite marker panels may not be suitable in Asian populations.

In general, IHC screening is simple, fast and cost-effective with little added facilities required and are thus more cost-effective. One additional advantage is that the mutated MMR gene can be pinpointed through a loss of protein staining. IHC in our population may thus be superior to MSI as a screening tool for deficient MMR.

Future of Singapore Polyposis Registry (SPR) and Genetic Testing

Twenty-five years on, the SPR continues to strive to improve outcomes in hereditary colorectal cancer patients and their family members. The impact of predictive gene testing for hereditary cancer risk in clinical practice has allowed evolution of changes in care. Risk-reducing surgery and aggressive surveillance allows reduction in morbidity and mortality of patients. The decision for genetic testing however is a multistep process that requires several decision points. For the individual, this includes: whether to seek counselling; undergo mutation testing; receipt of test results and finally to accept surveillance protocols or possible prophylactic surgery. The other big decision conundrum will be whether and when to share results with family members. It is important as knowledge evolves that awareness of the various psychological outcomes after genetic testing remain paramount to these test individuals especially after disclosure to mutation carriers. Sharing of information to relatives is also sensitive and efforts will continue to encourage individuals to communicate test results to various family members. Future research will be to address current gaps in knowledge, provide informative strategies for facilitating optimal delivery of clinical services for high-risk populations in the nation, as well as provide greater awareness on a national level.

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Disseminated *Bacillus Calmette-Guérin* and Susceptibility to Mycobacterial Infections—Implications on *Bacillus Calmette-Guérin* Vaccinations*

Pamela PW Lee, ¹MBBS, FHKAM, FHKCPaed

Abstract

Bacillus Calmette-Guérin (BCG) is a live vaccine and has the potential to cause local disease and systemic dissemination in immunocompromised hosts, including infants who are infected with human immunodeficiency virus (HIV) through vertical transmission, and patients with primary immunodeficiencies (PID) such as severe combined immunodeficiency (SCID), chronic granulomatous disease (CGD), hyper-IgM syndrome, and defects of the IL12-IFN γ axis (Mendelian susceptibility to mycobacterial diseases, MSMD). Disseminated BCG is extremely difficult to treat. The chance of complete eradication is low unless functional immune response is restored by haematopoietic stem cell transplant. Prolonged use of anti-mycobacterial drugs often causes organ toxicities and drug resistance. Inflammatory complications which develop upon immunoreconstitution post-transplant may necessitate immunosuppressive treatment, which adversely affect immune recovery and increases risks of opportunistic infections. Multiple BCG reactivations can occur in patients with CGD and MSMD, and BCG can remain latent until reactivations take place in adulthood and manifest as disease. It is important for neonatologists, general practitioners, primary care clinicians and nurses working in maternal and child care centers to be aware of BCG-related complications, which may be the first sign of an underlying immunodeficiency. As neonatal BCG is included in standard vaccination schedule in many countries, it is a challenge to identify and avoid administration of BCG to infants who potentially have PIDs. Deferring BCG vaccination is recently advocated to protect highly vulnerable populations, but the appropriate strategy is yet to be determined. Newborn screening for SCID offers a potential to avoid this complication, if an integrated system of screening and vaccination can be organised.

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Key words: BCG, Newborn screening, Primary immunodeficiency

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Introduction

Tuberculosis (TB) is endemic in Asia. Out of 22 countries with high TB burden, 10 are Asian countries.¹ *Bacillus Calmette-Guérin* (BCG) vaccine is one of the world's most widely used vaccine. It is a live vaccine made from attenuated strains of *Mycobacterium bovis* (*M. bovis*). BCG vaccine has consistently shown high efficacy against childhood tuberculous meningitis and miliary TB, while efficacy against adult pulmonary TB and other mycobacterial diseases is more variable. In a study performed by Trunz et al, it was estimated that 100 million doses of BCG vaccine given to children every year prevent about 40,000 cases of TB meningitis and miliary TB before these children

reach their fifth birthdays, or roughly 1 case prevented for every 2500 inoculations. In particular, the numbers of cases prevented would be highest in Southeast Asia (46%), compared with 27% in sub-Saharan Africa and 15% in the Western Pacific region.² The coverage rate of BCG vaccine in Southeast Asia and Western Pacific is 88% and 97%, respectively.³

Common Complications after BCG Inoculation

In most countries, BCG vaccine is administered to neonates soon after birth by intradermal inoculation, typically over the left upper deltoid region. Inoculation site reaction commonly begins with an erythematous induration of 5 mm to 15 mm, followed by formation of a bluish-red

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pustule 2 to 3 weeks later. Ulceration, drainage and exudative crust formation may occur after 4 to 6 weeks, and finally heals with central crusting leaving a small residual scar. Sometimes, complications such as local abscess, ulcers or regional suppurative lymphadenitis may occur, reported to affect approximately 0.1 to 0.5/1000 vaccinees.⁴ These are related to age of the recipient, inoculation technique, strain and reactogenicity of the BCG in use. In most cases, these local complications resolve without intervention or sequelae. Suppurative lymphadenitis can be treated with needle aspiration, while surgical excision may be required if there are multiloculated or matted lymph nodes. Incision and drainage is not recommended, as this is prone to cause sinus formation, persistent discharge, poor wound healing and scarring.⁵⁻⁸

Disseminated BCG

The estimated incidence of disseminated BCG is 1 to 3.4 per million.⁹⁻¹¹ Distant BCG, defined as involvement of any site beyond a local or regional ipsilateral process, includes any of the following: BCG confirmed from at least 1 distant site beyond the vaccination site e.g. pulmonary secretions (gastric aspirate, tracheal aspirate), cerebrospinal fluid, urine, osteitis and distant skin lesions.¹⁰ The diagnostic criteria for disseminated BCG as defined by the European Society for Immunodeficiency (ESID) can be found on the ESID website.¹² Distant and disseminated BCG should be regarded as an indicator for underlying immunodeficiency until proven otherwise. The estimated incidence of disseminated BCG in HIV-infected babies who receive BCG vaccination is nearly 1/100.¹³ If HIV is excluded, primary immunodeficiency disorders (PIDs) should be suspected.

Disseminated BCG in Patients with Primary Immunodeficiencies

Innate immunity, particularly phagocytic oxidative burst activity and the interleukin-12/interferon-gamma (IL-12/IFN- γ) axis, as well as T-cell immunity are crucial in protective immune response against mycobacteria. PIDs which are associated with disseminated BCG include severe combined immunodeficiency (SCID), chronic granulomatous disease (CGD), X-linked hyper-IgM syndrome (CD40 ligand deficiency) and Mendelian susceptibility to mycobacterial diseases (MSMD).¹⁴ The clinical features and laboratory findings are listed in Table 1.

Disseminated BCG: Clinical Features

All infants presenting with BCG complications should be thoroughly examined for signs of distant or disseminated disease. Clinicians should look for enlarged lymph nodes

in the neck, supraclavicular region and groin. The liver and spleen are often enlarged in disseminated disease. Abnormal respiratory signs may suggest pulmonary involvement, and chest x-ray should be performed to look for abnormal opacities or infiltration. Dissemination to skin often manifest as erythematous papules or indurations (Fig. 1a). Bone involvement may present as swelling or tenderness. Osteolytic lesions may be seen on the x-ray (Fig. 1b), and the extent can be evaluated by bone scan. Bone marrow involvement should be suspected if there is anaemia, and a microbiologist should be consulted as special culture medium and prolonged culture is required to maximise the yield of BCG. One should carefully examine for neurological deficits, and brain imaging and lumbar puncture should be performed to exclude BCG meningitis.

Infants with disseminated BCG are often susceptible to other infections, and the clues to the possibility of an underlying PID may be identified by thorough physical examination. First, they often fail to thrive as a result of repeated episodes of infection and poor feeding. Mucocutaneous candidiasis is suggestive of T-cell deficiency or gain-of-function STAT1 defect. In addition to BCG, infants with SCID are susceptible to *Pneumocystis jiroveci pneumonia* (PCP), viral (respiratory viruses and cytomegalovirus) and fungal pneumonia (*Aspergillus*), and bronchoalveolar lavage should be considered for microbiological documentation. Patients with CGD often have pyogenic infection such as perianal abscess, lymphadenitis, osteomyelitis and hepatosplenic abscess.

Immune Evaluation for Infants with Disseminated BCG

While definitive diagnosis of PIDs may require sophisticated functional and molecular studies, a significant proportion of PIDs can be picked up by simple tests such as full blood count and serum immunoglobulin levels. The fact that absolute lymphocyte count (ALC) $<2.5 \times 10^9/L$ is abnormal in infants is often under-recognised. An infant presenting with recurrent infections and failure to thrive with lymphopenia on multiple occasions, together with absent thymic shadow on chest x-ray should lead to a high suspicion of SCID.¹⁵ Early referral to immunologists for diagnostic evaluation is essential, and this has a significant impact on the survival and prognosis of PIDs. Timely commencement of treatment will prevent organ damage and preserve the best outcome for patients whose disease is amenable to cure by haematopoietic stem cell transplant (HSCT).

Basic immune evaluation includes lymphocyte subset for enumeration of T-cells, B-cells and NK-cells, immunoglobulin levels (IgG, A, M) as well as evaluation of neutrophil oxidative burst by nitroblue tetrazolium test (NBT) or dihydrorhodamine test (DHR). Functional analysis of the IL12-IFN γ axis is only available in specialised

Table 1. Clinical and Immunological Features of Primary Immunodeficiency Predisposing to BCG Disease

PID	Inheritance	Infections Other Than BCG Disease	Non-infective Manifestations	Immunological Investigations
Severe combined immunodeficiency	X-linked: IL2RG deficiency Autosomal recessive: T-B+ SCID: e.g. <i>JAK3</i> , <i>IL7R</i> T-B- SCID: e.g. <i>RAG1</i> , <i>RAG2</i> , <i>Artemis</i> , <i>LIG4</i> ADA deficiency Complete DiGeorge syndrome Cartilage hair hypoplasia	Bacterial sepsis Respiratory viruses (RSV, parainfluenza, influenza) CMV, EBV Fungal: mucocutaneous candidiasis, candidemia, aspergillosis <i>Pneumocystis jiroveci</i> pneumonia Complications associated with other live vaccines: rotavirus, varicella zoster, oral polio vaccine	Autoimmunity associated with Omenn syndrome (leaky SCID) Maternal engraftment syndrome resulting in graft-versus-host disease Dysmorphism in some forms of SCID e.g. cartilage hair hypoplasia, <i>LIG4</i> deficiency	IgG/A/M Lymphocyte subset Lymphocyte proliferation Measures of thymic output: naïve T-cells, TRECs Molecular confirmation: Flow cytometry for protein expression (e.g. γ c, <i>JAK3</i> , <i>IL7R</i>) FISH for 22q11.2 deletion Genetic test
Chronic granulomatous disease	X-linked: CYBB deficiency Autosomal recessive: e.g. <i>CYBA</i> , <i>NCF1</i> , <i>NCF2</i>	Bacterial sepsis (e.g. <i>S. aureus</i> , <i>E. coli</i> , <i>Salmonella</i> , <i>Klebsiella</i> , <i>Pseudomonas</i>) <i>Nocardia</i> , <i>Serratia</i> , <i>Aspergillus</i> , <i>Burkholderia cepacia</i> , <i>B. pseudomallei</i>	Granuloma formation	Nitroblue tetrazolium test or dihydrorhodamine test Genetic test
Mendelian susceptibility to mycobacterial diseases	Autosomal recessive: <i>IL12B</i> , <i>IL12RB1</i> , <i>IFNGR1</i> , <i>IFNGR2</i> , loss-of-function <i>STAT1</i> , <i>NEMO</i> , <i>TYK2</i>	Non-tuberculous mycobacteria Tuberculosis Non-typhoidal salmonellosis	NEMO: anhidrotic ectodermal dysplasia	Functional studies of the <i>IL12/IFN-γ</i> axis Genetic test
Gain-of-function <i>STAT1</i> defect	Autosomal dominant	Chronic mucocutaneous candidiasis Invasive mycosis e.g. aspergillosis, coccidioidomycosis, histoplasmosis, <i>Penicillium marneffe</i> Non-tuberculous mycobacteria Viral infections e.g. CMV, EBV	Autoimmunity e.g. type 1 diabetes mellitus, hypothyroidism, enteropathy	Flow cytometry for phosphorylated <i>STAT1</i> expression Genetic test
<i>CD40</i> ligand deficiency	X-linked	Encapsulated bacteria e.g. <i>S. pneumoniae</i> Cryptosporidium <i>Toxoplasma gondii</i> <i>Pneumocystis jiroveci</i>	Autoimmunity	IgG/A/M <i>CD40</i> ligand expression by flow cytometry Genetic test

ADA: Adenosine deaminase; CMV: Cytomegalovirus; EBV: Epstein-Barr virus; FISH: Fluorescent in situ hybridisation; RSV: Respiratory syncytial virus; SCID: Severe combined immunodeficiency; TREC: T-cell receptor excision circles

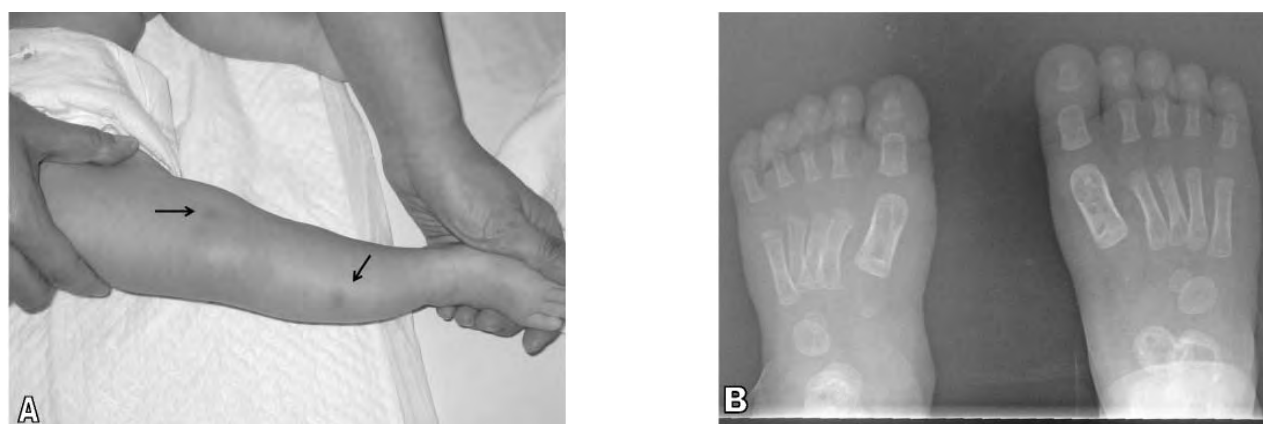


Fig. 1. Disseminated BCG in SCID patients, manifesting as erythematous skin nodules in A and osteolytic bone lesions in B. Multiple moth-eaten lesions can be seen in the metatarsal bones.

laboratories. Genetic testing provides definitive diagnosis and useful information for family screening and future prenatal diagnosis.

Challenges in the Management of BCG Disease in Patients with PID

It is recommended that anti-TB treatment including 4 or more anti-TB drugs should be given to patients with PID until complete recovery.¹² Then, a prophylactic regimen with 2 drugs should be continued, until complete immunological reconstitution after HSCT is achieved. It should be noted that BCG strains are inherently resistant to pyrazinamide. Commonly used anti-BCG regimen consists of a backbone of 3 first-line anti-TB drugs (isoniazid, rifampicin and ethambutol), plus an additional agent to which BCG is also susceptible, such as quinolone (e.g. ciprofloxacin, levofloxacin), aminoglycoside (e.g. amikacin, streptomycin) and clarithromycin.¹⁶ Low-level isoniazid resistance was observed in the Denmark strain (SSI 1331) and Connaught strain.^{16,17} The regimen should be reviewed when culture and sensitivity results are available, and modified when side effects arise.

BCG disease does not only contribute to morbidity and mortality for infants prior to transplant, but also complicates post-transplant course and adversely affect recovery and quality of life. Manifestations range from fever, skin papules, ulceration and discharge from the BCG inoculation site, hepatosplenomegaly and osteomyelitis, often become more severe after transplant as the donor graft begin to mount an immune response in an attempt to clear the BCG. Enlarged intra-abdominal and groin lymph nodes can result in lymphedema. Immunoreconstitution syndrome (IRIS) presenting as fever, hypercalcaemia and renal impairment requires treatment with corticosteroid. Disseminated BCG disease is difficult to control, and surgical drainage of abscess might be needed. Drug resistance could also occur, and patients might require prolonged course of antimycobacterial treatment with potential toxicities.¹⁸⁻²⁴

Disseminated BCG Disease—Is It Preventable?

According to a multi-centred survey on 349 BCG-vaccinated SCID infants, 51% had BCG-associated complications and two-thirds of them had disseminated BCG, which was a 33,000-fold increased risk over the general population.²⁵ In another cohort of 40 Iranian infants with SCID, the incidence of disseminated BCG was as high as 45%.²⁶ In many countries, BCG is incorporated into standard neonatal vaccination schedule and infants are often vaccinated during the newborn period. Infants with undiagnosed PID might have received BCG, which would be contraindicated should their condition been known. It was

advocated that careful family history should be taken prior to neonatal vaccination.^{23,27} Practically, the awareness of a family history of vaccine-related complications should be promoted among pregnant women in their antenatal visits, so that sufficient opportunity for detailed evaluation and counselling is possible. However, a positive family history will be present in <10% of SCID cases.

The study by Marciano et al (2014) showed that infants with SCID who received BCG beyond the first month of life had lower prevalence of BCG-associated complications compared to those vaccinated within the first month (38% vs 55%), and none of them died of BCG-related complications (0% vs 18%). It was suggested that deferring universal BCG vaccination until after 1 month of age would diminish BCG-associated complications in these vulnerable immunodeficient individuals. This is even more relevant to countries which are planning to implement newborn screening (NBS) for SCID, as it is logical to administer BCG only after NBS result is available and to exclude infants who are screened positive from BCG vaccination. Such a policy change may give rise to concerns about a decrease in coverage due to “missed opportunity” of vaccinating patients after birth and the potential increased risk of BCG-preventable diseases during the “unprotected” intervals. The counter-argument is that BCG vaccine has a documented protective effect against meningitis and disseminated TB in children, but it does not prevent primary infection and reactivation of latent pulmonary TB, which is the principal source of bacillary spread in the community.

Vaccine coverage is another potential concern. In Western Pacific, the coverage of BCG at birth (97%) is similar to the coverage of the third dose of diphtheria-pertussis-tetanus (DTP) vaccine (96%), which is a key indicator of immunisation programme performance.³ This implies that delaying BCG vaccination will not compromise the coverage rate in a robust public healthcare system. However, it should be noted that in Southeast Asia, the coverage of BCG vaccine at birth is 90% while that of the third dose of DTP vaccine is only 77%.³ The appropriate strategy to prevent administering live vaccines to susceptible infants and yet achieving satisfactory coverage of BCG vaccine is yet to be determined.

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Does Limited Tourniquet Usage in Primary Total Knee Arthroplasty Result in Better Functional Outcomes?

Dear Editor,

Pneumatic tourniquet use has gained popularity in peripheral limb operations since its advent in 1904 by Harvey Cushing, permitting tourniquet pressure to be monitored and manually controlled throughout surgery. In total knee arthroplasty (TKA), application of a mid-thigh pneumatic tourniquet offers the possible benefits of providing a dry operating field, reducing intraoperative blood loss, allowing cement to set in an optimal and completely dry environment thus potentially improving bone-cement inter-digitation and reducing operative time.¹⁻⁴

There is ongoing controversy with regard to risks associated with tourniquet use in TKA surgery.^{2,5-10} Many authors have advocated that tourniquet usage compromises the ability to perform early straight-leg-raise and knee flexion after surgery, increases risks of nerve, muscle and vascular injury, as well as postoperative swelling, stiffness and pain.^{5,11-13} Cardiorespiratory function may be affected by tourniquet inflation and deflation, which could lead to intraoperative hypoxia, pulmonary embolic events and cardiac arrest.⁹ Reactive hyperaemia has also been noted on tourniquet deflation, which may cause substantial bleeding and affect operation time.^{2,8,10} Tourniquets may also be related to an increased incidence of early infection and wound healing disorders due to perioperative hypoxia and reduced postoperative tissue perfusion.^{5,7,8} Several authors have correlated tourniquet use and an increased incidence of venous thromboembolic phenomena.^{6,7,8}

Arthroplasty surgeons continue to face a dilemma on the ideal time of tourniquet release. The relationship between duration of tourniquet use and development of postoperative complications also remains controversial. This has resulted in variations in the profile of tourniquet use; either throughout the procedure, use for part of the procedure, or none at all. There have been few prospective studies evaluating clinical outcomes of partial or limited tourniquet use in TKA surgery. There is scant literature on the effects of cement inter-digitation, or long-term outcomes of implant loosening or radiolucency between patients who received conventional tourniquet compared to limited tourniquet and non-tourniquet assisted TKA surgery. The aim of this study is to determine whether the limited use of a tourniquet during TKA surgery affects predefined clinical outcomes.

Materials and Methods

We reviewed 121 cases of TKA performed by single surgeon (senior author) over a 4-year period between 2005 to 2008 and had been prospectively entered into a database. Using preliminary data, we calculated that our group sizes are adequately powered to detect an effect size of 0.4 at 80% power. All operations were done for osteoarthritis. Only unilateral surgeries were performed. Patients with inflammatory arthritis on potential procoagulant medications were excluded. There were no anticoagulated patients in this cohort. The first 68 patients underwent surgery with limited tourniquet use and the subsequent 53 patients underwent surgery with conventional tourniquet use. Tourniquets used were automatic pneumatic tourniquets and pressures were standardised at 300 mmHg. We defined "limited tourniquet use" as deflation of tourniquet until cementation of implants and "conventional tourniquet use" as tourniquet use throughout surgery and deflation after wound closure and application of initial dressings. The following data parameters were recorded: age, gender, race, coexisting medical problems, type of anaesthesia, use of tourniquet and tourniquet time, operating time and drain volumes postoperatively. Institutional review board (IRB) approval was obtained for the study and informed consent was taken from all patients.

The 2 groups were well-matched in terms of age, gender, ethnicity, comorbidities, use of aspirin, type of anaesthesia, and preoperative range-of-motion. Postoperative incidence of deep vein thrombosis (DVT) was evaluated. Preoperative and postoperative pain scores using the visual analogue scale (VAS), joint range-of-motion, and radiographic evidence of implant loosening, were assessed over a period of 2 years postoperatively.

Surgical Technique and Postoperative Rehabilitation

A longitudinal midline incision and medial parapatellar arthrotomy was performed in all operations. The prostheses used were all cemented implants (Scorpio NRG, Stryker, Mawah, New Jersey). All knee prostheses were posterior-stabilised and the patella was not resurfaced. With reference to tourniquet use, there were 2 subsets of patients namely Group A (conventional tourniquet), who had the tourniquet inflated until cementation of the implants, wound closure and

Table 1. Summary of Patient Cohort

Characteristics	Group A: Tourniquet Up Throughout Surgery (n = 53)	Group B: Tourniquet Deflated After Cementation (n = 68)
Mean age	66.7 ± 7.08	66.4 ± 7.2
Gender		
Male	14	19
Female	39	49
Race		
Chinese	49 (92.4%)	53 (77.9%)
Indian	2 (3.8%)	8 (11.8%)
Malay	1 (1.9%)	5 (7.4%)
Others	1 (1.9%)	2 (2.9%)
Comorbidities		
Hypertension	43 (81.1%)	51 (75%)
Diabetes mellitus	19 (35.8%)	18 (26.4%)
Prior cerebral vascular accident	6 (11.3%)	2 (2.9%)
Taking aspirin	7 (13.2%)	7 (10.3%)
Type of anaesthesia		
General anaesthesia	50 (94.3%)	59 (86.8%)
Regional anaesthesia	3 (5.7%)	9 (13.2%)
Mean operative time (min)	81.6 ± 11.8	97.7 ± 21.9
Mean tourniquet time (min)	84.8 ± 11.2	48.7 ± 12.7
Mean postoperative drain volume (mL, 24 hours after operation)	235.3	224.6

application of initial dressings, and Group B, who had the tourniquet deflated just after cementation of implants (limited tourniquet). In the subgroup with limited tourniquet usage, compression bandaging was performed after implantation of the cemented endoprosthesis (both femoral and tibial components) and the cement was allowed to set with the knee in full extension. The tourniquet was deflated at this point. This adoption of an event-based criterion ensured consistency in defining the use of the tourniquet.

All patients received a single dose of intravenous cefazolin (first-generation cephalosporin) prior to anaesthesia and 2 subsequent doses postoperatively, 8 hours apart. All patients had 1 Radovac drain placed for 24 hours and drainage volumes were charted. All patients were given mechanical calf pumps for DVT prophylaxis. Physiotherapy for ambulation with a walking frame was commenced on postoperative day 1. A routine Doppler ultrasound examination for DVT was performed on postoperative day 5.

Patients were followed up in the outpatient clinic at 2 weeks, 6 weeks, 3 months, 6 months, 1 year and 2 years postoperatively. During the follow-up visits, wound

examination was done by the senior author, and range-of-motion measurements were performed using goniometers. Radiographs were taken on postoperative day 1, at 6 weeks postoperatively, 3 months, 6 months, 1 year and 2 years postoperatively.

Statistical Analysis

All data was analysed with the use of SPSS version 18.0. All categorical variables were evaluated by the Fisher's exact test. Continuous variables were evaluated by Student's t-test. The level of significance was established at 95% confidence limits ($P < 0.05$).

Results

There were 88 female and 33 male patients. Of these, 102 were Chinese, 10 were Indians, 6 were Malays and 3 were classified under other ethnic origins. The mean age was 66.5 years. There were 53 patients in Group A (conventional tourniquet use) and 68 patients in Group B (limited tourniquet use). There were no significant differences in age, gender, race, presence of comorbidities and usage of aspirin between the 2 groups.

There was no significant difference in type of anaesthesia, type of implant, and postoperative haemoglobin as well as postoperative drain volumes between the 2 groups. The mean operating time was significantly higher in Group B as compared to Group A (97.7 vs 81.6 minutes, $P = 0.00$). Table 1 shows the summary of the patient cohort.

There was no significant difference in the postoperative range-of-motion ($P = 0.754$) between the 2 groups. The improvement in the knee range-of-motion postoperatively in the 2 groups is summarised in Table 2 and Figure 1.

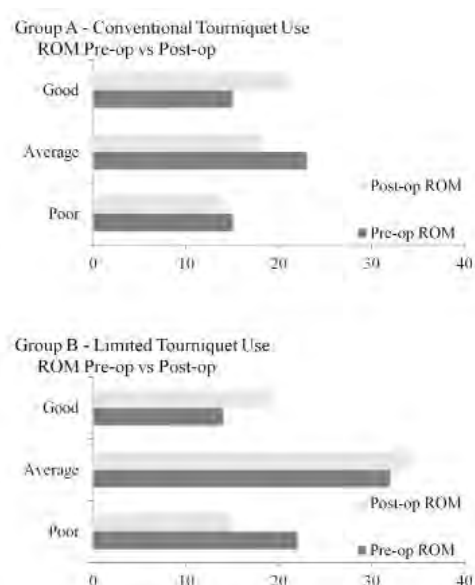


Fig. 1. Comparison of range-of-motion among the 2 groups. Poor: <90 degrees; Average: 90-110 degrees; Good: 110 degrees.

Table 2. Knee Range-of-Motion at 2 Year Follow-up

Characteristics	Group A: Tourniquet Up Throughout Surgery (n = 53)	Group B: Tourniquet Deflated After Cementation (n = 68)
Preoperative ROM	97.2° +/- 19.2°	93.0° +/- 22.4°
Postoperative ROM	117.3° +/- 15.2°	116.5° +/- 12.6°
Mean difference	20.1°, 95% CI, (14.8°, 25.4°)	23.5°, 95% CI, (18.2°, 28.8°)
Improvement in ROM in %	25.1%	30.3%

ROM: Range of motion

The most important predictor of postoperative range-of-motion was preoperative range-of-motion. The observation was the same at 2 years follow-up.

There was a significant difference ($P = 0.04$) in the overall (proximal and distal DVT) incidence of postoperative DVT when the tourniquet was used in a limited fashion as compared with conventional tourniquet use. In the subgroup of patients whom tourniquet use was done in the conventional manner, there were 12 cases (23%) of overall postoperative DVT. In the subgroup of patients whom tourniquet use was done in a limited fashion, the incidence of overall postoperative DVT was 9% (6 cases). This difference was statistically significant ($P = 0.04$). However, the incidence of proximal DVT in both groups was similar (5.6% and 4.4%, $P = 0.13$, Fig. 2).

One month after surgery, there were 24 patients (45%) in Group A who reported postoperative pain needing daily painkillers and occasionally limiting rehabilitation, and 18 (26.5%) patients in Group B reported the same. At the 2 year follow-up outpatient review, most patients reported mild or no pain (Fig. 1). Nine (17%) patients in Group A reported postoperative pain, while 6 (8.5%) in Group B reported postoperative pain (Fig. 3). The difference in prevalence of postoperative knee pain at 2 year postoperatively between the 2 groups was statistically significant ($P = 0.03$).

During the follow-up over 2 years, there was no case with radiographic evidence of implant loosening in both groups. We defined a strict criteria for radiographic implant loosening comprising of periprosthetic lucent lines, lucencies and

changes in implant position in an attempt to include early and subtle cases of loosening. All radiographs were reviewed by the senior author and a consultant musculoskeletal radiologist.

Of the 121 patients that underwent total knee replacement, none experienced complications such as dislocation, wound infection or dehiscence, nerve or vascular injury and periprosthetic fractures.

Discussion

Limited or no tourniquet use has often been suggested to increase operative time and blood loss. Our approach has been postulated to cause a compromised cementation field and suboptimal implant fixation, affecting the surgical and functional outcomes. In our study, however, we did not encounter any technical difficulties in limited tourniquet use. The complication rate was not increased and the functional outcomes of the patients postoperatively were not affected. Rather, the limited tourniquet subgroup fared better in terms of pain scores at 2 year follow-up and had a lower overall incidence of DVT in the immediate postoperative period.

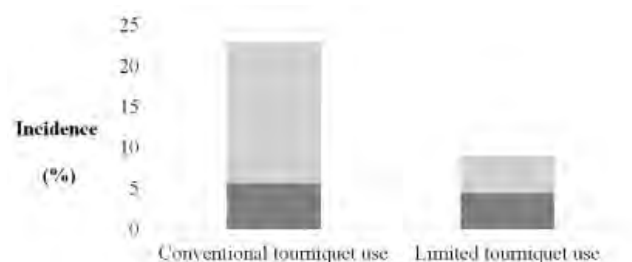


Fig. 2. Incidences of overall and proximal DVT among patients with limited tourniquet use and conventional tourniquet use (dark shaded areas represent incidence of proximal DVT).

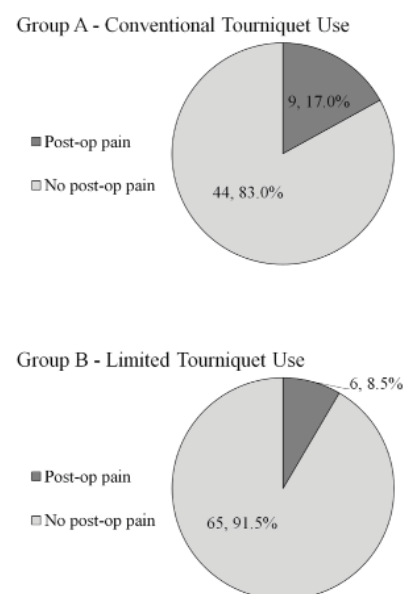


Fig. 3. Comparison of knee pain between patients with conventional and limited tourniquet use at 2 year follow-up.

Over the 2 year follow-up period of the subjects, there was no radiographic evidence of implant loosening which may be attributed to blood at the cement-bone interface leading to compromised cement interdigitation. The cement itself has probably played a hemostatic role on bleeding bone surfaces.¹⁴ It is therefore unlikely that the bone-cement interdigitation process will be compromised in the absence of a tourniquet.

Moreover, patients with limited tourniquet use experienced good surgical and functional results. Our findings were in agreement with Abdel-Salam and Eyres,⁷ in which patients with limited tourniquet use experienced less postoperative pain. We attribute the reduction in postoperative knee pain as a result of reduced direct pressure and injury to the nerves and soft tissue from limited tourniquet use.⁸

Limb swelling from reactive hyperemia from tourniquet use could increase the tension of the soft tissue around the knee, hinder knee flexion movement, reduce knee range-of-motion and pain.^{5,11-13} In our study, there was no significant difference in postoperative range-of-motion between patients who had undergone surgery with conventional tourniquet usage and those with limited tourniquet usage. With the exclusion of tourniquet during the cementation phase, the knee range-of-motion postoperatively is not significantly affected, disputing the belief by some authors that the process of bone-cement interdigitation would be compromised and leading to worse functional outcomes and knee range-of-motion.

The effect of tourniquet use on blood loss has been reported in several studies. Smith TO and Hing CB,² in a meta-analysis of 15 studies, have also found that there is no advantage to using a tourniquet in TKA for the reduction of transfusion requirements or reducing total blood loss. Several publications have also shown that there was no difference in total blood loss and transfusion requirements.^{3,10,13-15} Bin Li et al¹² has concluded that postoperative wound blood loss, the hidden blood loss and the calculated total blood loss were significantly greater in tourniquet usage. Although there could be some advantage in reducing intraoperative blood loss with the use of tourniquet, this could be offset by significantly greater overall blood loss as a result of reactive hyperemia.⁸ In our study, there were no significant differences in drain volumes between the 2 subgroups.

There is a significant reduction in overall risk of postoperative DVT through limited tourniquet use in TKA, without compromising the postoperative results. The overall incidence of postoperative DVT is significantly lower with limited tourniquet use ($P = 0.04$). Chung et al¹⁶ reported that the incidence of DVT appeared to be increased in patients with a higher tourniquet time but their results did not show statistical significance. We believe that shortening the tourniquet time leads to a lower incidence of DVT by reducing the duration of venous stasis. Eliminating tourniquet usage completely may increase surgical times further thereby placing the patient at

higher risk of infection and DVT. Usage of the tourniquet in a limited fashion is a reasonable compromise.

Limitations of our study include the lack of comprehensive assessment of subjective symptoms of pain and it not being a randomised controlled study. It is a prospective cohort study in which the first 68 patients underwent surgery with limited tourniquet use and the subsequent 53 patients underwent surgery with conventional tourniquet use. Moreover, being a single-surgeon study, variability in surgical technique affecting the results has been minimised. The distribution of patients in both groups was even so as to eliminate confounding factors. The relative proportion of regional and general anaesthesia differed slightly between the 2 groups, but this difference was not of statistical significance.

There exists significant controversy in thromboprophylaxis protocols for patients undergoing TKA in the Asian context. Chung et al¹⁶ mention that no often regular prophylaxis is given in Asia, compared to Western countries due to perceived differences in the incidence of venous thrombo-embolic phenomena. The duration of chemical thromboprophylaxis remains controversial.^{17,18} In addition, duplex ultrasound may overestimate the incidence of DVT. In our cohort of patients, routine chemical thromboprophylaxis was not administered (in keeping with institutional guidelines at that time). All patients received mechanical calf pumps and patients with above knee DVT received anticoagulation for 6 months. However this is unlikely to affect the results as routine chemoprophylaxis may lower the overall incidence of DVT but the difference in DVT incidence due to duration of tourniquet usage will not be affected as long as the 2 groups remain consistent.

Conclusion

In this study, patients who had undergone TKA with limited tourniquet use had lower overall incidences of postoperative DVT and fared better in terms of postoperative pain at 2 years of follow-up. Clinical outcomes of joint range-of-motion and radiographic evidence of implant loosening were comparable in both groups. The routine use of tourniquet in TKA surgery should probably be re-evaluated.

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A Tribute to Tham Cheok Fai, “Founding Father” of Singapore Neurosurgery

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Introduction

In 1960, Dr Tseng Yuen Lin returned home as the first formally trained Singaporean neurosurgeon after a 2-year stint at St Vincent's Hospital, Sydney. He unfortunately passed away of perforated appendicitis in 1962. Dr Tham Cheok Fai (Figs 1a and 1b) (commonly referred to by his trainees as “Mr Tham”), an aspiring spine surgeon at Orthopaedic ‘C’ Unit, Singapore General Hospital (SGH), was selected to succeed Dr Tseng after his passing. Mr Tham trained at Guy's Maudsley and King's College Hospital under world-renowned epilepsy neurosurgeon Professor Murray Alexander Falconer from 1963 to 1965. He returned to establish the first section of neurosurgery in 1965 at the Thomson Road Hospital (TRH). The fledgling neurosurgical service proved to be a resounding success by nearly doubling its operative volume by the early 1970s and receiving training accreditation by the Royal Australasian College of Surgeons (RACS). In 1972, the Singapore government decided to develop specialisation in the neurosciences and commissioned the building of a new neuroscience department at Tan Tock Seng Hospital (TTSH). In 1973, the Department of Neurology and Neurosurgery was opened, with Mr Tham as the Head of Department. From 1974 to 1976, the department received much needed support from American and Canadian neurosurgeons through the Foundation for International Education in Neurological Surgery (FIENS), and by late 1975, was pronounced a “mature” unit according to standards of the FIENS programme. By the mid-1980s, the neurosurgical unit at TTSH already sported 8 fully qualified neurosurgeons as well as the latest state-of-the-art operative and imaging equipment.

The Early Days of Neurosurgery in Singapore

In 1956, Sir Ian Douglas Miller, founding member of the Neurosurgical Society of Australasia (NSA) and Chairman of the RACS, visited (SGH) to promote the specialty of

neurosurgery. Prior to his visit, general surgeons dealt with surgical pathologies of the central nervous system, often with morbid outcomes.¹

“The results of brain surgery in Singapore were nearly 100% mortality at that time. Actually, for brain tumour, I saw only one who lived,” recalled Mr Tham in his interview with the National Archives of Singapore.²

Out of goodwill, Sir Douglas Miller offered to train Dr Tseng Yuen Lin (Fig. 2), then a resident at the SGH Surgical Professorial Unit, in the specialty of neurosurgery. From 1958 to 1960, Dr Tseng studied under Sir Douglas Miller at the St Vincent's Hospital in Sydney, and returned as Singapore's first fully qualified neurosurgeon after passing the Fellowship of the Royal Australasian College of Surgeons (FRACS) examinations. Upon returning, Dr Tseng took over the management of all patients with intracranial surgical pathologies, but seconded spinal cord pathologies to Professor Donald Rickson Gunn from the Orthopaedic Surgery Unit after a mutual agreement between the 2 of them was reached. In 1962, Dr Tseng passed away from perforated appendicitis prematurely. Mr Tham, who was then Professor Donald Gunn's senior registrar, was chosen to succeed Dr Tseng as Singapore's only neurosurgeon. From 1963 to 1965, Mr Tham trained at the Guy's Maudsley and King's College Hospitals in London under world-renowned Professor Murray Alexander Falconer, an authoritative figure on epilepsy surgery at that time.

Mr Tham described Professor Murray Falconer to be a surgeon of exacting standards who would “brook no nonsense”.² Taking a history and performing a physical examination for a patient admitted under Professor Murray Falconer often meant investing more than an hour. The Guy's Maudsley Hospital at that time boasted one of the best seizure surgery programmes in the world with a strong emphasis on teaching activities. The training at Guy's Hospital gave Mr Tham a sound foundation in the neurosciences and an excellent example which the future

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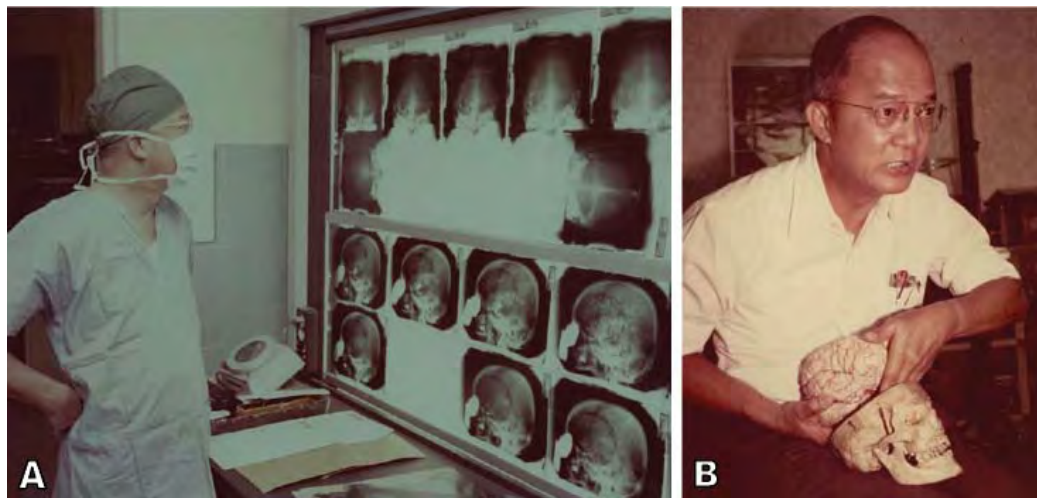


Fig. 1. Portraits of Tham Cheok Fai. In A, he is seen looking at cerebral angiograms preoperatively. Having been trained by Professor Murray Falconer, he was described by his trainees to be “Germanic” in his approach to neurosurgery. An extremely careful surgeon, he was even known to perform his patients’ removal of stitches by himself. In B, Mr Tham at a teaching session using his own brain and skull models. He was a strong advocate of continuing education and placed great emphasis on having a structured clinical teaching programme for the medical officers and nurses in his department.



Fig. 2. Photograph of SGH Surgical Professorial Unit taken in 1956. In the front row, Dr Tseng Yuen Lin is seated on the extreme left, Professor Yeoh Ghim Seng at the centre, and Mr Tham on the extreme right. Dr Tseng would train under Sir Douglas Miller at the St Vincent’s Hospital in Sydney from 1958 to 1960 and become Singapore’s first fully qualified neurosurgeon. After his premature death in 1962, Mr Tham would succeed him as the only neurosurgeon in Singapore at that time.



Fig. 3. Photograph of the Department of Surgery, Thomson Road Hospital (TRH) taken in 1966. This photograph was part of Mr Tham’s personal collection. Mr Tham is seated in the front row, fourth from the left and to his immediate right is Mr Choo Jim Eng (fondly known as “Jimmy Choo”), the Head of the Department. When the section of neurosurgery became successful in the late 1960s, Jimmy Choo gave Mr Tham an additional operating list each week to cope with the increasing demand for his services.

Singapore neurosurgical unit could be modelled after. He returned in 1965 to establish the first section of neurosurgery under the Department of Surgery of TRH (Fig. 3).

Starting out humbly with only a single neurosurgeon with 1 operating list a week, the TRH neurosurgical section grew from strength to strength and by the early 1970s had managed to double its workload. Mr Tham collaborated with several specialists from other fields to introduce neuro-radiology and neuro-anaesthesia to the fledgling service. For their contributions to neurosurgery, Dr Oon Chong Lin (radiologist)³ and Drs Chin Mun Kui, Siva Kanagaratnam, and Lillian Lee (anaesthetists) deserve special mention.⁴ In the late 1960s, the unit received accreditation by the RACS for 2 years of training under the FRACS (Neurosurgery) programme at the courtesy of Western Australian neurosurgeon Dr John Lekias, an important milestone for the training of subsequent Singaporean neurosurgeons.³ In 1971, Dr Gopal Baratham, a Singaporean-born doctor who completed neurosurgical training in the United Kingdom, joined the service as Singapore's second neurosurgeon. During these early years, several international experts from across the British Commonwealth visited the unit to offer advice on its development; amongst them were Professor Norman M Dott from Edinburgh (Fig. 4), Professor Murray A Falconer from Guy's Hospital, Dr Francis P Morgan from Melbourne, Dr John Lekias and Dr Bryant Stokes of Western Australia, Dr Anthony James from Wellington, Dr Trevor Dinning from Adelaide, and Dr John Yelland and Dr Kenneth Jamieson of Brisbane.⁵

In 1972, the government decided to expand neurosurgery under the medical specialisation scheme.⁶ The Department of Neurology and Neurosurgery, a centralised unit dedicated to the diseases of the nervous system, was built at TTSH and opened its doors in 1973. Mr Tham supervised most of



Fig. 4. A photograph from Mr Tham's personal collection that was taken during Professor Norman M Dott's visit to Singapore in the 1960s. From left to right: Mr Tham Cheok Fai; Professor Yahya Cohen, general surgeon at Singapore General Hospital (SGH); Professor Norman McOmish Dott from the University of Edinburgh; Sir Gordon Arthur Ransome, physician at SGH. A resident of Harvey Cushing and amongst the 3 pioneers of modern-day neurosurgery in Great Britain (the other 2 being Geoffrey Jefferson from Manchester and Sir Hugh Cairns at Oxford), Professor Dott had made several landmark contributions to the field of neurosurgery – the best known of which were being the first to surgically treat a patient with a ruptured intracranial berry aneurysm and being amongst the earliest to popularise the transphenoidal approach to the sella turcica.¹¹ Professor Norman Dott spent 2 months with Mr Tham at the section of neurosurgery in TRH to guide the service when it was still in its infancy.⁵

the unit's construction himself and modelled the department after the Guy's Hospital neurosurgical unit in London.⁷ Through a fortuitous arrangement by Professor Donald Gunn, who had moved to join the University of Washington in Seattle, Singapore managed to engage the expertise of North American neurosurgeons through the FIENS programme.⁸ From 1974 to 1976, 11 North American and 1 Canadian neurosurgeon rotated on a 1-to-3 month basis to teach and operate at TTSH (Table 1).⁹ In late 1975, Professor

Table 1. Visiting Neurosurgeons under FIENS (1974 – 1976)⁹

Surgeon	Time of Visit	Affiliation
Wallace Nelson, MD	January – February 1974	Seattle, Washington
W Craig Grant, MD	March – May 1974	Memphis, Tennessee
John R Clark, MD	June 1974	Chico, California
Stanley A Radvan-Ziemnowicz, MD	July 1974	Washington, DC
Robert J Goodall, MD	August 1974	Houston, Texas
Joseph E Gelety, MD	September 1974	Fort Lauderdale, Florida
John C Gibson, MD	October – November 1974	Seattle, Washington
H Harvey Gass, MD	December 1974	Detroit, Michigan
Edward L Katz, MD	January – March 1975	Portland, Maine
Franz E Glasauer, MD	April – June 1975	Buffalo, New York
William D Stevenson, MD	September – December 1975	Halifax, Nova Scotia, Canada
Stanley A Radvan-Ziemnowicz, MD	January – February 1976	Washington, DC
Harold C. Voris, MD	Late 1976	Chicago, Illinois

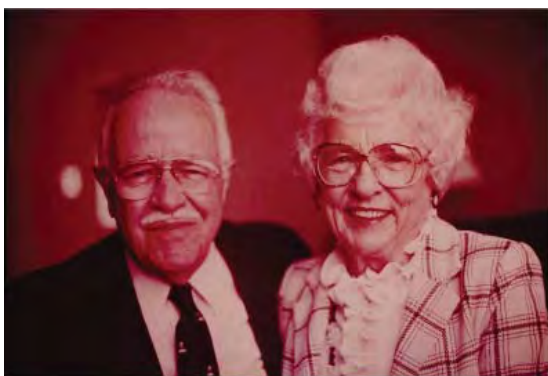


Fig. 5. Photograph from Mr Tham's personal collection of Professor William D Stevenson and his wife when they visited Singapore in 1975. Professor Stevenson, the first neurosurgeon of Atlantic Canada, is credited for building the neurosurgical service at the Victoria General Hospital in Halifax, Nova Scotia from scratch.¹² He was impressed by the standard of neurosurgery at the TTSH unit and pronounced it to be a mature unit, which led to the FIENS withdrawal in early 1976.

William D Stevenson (Fig. 5), a senior neurosurgeon from the FIENS programme, pronounced the TTSH unit mature.⁴ In the same period, 2 new neurosurgeons trained under the FRACS (Neurosurgery) programme in Western Australia joined the department – Dr James Khoo joined in 1975 and Dr Ong Peck Leong in 1977. In 1977, TTSH procured the most advanced neuroimaging equipment of that time, the Siemens Siretome 2000 computer tomography (CT) scanner, and became the first unit in Southeast Asia to offer CT imaging for intra-cranial pathologies.¹⁰

Although withdrawn in 1976, the FIENS had given Singapore a direct link with North American neurosurgery. Following its withdrawal, several eminent American neurosurgeons still continued to visit the unit as guest lecturers, including great personalities like Dr Thoralf Sundt, Professor Charles G Drake, Dr James Ausman and Professor Peter Janetta. Deserving special mention is Dr Ralph B Cloward from the University of Hawaii (Fig. 6), who performed his trademark 'Cloward's procedure' on Mr Lim Kim San, then the Minister of Finance. Through interaction with our American counterparts, several Singaporean doctors obtained the opportunity to undergo neurosurgical residency training in the United States.

By the mid-1980s, the TTSH unit was staffed with 8 neurosurgeons and equipped with the latest operative and imaging equipment on the market. A full range of neurosurgical procedures were being performed on a regular basis. Mr Tham stepped down as Head of Department in 1985 and retired from clinical practice in 1991. For his contributions to Singapore neurosurgery, he was awarded the Public Administration Medal (Gold) and inaugurated into



Fig. 6. Photograph from Mr Tham's personal collection of Dr Ralph Bingham Cloward from the University of Hawaii (seated on the extreme right) being hosted by Mr Tham at his house. Mr Tham is seated on the extreme left in this photograph. Dr Cloward was famous for being the only neurosurgeon in theatre during the Japanese assault on Pearl Harbour in World War II,¹³ as well as for pioneering the anterior cervical discectomy and fusion (ACDF) procedure for cervical degenerative disc disease.¹⁴

the RACS and the American Association of Neurological Surgeons (AANS).¹⁰ He passed away in 2013 at the age of 88, leaving behind a legacy of neurosurgery in Singapore. For his work, he would be fondly remembered by the neurosurgical community in Singapore as Singapore's "Father" of Neurosurgery.

Acknowledgement

The authors wish to thank Mr Tham's daughters, Drs Tham Suet Lan and Tham Mae Lan, for providing them with Mr Tham's personal photograph collection.

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Dr Tan Ngoh Chuan (1931 – 2015)

Dr Tan Ngoh Chuan (NC Tan) is a pioneer who contributed greatly to the development of cardiothoracic surgery in Singapore. He was born on July 7, 1931 in Penang, Malaysia and obtained his MBB degree from the University of Malaya in Singapore in 1955. He was awarded a Senior Colombo Plan Fellowship in Surgery and was based in Sydney and Melbourne, Australia from 1959 to 1961.

He was a consultant and head of the Department of Cardiothoracic Surgery at the Tan Tock Seng Hospital (TTSH) from 1965 to 1980 and senior consultant and head of cardiothoracic surgery at the Singapore General Hospital from 1981 to 1988. In 1967, he began a programme in open cardiac surgery at TTSH.

A gifted scholar, Dr Tan was the recipient of several academic awards including the Soon Eng Kong Scholarship (Merit) in 1948, Soon Eng Kong Gold Medalist (1948), the Senior Colombo Plan Fellowship (1959 – 1961), the World Health Organization (WHO) Travelling Fellowship in Cardiothoracic Surgery (1977) and a Health Manpower Development Programme (HMDP) Fellowship for Coronary Artery Bypass Surgery (1982).

Dr Tan was awarded a Public Administration Medal (Gold) by the Government of Singapore in 1977. He played a very active role in professional organisations in his field, and held, at various points in his career, the offices of:

Master, Academy of Medicine, Singapore	1987 – 1990;
Chairman, Chapter of Surgeon	1976 – 1978;
Editor-in-Chief, Annals	1975 – 1989;
President of the Cardiac Society, Singapore	1975 – 1976, 1978 – 1979.

He was also the Past President of the Association of Cardiothoracic Surgeon of Asia and the ASEAN Federation of Cardiology.

Due to his contributions, he is regarded as the doyen of cardiothoracic surgery in Singapore and his efforts have spawned subsequent generations of cardiothoracic surgeons in Singapore.



Unlike most cardiothoracic surgeons, Dr NC Tan is a very gentle person (and a gentleman) known for his meticulous surgical work and his small, numerous, verbal “thank you” acknowledgements to his assistants who helped him throughout the whole operative procedure. At the end of each operation, which could be 6 hours or longer, he would close the pericardium (the covering of the heart) as meticulously as he could, like with a needle doing a ‘Chinese Darning’. It was revealed to us, later on in a re-operation, a proper closure of that kind will protect the heart well from the sternal saw during the repeat cutting of the sternum. If this was an old case of Dr NC Tan, we would be reassured the pericardium is securely closed and the repeat sawing of the sternum would be safe.

He would always type his operative notes at the end of each operation with his portable typewriter, which he fished out from his bag that he carried all the time. We would always hear the tit-tat, tit-tat in the background while arranging for the patient to be transferred to the intensive care unit (ICU) bed from the operating table.

Nowadays, for electronic medical records and clarity purposes, we are encouraged to type our operative note on the computer in the operating theatre. He was ahead of his time.

When I was under his tutelage as a young surgeon in training, he took me through my first open aortic valve replacement. I would always remember it because during my HMDP fellowship in New York University (NYU) Medical Centre, New York, I was tested on my surgical ability and I was able to impress my attending surgeon with my aortic valve replacement. He had thought that I was doing it for the first time. I kept quiet about it but deep down in my heart I remembered NC and was emotionally touched by him. It was a good omen and the rest of the fellowship was smooth sailing from then on.

Dr NC Tan was also a family man who had 2 lovely daughters and 2 autistic sons. They received solid support from him and his wife, Dr Dixie Tan. They were a good team and shared the same values. In spite of already having 4 children, he supported his wife in taking her MMed exam, her voluntary work and founding of the

Association of Educationally Subnormal Children, as well as her career in politics and service for the nation. On weekends, he spent time with his family on outings to MacRitchie Reservoir, Botanic Gardens, Mount Faber, as well as playing tennis. These simple get-togethers are fondly remembered by their children. They also sung in the church choir together. When his daughters were in the later stages of their adult lives, he supported them wholeheartedly in their career choices and was proud of their achievements. Likewise, the children are equally proud of their parents.

Dr NC Tan will always be respected, admired and remembered as a friend, colleague and teacher in our heart and as one of us.

James Wong

Chapter of Cardiothoracic Surgery, Singapore
