Predictive Factors for Sperm Retrieval in Males With Non-obstructive Azoospermia

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Abstract

The aim of the current study was to investigate the predictive markers for males with non-obstructive azoospermia (NOA) before they received conventional testicular sperm extraction (cTESE) or microdissection testicular sperm extraction (microTESE). Between January 2010 and December 2020, a total of 56 patients who received cTESE or microTESE surgery at the Urology department of the MacKay Memorial Hospital were included. Our univariate analysis revealed that the following parameters was associated with sperm retrieval: Follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, testicular volume, histopathology of maturation arrest and Sertoli cell-only. The multivariate analysis showed that Sertoli cell-only was significantly less likely to harvest spermatozoa than normal spermatogenesis (OR = 0.03 (0.002-0.42); p = 0.01). A comparison of cTESE and microTESE revealed that the overall successful sperm retrieval rate was not significantly different between the two methods (74.1% vs. 58.6, p = 0.22). This study demonstrated that lower levels of FSH, LH and prolactin, and a higher testicular volume and better histopathology were associated with a higher sperm retrieval rate in the univariate analysis. In the multivariable analysis, only Sertoli cell-only syndrome appeared to have a significantly negative effect on the successful harvesting of sperm when compared with normal spermatogenesis.

Introduction

Azoospermia is defined as the absence of sperm in the ejaculated semen, and it is found in 10–15% of infertile men[1]. The most common causes of male infertility include non-obstructive azoospermia (NOA) and obstructive azoospermia (OA), followed by azoospermia factor (AZF) microdeletion on the Y chromosome and Klinefelter syndrome[2].

Testicular sperm retrieval combined with intracytoplasmic sperm injection (ICSI) increases pregnant outcomes for patients with NOA[3]. Conventional testicular sperm extraction (cTESE) and microdissection testicular sperm extraction (microTESE) are techniques used to retrieve sperm in men with NOA. MicroTESE is reported to have a higher sperm retrieval rate than cTESE, especially when there is small orchidometry and/or a high Follicle-stimulating hormone (FSH) concentration, however the procedure is more expensive in terms of surgical time and equipment required[4]. Therefore, choosing the appropriate NOA patients is important before undergoing microTESE.

Parameters such as hormone level, age, testicular volume and chromosome profile have been previously analyzed as predictors of the sperm retrieval rate, however different results have been reported[2,5,6]. The aim of this study was to investigate the predictive value of different factors for males with NOA before they received cTESE or microTESE.

Results

Patient characteristic

The mean age of the 56 men included in the current study was 36.64 ± 5.17 years with a mean body mass index (BMI) of 25.66 ± 3.85. Histopathological examination diagnosed 41.1% (23/56), 30.4% (17/56), 5.3% (3/56) and 23.2% (13/56) of patients with normal spermatogenesis, hypospermatogenesis, maturation arrest and Sertoli cell-only, respectively. In total, spermatozoa were successfully retrieved in 66.1% of the 56 NOA patients.

The demographic data of the 56 patients, according to their histopathological features and sperm retrieval is shown in Table 1. Spermatozoa were recovered from 91.3% (21/23) of patients with normal spermatogenesis, 82.3% (14/17) with hypospermatogenesis, 33.3% (1/3) with maturation arrest and 7.1% (1/13) with Sertoli-cell only. Statistical differences in the sperm retrieval rate were observed with an increasing trend from Sertoli-cell only, maturation arrest, hypospermatogenesis and normal spermatogenesis (p < 0.001).

Table 1
Patient characteristic between the successful and unsuccessful sperm retrieval groups

	NormoS			HypoS			MA			SCO			Total
Characteristic	Successful SR	No SR	*p* value	Successful SR	No SR	*p* value	Successful SR	No SR	*p* value	Successful SR	No SR	*p* value	Suc SR
	n = 21	n = 2		n = 14	n = 3		n = 1	n = 2		n = 1	n = 12		N =
Age (years)	36.48 ± 5.14	45.00 ± 2.83	0.03*	37.71 ± 5.34	40.00 ± 4.00	0.50	29.00	37.5 ± 9.19	0.59	36.00	34.00 ± 3.22	0.56	36.7 5.2(
BMI (kg/m^2)	24.90 ± 4.22	26.37 ± 4.79	0.65	26.29 ± 3.77	27.37 ± 6.39	0.69	24.34	23.28 ± 4.22	0.87	32.19	25.66 ± 2.46	0.03*	25.6 4.09
Smoking (%)	2 (9.5)	0 (0)	1.00	3 (21.4)	2 (66.7)	0.19	0 (0)	2 (100)	0.33	1 (100)	3 (25)	0.31	6 (1
FSH (mIU/ml)	6.32 ± 4.27	12.26 ± 4.21	0.07	12.20 ± 12.41	20.98 ± 8.90	0.27	7.16	19.30 ± 12.65	0.58	17.45	19.33 ± 7.67	0.82	8.87
LH (mIU/ml)	4.59 ± 3.26	6.96 ± 0.28	0.33	6.03 ± 4.50	5.49 ± 1.12	0.84	7.06	11.77 ± 4.66	0.56	4.69	9.39 ± 7.68	0.57	5.2(
Prolactin (ng/mL)	7.18 ± 3.44	7.48 ± 0.69	0.91	5.87 ± 2.51	7.62 ± 2.05	0.28	8.72	10.86 ± 4.96	0.79	8.59	9.46 ± 4.10	0.84	6.76
Testosterone (ng/mL)	4.36 ± 1.96	3.25 ± 0.32	0.44	4.68 ± 2.21	4.07 ± 0.84	0.65	11.66	6.53 ± 0.63	0.10	1.96	4.28 ± 3.03	0.48	4.61
Right testis vol. (ml)	9.86 ± 3.22	4.71 ± 0.12	0.07	8.00 ± 5.72	5.64 ± 2.76	0.51	12.99	6.33 ± 1.66	0.19	4.90	4.41 ± 2.48	0.85	9.1(
Left testis vol. (ml)	9.69 ± 3.22	4.99 ± 0.33	0.06	8.29 ± 5.69	5.17 ± 1.90	0.37	7.07	6.60 ± 0.99	0.77	5.30	4.67 ± 2.31	0.80	8.97
Varicocele (%)	12 (57.1)	2 (100)	0.50	7 (50)	1 (33.3)	1.00	1 (100)	2 (100)	-	1 (100)	5 (41.7)	0.46	21 (
Microcalcification (%)	1 (4.8)	0 (0)	1.00	2 (14.3)	0 (0)	1.00	0 (0)	0 (0)	-	1 (100)	1 (8.3)	0.15	4 (1
cTESE / MicroTESE	13/8	0/2	0.18	6/8	2/1	0.58	1/0	0/2	0.33	0/1	5/7	1.00	20/
Complication	4 (19)	0 (0)	1.00	1 (7.1)	0 (0)	1.00	0 (0)	1 (50)	1.00	0 (0)	0 (0)	-	5 (1
NormoS (%)													21 (
HypoS (%)													14 (
MA (%)													1 (2
SCO (%)													1 (2
46, XY	18 (85.7)	2 (100)	1.00	14 (100)	3 (100)	-	1 (100)	2 (100)	-	1 (100)	10 (83.3)	1.00	34 (
46, XY, 15 dup	3 (14.3)	0 (0)		0 (0)	0 (0)		0 (0)	0 (0)		0 (0)	2 (16.7)		3 (8
47, XXY	0 (0)	0 (0)		0 (0)	0 (0)		0 (0)	0 (0)		0 (0)	0 (0)		0 (0
AZF deletion	0 (0)	0 (0)	-	1 (7.1)	1 (33.3)	0.33	0 (0)	0 (0)	-	0 (0)	1 (8.3)	1.00	1 (2

NormoS normal spermatogenesis, HypoS hypospermatogenesis, MA maturation arrest, SCO Sertoli cell-only, SR sperm retrieval, BMI body mass index, FSH fo hormone, LH luteinizing hormone, vol. volume, cTESE conventional testicular sperm extraction, microTESE microdissection testicular sperm extraction, AZF a

*mean $p < 0.05$

Parameters which were significantly different between successful and non-successful sperm retrieval patients included FSH (8.87 ± 8.72 vs. 18.84 ± 7.77, $p < 0.001$), luteinizing hormone (LH) (5.20 ± 3.72 vs. 8.77 ± 6.38, $p = 0.01$), prolactin (6.76 ± 3.08 vs. 9.11 ± 3.64, $p = 0.01$), right testicular volume (9.10 ± 4.60 vs. 4.84 ± 2.29, $p < 0.001$), left testicular volume (8.97 ± 4.29 vs. 4.98 ± 2.02, $p < 0.001$) and histopathology ($p < 0.001$). Higher FSH, LH and prolactin values were indicative of lower sperm retrieval rates, whereas lower testicular volume and better histopathology indicated unsuccessful sperm retrieval. Age, BMI, smoking

history, testosterone level, presence of varicocele or microcalcification, cTESE or microTESE, complications, chromosome profile or AZF microdeletion revealed no significant difference between the successful and unsuccessful sperm retrieval groups ($p > 0.05$). In the subgroup analysis, younger age in the normal spermatogenesis subgroup (36.48 ± 5.14 vs. 45.00 ± 2.83, $p = 0.03$) and a higher BMI in the Sertoli cell-only subgroup (32.19 vs. 25.66 ± 2.46, $p = 0.03$) were found to be significantly associated with a higher sperm retrieval rate.

Overall, three patients were found to have AZF microdeletions, including one patient with AZFa/AZFb microdeletions whose procedure successfully discovered spermatozoa with a hypospermatogenesis histology, whereas the other two patients, one with a AZFa microdeletion and the other also with AZFb/AZFc microdeletions failed to retrieve spermatozoa with hypospermatogenesis and Sertoli cell-only, respectively.

Univariate and multivariate analysis

Univariate and multivariate analysis were performed to investigate the correlation between the various factors and sperm retrieval (Table 2). The results of the univariate analysis revealed that the following factors were significantly associated with sperm retrieval: FSH (OR = 0.88 (0.82–0.95); $p = 0.001$), LH (OR = 0.86 (0.75–0.98); $p = 0.026$), prolactin (OR = 0.81 (0.68–0.97); $p = 0.024$), right testicular volume (OR = 1.34 (1.11–1.61); $p = 0.002$), left testicular volume (OR = 1.45 (1.14–1.85); $p = 0.002$), histopathology of maturation arrest (OR = 0.048 (0.003–0.79); $p = 0.03$) and Sertoli cell-only (OR = 0.008 (0.001-0.10); $p < 0.001$). Multivariate analysis revealed that a histopathology of Sertoli cell-only was less likely to harvest spermatozoa than normal spermatogenesis (OR = 0.03 (0.002–0.42); $p = 0.01$).

Table 2
Univariate and multivariate logistic regression analysis of different parameters as predictors of successful sperm retrieval

Parameter	Univariate		Multivariate	
	P value	OR (95% interval)	P value	OR (95% interval)
FSH (mIU/ml)	0.001*	0.88 (0.82–0.95)	0.39	0.95 (0.84–1.07)
LH (mIU/ml)	0.026*	0.86 (0.75–0.98)	0.79	1.03 (0.83–1.28)
Prolactin (ng/mL)	0.024*	0.81 (0.68–0.97)	0.89	0.98 (0.75–1.29)
Right testis volume (ml)	0.002*	1.34 (1.11–1.61)	0.76	1.07 (0.70–1.63)
Left testis volume (ml)	0.002*	1.45 (1.14–1.85)	0.46	1.21 (0.73–1.98)
NormoS (%)	0a		0a	
HypoS (%)	0.41	0.44 (0.07–3.01)	0.86	1.28 (0.09–18.57)
MA (%)	0.03*	0.048 (0.003–0.79)	0.11	0.07 (0.003–1.79)
SCO (%)	< 0.001*	0.008 (0.001-0.10)	0.01*	0.03 (0.002–0.42)

FSH follicle stimulating hormone, LH luteinizing hormone, NormoS normal spermatogenesis, HypoS hypospermatogenesis, MA maturation arrest, SCO Sertoli cell-only,*mean $p < 0.05$

cTESE vs. microTESE

A comparison of the sperm retrieval rate via cTESE and microTESE was performed (Table 3). Overall, the successful sperm retrieval rate was not significantly different between cTESE and microTESE (74.1% vs. 58.6%; $p = 0.22$). In addition, we found no statistically significant differences in the sperm retrieval rate after nonsignificant characteristics in the subgroups of hypospermatogenesis (75% vs. 88.9%; $p = 0.58$), maturation arrest (100% vs. 0%; $p = 0.33$) and Sertoli cell-only (0% vs. 12.5%; $p = 1.00$).

Table 3
Patient characteristic between the cTESE and microTESE groups

	NormoS			HypoS			MA			SCO		
Characteristic	cTESE	micro TESE	P value	cTESE	micro TESE	P value	cTESE	micro TESE	P value	cTESE	micro TESE	P value
	n = 13	n = 10		n = 8	n = 9		n = 1	n = 2		n = 5	n = 8	
Age (years)	35.15 ± 5.63	39.90 ± 4.25	0.038*	35.13 ± 4.45	40.78 ± 4.21	0.017*	29.00	37.50 ± 9.19	0.59	33.60 ± 4.62	34.50 ± 2.07	0.90
BMI (kg/m^2)	24.62 ± 2.89	25.57 ± 5.56	0.63	25.14 ± 2.99	27.67 ± 4.74	0.22	24.34	23.28 ± 4.21	0.87	25.56 ± 1.99	26.55 ± 3.52	0.58
Smoking (%)	0 (0)	2 (20)	0.18	1 (12.5)	4 (44.4)	0.29	0 (0)	2 (100)	0.33	0 (0)	4 (50)	0.11
FSH (mIU/ml)	5.52 ± 3.40	8.54 ± 5.35	0.11	16.68 ± 13.97	11.15 ± 10.34	0.36	7.16	19.30 ± 12.65	0.58	18.58 ± 10.37	19.56 ± 5.57	0.83
LH (mIU/ml)	3.43 ± 1.55	6.59 ± 3.92	0.015*	4.34 ± 1.84	7.34 ± 5.05	0.13	7.06	11.77 ± 4.66	0.56	6.45 ± 7.68	10.65 ± 7.35	0.35
Prolactin (ng/mL)	6.45 ± 2.39	8.20 ± 4.10	0.21	6.12 ± 2.59	6.22 ± 2.53	0.94	8.72	10.86 ± 4.96	0.79	10.16 ± 5.19	8.91 ± 3.22	0.60
Testosterone (ng/mL)	5.00 ± 2.14	3.30 ± 0.93	0.02*	4.95 ± 1.62	4.24 ± 2.38	0.49	11.66	6.53 ± 0.63	0.10	5.21 ± 4.36	3.41 ± 1.67	0.31
Right testis vol. (ml)	11.51 ± 3.19	6.68 ± 2.77	0.001*	8.02 ± 5.75	7.19 ± 5.24	0.76	12.99	6.33 ± 1.66	0.19	5.90 ± 2.56	3.54 ± 1.88	0.08
Left testis vol. (ml)	10.82 ± 2.64	7.28 ± 3.22	0.001*	8.65 ± 4.81	6.93 ± 5.89	0.52	7.07	6.60 ± 0.99	0.77	4.54 ± 2.00	4.83 ± 2.47	0.84
Varicocele (%)	7 (53.8)	7 (70)	0.67	5 (62.5)	3 (33.3)	0.35	1 (100)	2 (100)	-	3 (60)	3 (37.5)	0.59
Microcalcification (%)	0 (0)	1 (10)	0.44	1 (12.5)	1 (11.1)	1.00	0 (0)	0 (0)	-	0 (0)	2 (25)	0.49
46, XY	12 (92.3)	8 (80)	0.56	8 (100)	9 (100)	-	1 (100)	2 (100)	-	1 (100)	6 (75)	0.49
46, XY, 15 dup	1 (7.7)	2 (20)		0 (0)	0 (0)		0 (0)	0 (0)		0 (0)	0	
47, XXY	0 (0)	0 (0)		0 (0)	0 (0)		0 (0)	0 (0)		0 (0)	2 (25)	
AZF deletion	0 (0)	0 (0)	-	2 (25)	0 (0)	0.21	0 (0)	0 (0)	-	1 (20)	0 (0)	0.39
Successful SR	13 (100)	8 (80)	0.18	6 (75)	8 (88.9)	0.58	1 (100)	0 (0)	0.33	0 (0)	1 (12.5)	1.00
Complication	2 (15.4)	2 (20)	1.00	0 (0)	1 (11.1)	1.00	0 (0)	1 (50)	1.00	0 (0)	0 (0)	-

NormoS normal spermatogenesis, HypoS hypospermatogenesis, MA maturation arrest, SCO Sertoli cell-only, cTESE conventional testicular sperm extraction, microTESE microdissection testicular sperm extraction, BMI body mass index, FSH follicle stimulating hormone, LH luteinizing hormone, vol. volume, AZF azoospermia factor, SR sperm retrieval

*mean $p < 0.05$

Discussion

ICSI was first established in 1992 to treat couples with infertility due to low sperm quantity and quality who had undergone failed *in-vitro* fertilization or subzonal insemination[7]. Several sperm retrieval methods, including testicular sperm aspiration, cTESE and microTESE were designed to harvest sperm from the testes of men with azoospermia-related infertility[3]. In men with NOA, the successful retrieval rate was reported as 30–60% in a recent series[5,8–10]. The current study displayed no significant deviation from the typically reported sperm retrieval rate following the TESE procedure (66.1%).

A recent systematic review compared the outcomes of cTESE and microTESE and the overall sperm retrieval rate was significantly higher in the microTESE group (42.9–63% vs. 16.7–45%, $p < 0.05$). According to testicular histology, Sertoli cell-only and hypospermatogenesis showed more favorable results in the microTESE group (41% in microTESE vs. 6.3–29% in cTESE)[11]. Another study reported that microTESE was 1.5 times more superior for successful sperm retrieval than cTESE; however, no subgroup analyses were performed[12]. In the current study, there was no statistically significant difference in the sperm retrieval rate between the cTESE and microTESE groups (74.1% vs. 58.6, $p = 0.22$). Sub-analysis of the sperm retrieval rate according to testicular histopathology also revealed no statistically significant difference in the hypospermatogenesis, maturation arrest and Sertoli cell-only subgroups. Normal spermatogenesis could not be effectively analyzed because there were significant variations in patient characteristics ($p < 0.05$).

Some parameters have been previously reported to predict sperm retrieval. Higher testicular volume, lower levels of FSH and better histological features were investigated as sperm retrieval predictors by Doroteja *et al.* However, after multivariable analysis, better semen and histopathology remained the only

predictive parameters[5]. A retrospective cohort analysis found that FSH level had a positive correlation between the success and failure groups in microTESE[13]. Yalcin Kizilkan *et al.* found that a previous successful testicular biopsy and higher testicular volume were predictive parameters for microTESE patients[6]. One study reported no predictors of successful outcomes, whereas tobacco usage was a predictor factor for patients with a negative TESE[2]. Another recent study concluded that there were no definite predictors for sperm retrieval, except for chromosome disease; however, Sertoli cell-only histopathology was associated with a reduced chance of harvesting spermatozoa[14]. Our univariate analysis revealed that lower levels of FSH, LH and prolactin, and higher testicular volume and better pathology were predictors of successful sperm retrieval. However, these parameters showed no statistically significant difference in the multivariate analysis except for the pathology result. Compared with normal spermatogenesis histopathology, Sertoli cell-only had lower sperm retrieval rates.

In addition, advanced paternal age is associated with male infertility caused by alternative reproductive hormone levels, sexual disorder or decreased sperm production[15]. Obesity can cause hormone alterations, increased systemic inflammation and increased testicular temperature, which can influence male fertility[16]. In the current study, in the group with normal spermatogenesis, those with a younger age showed higher rates of sperm retrieval (36.48 ± 5.14 vs. 45.00 ± 2.83, $p = 0.03$). In the Sertoli cell-only group, we found that those with a higher BMI had a higher rate of sperm retrieval (32.19 vs. 25.66 ± 2.46, $p = 0.03$). These findings could be due to the small number of patients included in the study which could have caused a sampling error.

Klinefelter syndrome, 47, XXY karyotype, is the most common genetic cause of male infertility and is found in 10% of azoospermia patients[17]. Until recently, it was considered to be a model of definite male infertility. A 54.5% – 72% sperm retrieval rate was reported in Klinefelter syndrome patients after microTESE[18–20]. However, Klinefelter patients with NOA still had a lower sperm retrieval rate than non-Klinefelter patients[14]. In the current study, spermatozoa were harvested from 0% of Klinefelter syndrome patients, which is not comparable with previous research; this is probably due to the low number of Klinefelter syndrome patients included. Y chromosome microdeletion was also a significant factor for NOA. The AZF located on the Y chromosome is the region for spermatogenesis and three overlapping regions named AZFa, AZFb and AZFc have been identified. AZFa or AZFb microdeletions are associated with maturation arrest or Sertoli cell-only syndrome[21,22]. Men with AZFa or AZFb microdeletions have minimal sperm retrieval success, whereas those with AZFc microdeletions have a reported success rate of approximately 50%[23,24]. The current study demonstrated that one patient with AZFa/AZFb microdeletions succeeded in recovering spermatozoa, whereas two other patients, one with a AZFa microdeletion and the other with AZFb/AZFc deletions failed to retrieve spermatozoa.

Complications such as hematoma, testicular fibrosis and testicular atrophy were less frequent in microTESE groups compared with cTESE groups[11]. The rates of hematoma and dehiscence were significantly different between the TESE-positive group and the TESE-negative group[2]. In the current study, the complication rate showed no significant difference between the cTESE and microTESE groups and there was also no significant difference in the subgroup histopathology analysis. A comparison of the positive and negative sperm retrieval groups also revealed no significant difference.

There were several limitations to the present study, including the fact that it was a single center study, its small cohort size and its retrospective nature. In addition, the current study only included Taiwanese patients and may not be comparable with Western populations or the entire NOA cohort. No standardized or long-term follow up was established. Finally, in future studies, predictor values of a successful pregnancy rate should also be analyzed rather than only those for sperm retrieval.

In conclusion, several parameters were found to predict the chance of successful spermatozoa retrieval in NOA patients in the univariate analysis, including lower levels of FSH, LH and prolactin, and a higher testicular volume and histopathology results. However, these parameters showed no statistically significant difference in the multivariate analysis except for histopathological features. The presence of Sertoli cell-only was a marker for a lower chance of sperm retrieval than normal spermatogenesis. A comparison of cTESE and microTESE revealed that there were no significant differences in the sperm retrieval rate for all patients, and the subgroups of hypospermatogenesis, maturation arrest and Sertoli cell-only.

Methods

From January 2010 to December 2020, 119 patients who underwent cTESE or microTESE were retrospectively analyzed at the Urology department of MacKay Memorial Hospital. The following parameters were recorded for each patient: medical history, physical examination, hormone profile, testicular volume, genetic variables and diagnostic testicular biopsy. We excluded patients with oligospermia, a history of surgery via the scrotum or inguinal area, a history of vasectomy, congenital absence of the vas deferens, retrograde ejaculation or incomplete data analysis. Testicular histopathology was classified into four groups: normal spermatogenesis, hypospermatogenesis, maturation arrest and Sertoli cell-only. Successful sperm retrieval was defined as the collection of any number of motile or immotile spermatozoa which can be used for sperm injection. After application of the exclusion criteria, a total of 56 males with NOA were included in the study.

The surgical procedure was performed under general anesthesia. Patients were placed in a supine position and the skin around the area of incision was fully disinfected. A midline incision was made into the scrotum and carried down to the dartos layer, exposing the bilateral testis from the surrounding fascia. A horizontal incision of the tunica albuginea was made with extrusion of the seminiferous tubules. For the cTESE, a scissors biopsy of about 5x5x5mm testicular tissue was performed, whereas for the microTESE the testicular tissue about 5x5x5mm was biopsied under an operative microscope at 20-25x magnification. We removed larger opaque tubules from the tissue, which were more likely to contain spermatozoa.

The SPSS software package was used to analyze the variables. All data were compared using an independent Student's t-test, a Chi-squared test and a Fisher's exact test. Univariate and multivariate logistic regression models were constructed to analyze the association of several parameters with successful sperm retrieval. Statistical significance was set at $p < 0.05$.

Declarations

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Author contributions

WHH: project development, data collection, data analysis and manuscript writing.

YZC: project development, data collection, data analysis

MC: project development, data analysis, and manuscript editing.

PKC: project development and data analysis

WKT: project development and data analysis

AWC: project development, data analysis, and manuscript editing.

All authors have read and approved the manuscript.

Competing interests

The authors declare no competing interests.

Data availability

Data is available on request to the authors.

Ethics declarations

The present study, including its research protocols and data collection, were approved by the Institutional Review Board of Mackay Memorial Hospital. All methods were performed in accordance with the relevant guidelines and regulations and were approved by the Institutional Review Board of Mackay Memorial Hospital. In view of the retrospective nature of the study, obtaining patients' informed consent was waived by the Institutional Review Board of Mackay Memorial Hospital.

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