

A Comparison of Death Domain-Associated Protein 6 in Different Endometrial Carcinomas Histotypes

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ABSTRACT

BACKGROUND: Death domain-associated protein 6 (DAXX) is involved in regulating apoptosis via subcellular localization. The presence of DAXX point mutations correlates well with loss of nuclear expression on immunohistochemistry (IHC). In this study, we sought to determine (1) whether DAXX expression pattern is the same across different uterine carcinoma subtypes, and (2) which uterine carcinomas show loss of nuclear DAXX IHC.

DESIGN: We studied 65 uterine carcinomas of the following histologic types: 30 endometrioid (12 FIGO [The International Federation of Gynecology and Obstetrics] grade 1, 12 FIGO grade 2, and 6 FIGO grade 3), 8 serous, 14 clear cell, and 13 undifferentiated/dedifferentiated type (UEC/DDEC). Nuclear DAXX IHC was assessed in each tumor and was graded semi-quantitatively as follows: 0% to 50%, 50% to 75%, and greater than 75% of lesional cells react.

RESULTS: A total of 61% (25/41) of high-grade carcinomas (FIGO grade 3, serous, clear cell, and UEC/DDEC) showed retained DAXX nuclear staining in >75% of lesional cells, compared with only 4.2% (1/24) of the low-grade carcinomas (FIGO grades 1 and 2) ($P = .0001$), where DAXX expression was cytoplasmic. In addition, in the 11 DDEC cases, all the differentiated components showed loss of nuclear DAXX compared with the undifferentiated components which retained nuclear DAXX expression.

CONCLUSIONS: We demonstrate that loss of nuclear DAXX is present in low-grade endometrial carcinomas and the differentiated components in UEC/DDEC, but not in high-grade ones, suggesting DAXX's role in tumor progression and its potential as a therapeutic target in high-grade endometrial carcinomas.

KEYWORDS: Endometrial carcinoma, uterine carcinoma, DAXX, histone chaperone

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Introduction

Uterine endometrial carcinomas (UECs) are the most common gynecologic neoplasms in the United States.¹ They represent a histologically diverse group of tumors with different pathological features. High-grade endometrial cancers include serous, clear cell, and undifferentiated/dedifferentiated carcinomas (UEC/DDEC), the latter being more likely to recur/metastasize while carrying the worst prognosis.² Currently, despite increased understanding regarding the molecular pathways involved in the pathogenesis of UEC, the prognosis for advanced disease is still poor, with options for targeted therapies still lacking.³ An expansion of prognostic and therapeutic markers in UECs is urgently needed: to reflect the complexity of the carcinomas, to provide a more accurate stratification, and to allow for more effective therapies.

Death domain-associated protein-6 (DAXX), a predominantly nuclear protein, was originally death receptor binding protein. It has been shown to be important for enhancing Fas-mediated apoptosis through its interaction with JNK (c-Jun N-terminal kinase).^{4,5} The physiological function of DAXX relies on its ability to shuttle between the nucleus and the cytoplasm.⁶ It has been reported that in neurons, the function of

DAXX varies according to its localization: for example, when DAXX is located in the nucleus, it is anti-apoptotic, whereas when it is localized in the cytoplasm, it is pro-apoptotic.⁷ Per contra, an example of the former is that of diffuse large B-cell lymphoma, where DAXX is considered a cancer-promoting (while retained in the nucleus). Therefore, patients who have high nuclear DAXX expression, in some tumors, tend to have a worse prognosis.⁸

In addition to this, numerous studies have shown that DAXX mutations are present in various types of tumors, such as oral cancers, pancreatic neuroendocrine tumors, urothelial carcinomas, prostate cancers, glioblastomas, ovarian cancers, and so on.^{9–14} While per contra to previously suggested, other studies have shown that loss of DAXX (nuclear) correlates with worse clinical behaviors.^{9–14} Furthermore, given the important regulatory role of DAXX in apoptosis, multiple preliminary studies have shown promising therapeutic value in DAXX-targeted cancer treatment (in vivo studies).^{15,16}

Despite this, there have been no studies examining the role of DAXX in endometrial carcinomas.

In view of these considerations, the aim of this study was to examine the subcellular localization of DAXX in tissue samples



Table 1. DAXX staining across the various types of endometrial carcinomas. (UEC/DDEC, clear cell, serous and endometrioid).

TUMOR TYPE (N=65)	PERCENTAGE OF LESIONAL CELLS WITH DAXX NUCLEAR POSITIVITY		
	0%–50%	50%–75%	>75%
UEC/DDEC (n=13)	0	2	11 (on UC component)
Clear cell carcinoma (n=14)	5	4	5
Serous carcinoma (n=8)	2	0	6
Endometrioid type carcinoma (n=30)			
FIGO G1 (n=12)	10	1	1
FIGO G2 (n=12)	11	1	0
FIGO G3 (n=6)	0	3	3
Benign endometrium (n=13)			
Secretory endometrium (n=5)	3	2	0
Proliferative endometrium (n=8)	2	0	6

Abbreviations: DAXX, death domain-associated protein 6; FIGO, The International Federation of Gynecology and Obstetrics; UEC/DDEC, undifferentiated/dedifferentiated type; G1, grade 1; G2, grade 2; G3, grade 3.

from various histotypes of endometrial carcinomas. This was performed to provide some novel insights into (1) whether loss of DAXX is present in uterine carcinomas and (2) whether DAXX expression is the same across all the uterine carcinoma subtypes.

Materials and Methods

Case selection and classification

A total of 65 uterine carcinomas were identified in our institutional pathology database from 2010 to 2017. A subset of 30 endometrioid type (12 FIGO [The International Federation of Gynecology and Obstetrics] grade 1, 12 FIGO grade 2, and 6 FIGO grade 3), 8 serous type, 14 clear cell type (CCC), and 13 undifferentiated/dedifferentiated type (UEC/DDEC) were reviewed independently by 2 senior gynecological pathologists to confirm the diagnosis and to identify the best tumor-containing slide. Another random selection of 13 benign endometrium biopsies were also included in the study for comparison. The endometrium specimens included 5 cases of secretory endometrium and 8 cases of proliferative endometrium. Patient ages at resection ranged from 39 years to 90 years old, with all specimens being hysterectomies. The tissues were prepared in paraffin-embedded blocks for pathological diagnosis.

Immunohistochemical analysis

Immunohistochemical stains for DAXX (Anti-DAXX [Sigma Life Science; HPA008736, 1:100]) were assessed on tissue sections. The staining protocol and controls are submitted in Supplemental material.

Loss of DAXX expression was semi-quantified as <50% nuclear labeling within the tumor cells, 50% to 75% (dominant pattern) and more than 75% (significant pattern). Tumors with absent DAXX expression were required to have positive internal controls (normal endothelial, stromal, or inflammatory cells) before being considered negative. All the staining was performed at the Immunopathology Laboratory of Long Island Jewish Medical Center (Northwell Health System, New Hyde Park, NY).

Statistics

Statistical analysis was performed using the 2-tailed Fisher exact test and Student t tests (vassarstats.net) depending on the type of variables being analyzed. This work was approved by the Institutional Review Board of Northwell Health Systems.

Results

DAXX staining was performed on a total of 65 cases: a subset of 30 endometrioid type (12 grade 1, 12 grade 2, and 6 grade 3), 8 serous type, 14 clear cell type, and 13 UEC/DDEC together with an additional 13 benign endometrium (5 secretory phase and 8 proliferative phase) for comparison (Table 1). The mean ages for endometrial carcinoma patients were 63.4 ± 13.4 (range = 55–90) years, while the ages for benign endometrium were 45.2 ± 2.5 (range = 39–48) years ($P < .05$).

All of the cases showed both nuclear and cytoplasmic staining. The percentage of nuclear staining was quantified in each case. A variable spectrum of nuclear DAXX expression was identified among different tumor histotypes (Figure 1).

Among the endometrial carcinoma cases, 2/65 (3.1%) were FIGO stage IV, 8/65 (12.3%) were FIGO stage III, 11/65

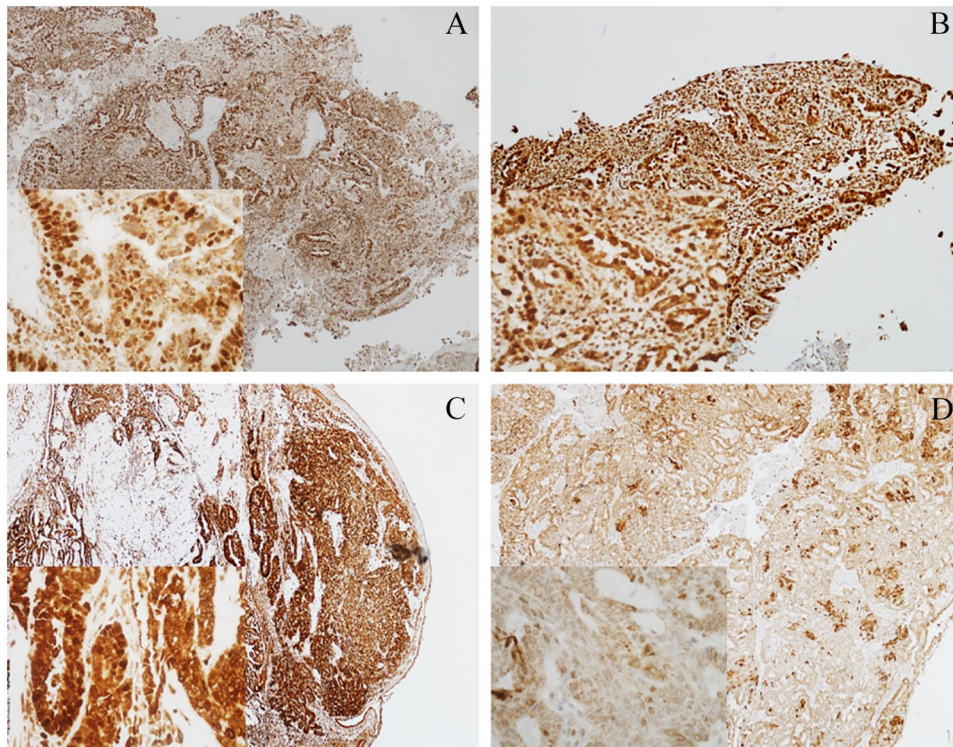


Figure 1. Immunostaining patterns of DAXX in different histotypes of endometrial cancers: (A) Serous type with low power at 4× and (inlet, 40×), (B) clear cell type with low power at 10× (inlet, 40×), (C) FIGO 3 endometrioid type with low power at 4× (inlet 40×), and (D) low-grade endometrioid type with low power at 4× and (inlet, 40×). At high power (40×), high-grade endometrial carcinomas (A, B, C) all show strongly retained nuclear staining of DAXX in tumor cells while low-grade endometrial carcinomas (D) mainly show loss of nuclear DAXX in tumor cells. Abbreviation: DAXX, death domain-associated protein 6; FIGO, The International Federation of Gynecology and Obstetrics.

Table 2. Comparison of immunostaining pattern of DAXX across endometrial carcinomas (low and high grade) with benign endometrium (secretory and proliferative).

GROUP	HIGH-GRADE ENDOMETRIAL CARCINOMAS (N=41)	LOW-GRADE ENDOMETRIAL CARCINOMAS (N=24)	SECRETORY ENDOMETRIUM (N=5)	PROLIFERATIVE ENDOMETRIUM (N=8)
DAXX nuclear > 50%	34	3	2	6
DAXX nuclear < 50%	7	21	3	2
P (compared with high grade)		<.001	.06	.63
P (compared with low grade)	<.001		.19	.0021

Abbreviation: DAXX, death domain-associated protein 6. Two-tailed Fisher exact test was used to compare between each group. $P < .05$ is considered statistically significant.

(16.9%) were FIGO stage II, and 44/65 (67.7%) were FIGO stage I. When using the cut-off value of 50%, the dominant (50%-75%) and significant pattern (>75%) nuclear DAXX expression were observed in 9/14 (64.3%) CCC, 6/8 (75%) serous carcinoma, and 6/6 (100%) FIGO grade 3 endometrioid carcinoma. In contrast, only 2/12 (16.7%) FIGO grade 1 and 1/12 (8.3%) FIGO grade 2 endometrioid carcinomas showed a (dominant or significant) nuclear staining pattern. Nuclear DAXX expression was found to be significantly higher in high-grade endometrial carcinomas (34/41) than in low-grade endometrial carcinomas (3/24) (82.9% vs 12.5%, $P < .0001$) (Table 2).

Especially, in the 11 DDEC cases, all the differentiated components (low grade) showed less DAXX staining (nuclear) retention compared with the undifferentiated components, where DAXX was mainly retained (Figure 2).

The normal endometrium interestingly showed different staining patterns (between secretory and proliferative types) (Figure 3). By using the cut-off of 50%, the proliferative endometrium had 6/8 (75%) cases show a dominant nuclear staining pattern; this was higher when compared with secretory endometrium (2/5, 40%). Ultimately, there was no statistically significant difference between the groups ($P = .29$). However, when a cut-off of 75% was used, significant nuclear DAXX

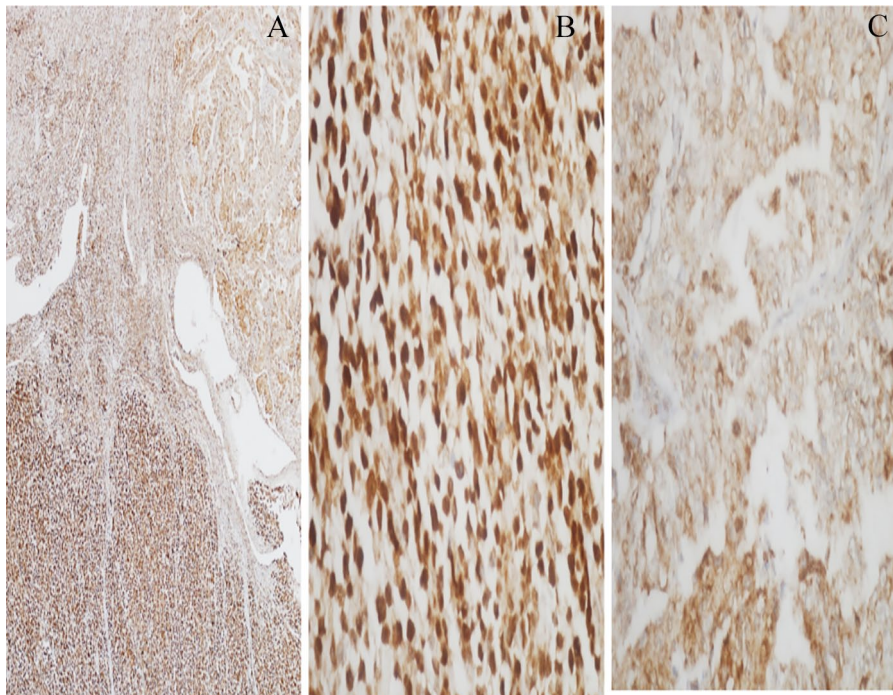


Figure 2. Dedifferentiated endometrial carcinoma at low power (4×) (A) showing 2 different histological patterns including undifferentiated component at the lower left and differentiated component at the lower right; at high power (40×) (B) showing the undifferentiated component predominantly with nuclear staining while the differentiated component (C) with loss of nuclear staining.

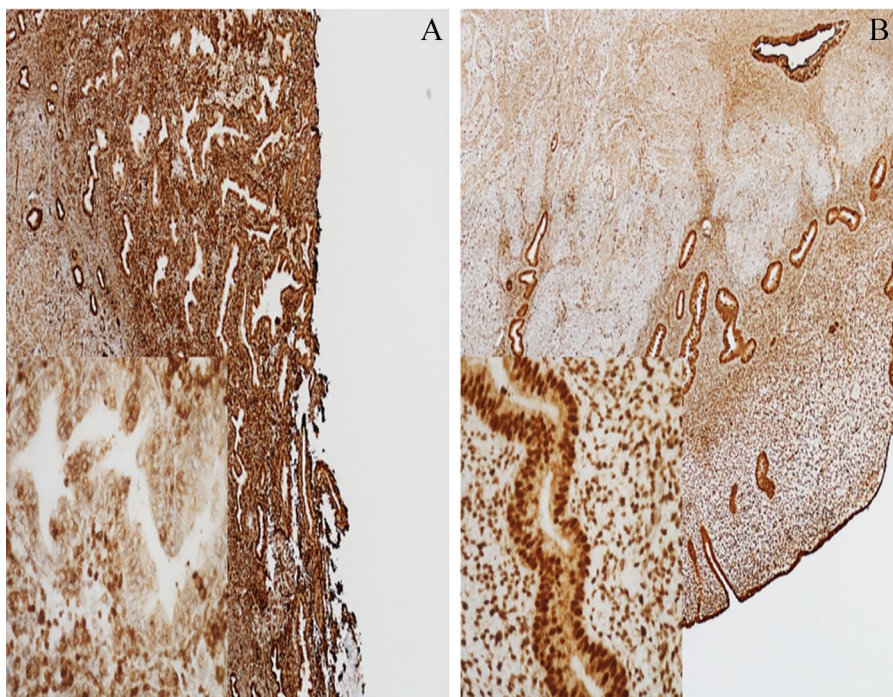


Figure 3. Immunostaining patterns of DAXX in normal endometrium: (A) Secretory endometrium with low power at 4× (inlet, 40×) and (B) proliferative endometrium with low power at 10× (inlet, 40×). The secretory endometrium (A) at high power shows loss of DAXX nuclear staining, while in contrast the proliferative endometrium (B) at high power shows retained nuclear DAXX staining.
Abbreviation: DAXX, death domain-associated protein-6.

staining was more frequently seen in proliferative endometrium when compared with secretory endometrium ($P = .021$). The secretory endometrium tended to have lower nuclear

DAXX than in high-grade cancers ($P = .06$), although there was no difference between high-grade cancers and proliferative endometrium ($P = .63$). In contrast, proliferative endometrium

showed higher nuclear DAXX than low-grade carcinoma groups ($P=.0021$), although there was no difference between low-grade carcinoma groups and secretory endometrium ($P=.19$). The results are summarized in Table 2.

Discussion

In our current study, DAXX analysis yielded variable patterns among the different endometrial histotypes. High-grade endometrial carcinomas tended to have higher percentage of nuclear staining (in tumor cells) than low-grade types. The normal endometrium also presented with different staining patterns, with more nuclear staining being seen in proliferative endometrium than secretory endometrium. Although expression of DAXX has been studied in a variety of tumors, no such studies have been performed in endometrial carcinomas.

DAXX was originally identified as pro-apoptotic factor,⁴ with its role in apoptosis being controversial and possibly related to its subcellular localization.⁵ It has long been known that protein and its subcellular localization can be closely related to tumor progression.⁵ For example, S100A4 is a prognostic variable in breast cancer, which varies depending on its subcellular localization.¹⁷⁻¹⁹ Similarly and as previously mentioned, while in neurons, the function of DAXX varies also according to its localization: when DAXX is located in the nucleus, it is anti-apoptotic, whereas when it is localized in the cytoplasm, it is pro-apoptotic.⁷ In addition, recent findings in gastric cancers showed that an increased DAXX (nuclear/cytoplasmic ratio) was significantly higher in cancer tissues than in adjacent normal tissues.²⁰ The possible anti-apoptotic role of DAXX is also implied in our observational study, which suggests that nuclear DAXX is higher in normal proliferative endometrium (possibly being anti-apoptotic) than in secretory endometrium (possibly being pro-apoptotic), while nuclear DAXX expression also showed higher expression in high-grade endometrial carcinomas than in low-grade carcinomas (using 2 different cut-off points). More convincingly, in dedifferentiated cases, almost all the tumors cells retained nuclear staining, while the adjacent differentiated components showed less nuclear staining.

Worth mentioning is that the nuclear expression of DAXX was not significantly different when comparing high-grade endometrial carcinomas and proliferative endometrium. This finding is not surprising as endometrial carcinomas are usually seen in the background of proliferative endometrium.¹ However, ultimately, in the progression to high-grade endometrial carcinomas, other proteins are also necessary (in addition to DAXX).

Interestingly, most of the low-grade endometrial carcinomas showed much less nuclear DAXX expression. Recently, with the advent toward molecular classification of endometrial carcinomas, genomic information has been gradually integrated into traditional histological classification systems.² It has been shown that endometrioid type carcinomas actually

represent a heterogeneous group of carcinomas with different molecular signatures, such as copy-number low variant, POLE (DNA Polymerase Epsilon, Catalytic Subunit) gene hotspot mutated, and MSI-H (microsatellite instability-high), while the high-grade endometrial carcinomas tend to be high copy-number variant.^{2,3} Therefore, low nuclear DAXX expression, in either low- or high-grade groups, may follow a different molecular pathway than those of high nuclear DAXX expression.

Whether loss of nuclear staining is due to DAXX mutations or protein relocation currently remains unknown. Studies have shown that loss of nuclear DAXX on IHC may be a surrogate marker for the presence of DAXX mutations,⁵ which may partly explain its pathogenesis in low-grade endometrial carcinomas. Conversely, in high-grade endometrial carcinomas, retained nuclear DAXX may stem from reduced nuclear-to-cytoplasm protein relocation, which establishes one of the crucial hallmarks of carcinogenesis (self-initiated continuous tumor proliferation). We did not perform molecular analysis, which is a limitation in our current study. Therefore, whether loss of nuclear staining is due to DAXX mutations, protein relocation, or something else remains to be further elucidated. Finally, regarding the DAXX staining pattern of the uninvolved biopsy-retrieved endometrium, in our study, strong nuclear staining was seen in the proliferative but not in secretory endometrium. This is possibly due to the anti-apoptotic/proliferative role of DAXX.²¹ Ultimately, however, only further research will allow us to fully understand its complete role.

Another newly established function of DAXX is its regulation of telomeres.⁵ Telomere elongation and maintenance is likely partially responsible for cancer progression.^{5,22} There are 2 main telomere maintenance mechanisms that have been described: telomerase dependent and telomerase independent (such as alternative lengthening of telomeres [ALT]).²² A common mechanism of carcinogenesis in telomerase-dependent pathway is mutations in the promoter of the telomerase reverse transcriptase (*TERT*) gene. Whereas in the telomerase independent pathway, an ALT (alternative lengthening of telomeres) phenotype, which uses homologous recombination for telomere length maintenance (instead of activation of the telomerase enzyme), is ultimately implicated in the progression of multiple cancer types (especially in brain tumors and sarcomas).^{23,24}

DAXX also interacts with ATRX (alpha-thalassemia/mental retardation syndrome X-linked) to form a protein complex, which can function as an H3.3-specific histone chaperone, while incorporating H3.3 into telomeres, thus contributing to the regulation of ALT.^{5,25} It has been reported that 80% of tumors with an ALT phenotype have mutations in either ATRX or DAXX.⁵ Loss of ATRX and/or DAXX has been associated with an ALT phenotype in multiple tumor types, such as sarcomas, pancreatic neuroendocrine tumors, gliomas, and uterine leiomyosarcomas.^{14,26-29} However, only up to 3% of

endometrial carcinomas harbor an ALT phenotype.²⁴ Therefore, the common loss of DAXX in low-grade endometrial carcinomas might not explain its role in contributing to the regulation of ALT.

Finally, endometrial carcinoma represents a heterogeneity of different histotypes. Each type may carry different histopathological features and genetic signatures.^{2,3} In our study, the heterogeneous DAXX expression pattern found in its different histotypes may suggest a potential role for target therapy. In vitro studies have already established its therapeutic value, as inhibiting DAXX was found to arrest the growth of glioblastoma in mice models.²⁹ In the therapeutic context, several approaches to target DAXX have been investigated, including using peptides or small molecules to disrupt DAXX interaction with chromatin-associated proteins.³⁰

Our study is not without limitations. The major concern is that we were not able to validate the specificity of the antibody by Western blot. Although we had positive and negative controls (IHC) for DAXX, ideally, a reaction with DAXX-rich and DAXX-deficient tissue on blots can give more convincing results. Second, we did not have precursor lesions in our study, including atypical endometrial hyperplasia with atypia. Including such cases would give us a clearer spectrum of DAXX expression in the pathogenesis of endometrial carcinomas.

In summary, we conclude that loss of DAXX protein expression is associated with low-grade endometrial carcinomas. With DAXX expression being found to be significantly higher in high-grade endometrial carcinomas, it is possible that future cancer therapies could target this pathway, which may have significant implications for treating patients with endometrial carcinomas.

Authors' Note

This study was presented at USCAP 2019 National Harbor as poster. It has never been published elsewhere.

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Author contributions

CJ and MN developed the theoretical formalism, performed the analytic calculations and performed the numerical simulations. Both CJ and SH contributed to the final version of the manuscript. MK contributed to the collection of the cases.

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Patients' Consent Waiver Statement

This is a retrospective study which involved only de-identified information. The consent is not required as per Northwell

Health Institutional Review Board (IRB) board. The IRB number for this study is 18-0890.

Supplemental Material

Supplemental material for this article is available online.

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