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Review Article

Mitochondrial Dysfunction in Patients with Urogenital Disease

Tzu-Yu Chuang[1], Te-Wei Chang[1], Shiou-Sheng Chen[1,2,3,4], Chan-Chi Chang[1], Wei-Ming Cheng[1,2], Yau-Huei Wei[5]*

[1]Division of Urology, Taipei City Hospital Zhongxiao Branch, [2]Department of Urology, School of Medicine, National Yang-Ming Chiao Tung University, [3]Commission for General Education, National Taiwan University of Science and Technology, [4]General Education Center, University of Taipei, Taipei, [5]Center for Mitochondrial Medicine and Free Radical Research, Changhua Christian Hospital, Changhua City, Taiwan

Abstract

Mitochondria are intracellular organelles responsible for the production of the majority of adenosine triphosphate (ATP). In addition to energy production, mitochondria also contribute to cellular apoptosis, the regulation of intracellular Ca^{2+} homeostasis, signaling through reactive oxygen species (ROS), and the coordination of the cell cycle. The prevalence rate of primary mitochondrial disease was estimated at nearly 1:5000. In this review, we have integrated recent evidence to discuss new insights into how mitochondrial dysregulation plays a role in bladder dysfunction, reproductive disorder and the correlation between mtDNA mutation and bladder cancer.

Keywords: Bladder, mitochondria, urology

Introduction

Mitochondria are intracellular organelles responsible for the production of the majority of adenosine triphosphate (ATP) and are thus called the powerhouse of mammalian cells.[1] In addition to energy production, mitochondria also contribute to cellular apoptosis, the regulation of intracellular Ca^{2+} homeostasis, signaling through reactive oxygen species (ROS), and the coordination of the cell cycle.[2] Mitochondrial dysfunction may result in a wide spectrum of human diseases, such as diabetes, autism, myopathy, optic neuropathy, gut dysmotility, cardiovascular disease, and neurological disorders.[3-5] The prevalence rate of primary mitochondrial disease was estimated at nearly 1:5000 and defined as disorders resulting from the mutations of either nuclear DNA (nDNA) or mitochondrial DNA (mtDNA).[6] Although some researchers have focused on bowel dysfunction in patients with a certain mitochondrial disease, more recent basic and clinical research have revealed that these patients have a higher prevalence rate of lower urinary tract symptoms (LUTSs) and sexual dysfunctions without an effective treatment available.[7] Besides, various studies have detected mtDNA mutations in bladder cancer.[8,9] In this review, we have integrated recent evidence to discuss new insights into how mitochondrial dysregulation plays a role in bladder dysfunction, reproductive disorder, and the correlation between mtDNA and bladder cancer.

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***Address for correspondence:** Dr. Yau-Huei Wei, Center for Mitochondrial Medicine and Free Radical Research, Changhua Christian Hospital, No. 176, 6th Floor, Zhonghua Road, Changhua 50046, Taiwan.
E-mail: yhweibabi@gmail.com

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Function of Mitochondria and the Mitochondrial Disease

Mitochondria are double-membrane bound organelles, which contain outer and inner membranes composed mainly of proteins and phospholipid layers. The matrix is the space within the inner membrane, and it is the important place for the biochemical reactions of the tricarboxylic acid (TCA) cycle (Krebs cycle) and β-oxidation of fatty acids that fuel nicotinamide adenine dinucleotide (NADH) or flavin adenine dinucleotide ($FADH_2$) into the respiratory chain for the production of ATP. Available evidence showed that tumor cells require massive ATP to synthesize proteins, lipids, and nucleotides for rapid cell growth.[10] Aerobic organisms as well as tumor cells acquire the majority of energy through the oxidative phosphorylation (OXPHOS) system, which is 13 times more efficacious than anaerobic fermentation.[11] The interference of the mitochondrial OXPHOS function could impair the cell cycle, which supports the hypothesis that mitochondria are one of the major players in uncontrollable cell proliferation.[12] Massari *et al*. found that bladder cancer cells used OXPHOS to provide most of the energy and also increased the transcription of genes required for glycolysis and fatty acid synthesis.[13]

One of the specific features of the mitochondrion is that it has its own genome, which is different from nDNA, and is thought to be derived from the circular genomes of aerobic archaebacteria engulfed by eukaryotic cells.[14] The mammalian mtDNA is a maternally inherited genome consisting of circular double-stranded DNA. As a consequence, diseases caused by a pathogenic mutation of mtDNA display a maternal mode of inheritance. Human mtDNA is much more susceptible to oxidative damage by ROS as compared with nDNA, resulting in point mutations or large-scale deletions. This is because mtDNA is intron-less and is located near the inner membranes embedded with mitochondrial respiratory enzymes, which generate ROS during electron transport and may attack mtDNA, lacking an efficient DNA repair mechanism.[15] DNA base excision repair (BER) is the major repair pathway for the repair of oxidative damage in mtDNA.[16] BER activity decreases with age, and this change may lower the DNA repair activity in mitochondria and increase the accumulation of mtDNA mutations.[17] Table 1 summarizes the urological diseases associated with mtDNA mutations.

Given the involvement of the multiorgan system, the diagnosis of the mitochondrial disease remains a challenge to clinicians. If the patients present with any symptoms, such as poor growth, muscle weakness, seizure, autism, visual or hearing impairments, developmental delays, gastrointestinal disorders, and thyroid dysfunction, the evaluation of mitochondrial biomarkers in the blood, urine, and spinal fluids should be performed.[18] First, lactate, pyruvate, and amino acids in the plasma and spinal fluids, plasma acylcarnitines, and urine organic acids should be measured. Then, the next-generation sequencing of nDNA and mtDNA in white blood cells should be performed. Whole exome sequencing and whole-genome sequencing have been considered as first- or second-line genetic tests for patients with suspected mitochondrial diseases.[19] The Mitochondrial Medicine Society has published clinical criteria to aid the diagnosis of mitochondrial disease.[18]

Patients with mitochondrial disease present with multisystem disorders, resulting in the need for multidisciplinary care. Current clinical care is developed using consensus-based recommendations from specialists due to the lack of high-level randomized trials.[20] Various organ involvements in mitochondrial disease should be treated cautiously with different protocols. The Mitochondria Medicine Society has also provided guidelines based on an international consensus of physicians with experience in managing mitochondrial diseases.[21]

Mitochondrial Dysfunction and Lower Urinary Tract Symptom

Benign prostate hyperplasia is a major problem associated with human aging. Such a change could lead to LUTS or bladder outlet obstruction and result in bladder over-distension, decreased compliance, and increased postvoid residual urine volume. Constant bladder over-distension could also induce a reduction of bladder blood flow and chronic ischemia. Repeated ischemia and reperfusion cycle may cause an overproduction of ROS and cause oxidative damage to the bladder tissues. ROS include superoxide anions, hydrogen peroxide, and hydroxyl radicals, which could interact with nitric oxide and cause irreversible damage to DNA. Mitochondria are both the primary source and target of ROS. Oxidative damage to mtDNA could lead to mitochondrial dysfunction and in turn trigger the inflammatory response.[22]

On the other hand, one of the causes of mitochondrial dysfunction is genetic disorder, which is caused by the mutation of mtDNA-encoded genes that impair OXPHOS and result in decreased ATP generation. Loss of smooth muscle contractility is one of its phenotypic characteristics. Previous studies have shown that bladder detrusor muscle contraction needs to be supported by abundant energy, mainly from intracellular ATP production via mitochondrial OXPHOS.[23,24] Moreover, an *in vitro* animal model showed that rabbits with partial outlet obstruction revealed defects in oxidative metabolism and decreased activities of enzymes participating in the TCA cycle. Nevel-McGarvey *et al*. demonstrated that the reversal of the partial outlet obstruction and bladder decompensation by surgical intervention could recover the mitochondrial function.[25] Thus, it has been suggested that mitochondria play an important role in bladder muscle function and coordinate the micturition cycle.

LUTS is a general term that refers to conditions affecting the bladder and urethra functions. Storage symptoms include urgency, urge incontinence, frequency, and nocturia. Voiding symptoms include slow stream, terminal dribbling,

Table 1: Urological diseases associated with mitochondrial DNA dysfunction

Urological diseases	Mitochondrial dysfunction	Description
LUTS	mtDNA mutation	Females experienced more OAB symptoms and greater severity Patients with mitochondrial disease: 81.5%: OAB 4.5%: Low urine stream 28.8%: SUI
Reproductive dysfunction	mtDNA mutation	Spermatogenesis defects: Meiotic arrest and teratozoospermia Aging males showed reduced number of mitochondria 24.8% patients with severe mitochondrial disease have lower reproductive success
Bladder cancer	C16069T, A4918G and T10464C mtDNA variants A15607G, G8697A, G14905A and C15452A polymorphisms D-loop region mutation and deletion in mtDNA Overexpression of 12-bp deletion of the Cyt*b* gene	Results focused on the copy numbers of mtDNA in patients with bladder cancer showed controversial results

mtDNA: Mitochondrial DNA, LUTS: Lower urinary tract symptoms, OAB: Overactive bladder, SUI: Stress urinary incontinence, Cyt*b*: Cytochrome *b*

hesitancy, and straining. Postmicturition symptoms include postmicturition dribbling and incomplete emptying.[26] Patients with mitochondrial dysfunction frequently experience LUTS. A study on adults with confirmed mitochondrial diseases revealed that 81.5% of the patients had overactive bladder (OAB) symptoms, 34.5% had low stream symptoms, and 28.8% had stress urinary incontinence.[7] Females with mitochondrial dysfunction also experienced more OAB symptoms and greater severity compared with the healthy control.[7] The findings of the study conducted by Feeney *et al*. also supported this point of view.[27] The Newcastle Mitochondrial Disease Adult Scale (NMDAS) was used to evaluate the mitochondrial disease severity. Patients with a higher NMDAS score were at a higher risk of bladder dysfunction. Approximately, 25% of male patients and 37.5% of female patients with mitochondrial disease developed nocturia. Urinary incontinence was also found in 30.4% of patients with mitochondrial disease, as compared to 8.2% of the general population.[27] Urodynamic studies revealed that the most common symptoms of patients with severe neurological disorders are detrusor overactivity and increased bladder sensation. Detrusor underactivity was also observed in 14.2% of patients with neurological disorders.[27] These findings suggest that more urological attention should be given to patients with mitochondrial diseases in clinical practice. Moreover, clinicians are advised to be cautious when managing patients with LUTS or any symptoms of mitochondrial disease. It is conceivable that the detrusor muscle with mitochondrial dysfunction is exposed to higher levels of ROS and imbalanced redox signaling due to increased electron leakage from the defective respiratory chain. However, the molecular mechanism by which mitochondrial dysfunction leads to LUTS needs to be investigated further.

Mitochondria and Reproductive Function

Several animal models revealed that mitochondrial respiration and OXPHOS function correlate with the reproductive cycle and that the reproductive activity in adult rats and cats declines with age.[28,29] Human subjects have also presented with a decrease with age in bone density and muscle mass and decreased body hair, hot flush, insomnia, and erectile dysfunction, which are associated with a gradual and progressive decline in the plasma level of testosterone.[30] Luo *et al*. found that the number of mitochondria of Leydig cells was reduced in aged males, which was correlated with the decrease of testosterone production.[31] The older Leydig cells produced much more mitochondria-derived ROS and showed an age-related decrease in the intracellular levels of antioxidants, and this is consistent with the theory that the ROS compromise the ability of old Leydig cells to produce testosterone.[31-33]

Oxidative stress is also known to be associated with infertility. It has been established that ROS have toxic effects on sperm function and the quality of sperm. Ames *et al*. showed that the males with a long smoking history displayed a decline in fertility and increase risk of genetic defects, which were related to the lower concentration of Vitamin C in the semen.[34] Patients with varicoceles were found to have higher plasma protein carbonyl and lower protein thiols, indicating that oxidative damage to blood proteins could affect fertility.[35] Spermatozoa are also susceptible to oxidative stress-induced damage due to high levels of polyunsaturated fatty acids in the plasma membranes and low concentrations of antioxidant enzymes in the cytoplasm.[36,37] Spermatozoa with defective mitochondria generate ATP in a less efficient way and simultaneously produce more free radicals to damage mtDNA, leading to a decline of motility and fertility. These damages could also accelerate germ cell apoptosis, resulting in the decrease in sperm count and male infertility.[38] Interestingly, Nakada *et al*. found that mtDNA mutations and respiratory chain defects could induce meiotic arrest and teratozoospermia, emphasizing the importance of mitochondrial function in spermatogenesis.[39] A higher frequency of 4977 bp deletion of mtDNA in the sperm was also found to be correlated with the lower motility of spermatozoa.[40] 8-Hydroxy-2'-deoxyguanosine (8-OHDG) is regarded as a biomarker to detect the oxidative DNA damage induced by ROS. Chen *et al*. have found that patients with 4977 bp deletion of mtDNA in the sperm had significantly

higher 8-OHdG contents in the leukocyte DNA of the spermatic vein.[41] These findings suggest that mtDNA is associated with poor sperm quality.

As for erectile dysfunction, smooth muscle cells account for 40%–52% of the cavernous muscle to maintain the penile erection.[42] In an animal model, the expression of F1-ATP synthase in the cavernosum smooth muscle cells appeared to be lower in diabetic mice with erectile dysfunction. Furthermore, research has shown that the upregulation of F1-ATP synthase expression could suppress the apoptosis of cavernosum smooth muscle cells by increasing endothelial NOS expression and the cyclic guanosine monophosphate levels.[43]

In humans, a strong association has been observed between mitochondrial dysfunction and impaired smooth muscle function. A recent study revealed that the reproductive function of men with mitochondrial disease was significantly compromised.[44] Men with more severe mitochondrial diseases were found to have lower reproductive success rate than patients who were mildly affected (24.8% as compared with 16.3% of the general population, $P = 0.027$).[44] This finding is not consistent with that of a previous study showing that the reproductive success of women with mitochondrial dysfunction does not differ from that of the general population.[45] Broadly speaking, mitochondrial dysfunction affects three main fertility factors in males, including the production of testosterone, spermatogenesis (lower sperm count and higher incidence of abnormal spermatozoa), and cavernous smooth muscle function. Further research is needed to address the detailed mechanisms leading to the impairment of reproductive function in males with mitochondrial disease.

Mitochondria and Bladder Cancer

Bladder cancer is a major public health problem and ranks ninth in terms of worldwide cancer incidence. In Taiwan, the age-standardized incidence of bladder cancer was 7.96/100,000 subjects in the general population, and the prevalence rate was higher than that of other Asian countries.[46] Environmental carcinogens, such as tobacco, aristolochic acid, and arsenic in drinking water, may contribute to the occurrence of nearly 50% of all bladder cancers. Molecular genetic studies concerning the inheritance were also conducted for a better understanding of the pathophysiology of bladder cancer.[47]

Various mtDNA mutations have been identified in different types of cancer. In recent years, somatic mtDNA mutations were found to be associated with bladder, lung, breast, kidney, colon, head and neck, stomach, and leukemic malignancies.[48,49] Apart from the high rates of mtDNA base substitutions, single base insertions, and D-loop deletions in human and rat bladder cancers, nDNA-encoded mitochondrial proteins and enzymes were also found to be associated with mitochondrial dysfunction in cancers.[50] The mtDNA mutations bring about the disruption of the electron transport chain (ETC), which produces more ROS followed by additional mtDNA mutations, finally culminating in a vicious cycle.[51] Shakhssalim *et al*. collected and analyzed the DNA samples from the blood, neoplastic tissues, and adjacent nontumoral tissues of 26 patients with bladder malignancy as well as DNA samples from the blood of 504 healthy controls and showed that the C16069T mtDNA variation plays an important role in bladder cancer.[52] Another study with 926 patients reported that A4918G and T10464C variations were associated with bladder cancer and low mtDNA content was also correlated with the increased risk of bladder cancer.[53] This decrease in mtDNA copy number from peripheral blood cells and tissue cells may suggest that the cancer cells shift to more glycolytic metabolism, which induces carcinogenic pathways, such as the PI3k-PTEN-AKT transduction pathway, to limit apoptosis and increase cancer cell survival. The Warburg effect was first described by Dr. Otto Warburg in 1927 who found that most tumor cells increase its uptake of glucose. This theory has influenced the development of the imaging tools used for the detection of tumors and monitoring of malignancy, such as ^{18}F-fluorodeoxyglucose positron emission tomography.[54] These researches support that germline mtDNA mutations have a correlation with the energy metabolism of bladder malignancy and may guide the development of markers for the prediction of the risk of developing bladder cancer.

The human mitochondrial genome consists of a circular DNA of 16,569 bp, which encoded 22 transfer RNAs, 2 ribosomal RNA, subunits of ETC (Complexes I–IV) and ATP synthase (Complex V), and a noncoding displacement-loop (D-loop) region, where the promotors for heavy and light strands of mtDNA and transcription and replication origins of mtDNA are located.[55] A wide spectrum of diseases are associated with mtDNA mutations, which cause alterations of the ETC complexes, for example, prostate cancer was found to have a correlation with D-loop mutations in Complex I.[56] These ETC complexes transport electrons from a higher energy state to a lower energy state with subsequent energy release for ATP synthesis. It is the electrochemical gradient that drives the synthesis of ATP by coupling with ATP synthase. Recent studies have shown that OXPHOS is utilized for the massive production of ATP by advanced malignancies and metastatic tumor cells.[57] Moreover, highly invasive and less invasive urothelial cancer cells were found to have intercellular mitochondrial transferring through tunneling nanotubes, which facilitate the progression, invasiveness, and reprogramming of the bladder cancer cells.[58]

Somatic mtDNA mutations have also drawn much attention in recent years and are regarded as tumorigenic and known to participate in tumor progression. The somatic mtDNA mutation rate is nearly ten times higher than that of nDNA.[59] The instability of mtDNA could affect energy metabolism and the generation of ROS in mitochondria, the initiation of apoptosis, and tumorigenesis.[60] Furthermore, the mtDNA genes lack introns, and mutations in coding sequences may directly alter amino acid sequences and protein structures. It is well established that the histological progression of bladder cancer is correlated with p53 tumor suppressor gene

mutation. Loss of p53 could lead to a significant increase in the vulnerability of mtDNA to damage and finally result in the increased frequency of *in vivo* mtDNA mutations.[9] Wada *et al*. found that the most frequent mutations in the D-loop region were observed in the polycytidine stretch between nucleotide position (np) 303–309, which is the most unstable microsatellite region in the mtDNA of the primary tumors.[61] The mtDNA genes *ATPase6*, *ND1*, and cytochrome *b* (CytB) and the D-loop region were analyzed in 30 patients with bladder neoplasms and 27 healthy individuals. It was found that A15607G, G8697A, G14905A, and C15452A polymorphisms were more frequent in patients with bladder neoplasm than those in healthy controls. It was suggested that these mtDNA mutations could be considered as a bladder cancer biomarker, which can also be examined in urine or saliva.[8] Among the mtDNA-encoded polypeptides, CytB is essential for the core function and assembly of Complex III. In human bladder cancer, overexpression of a 21-bp deletion in the *CytB* gene and increased production of ROS and lactate were documented. The mutation of the *CytB* gene also induced significant tumor growth *in vitro* and *in vivo* by triggering rapid cell cycle progression through the upregulation of the nuclear factor-KB2 signaling pathway [Table 2].[62] The D-loop region, regarded as the noncoding region, has been demonstrated to have a higher mutation rate than the coding regions of mtDNA in the tumor tissues of cancer patients. Moreover, somatic mutations in 7 out of 31 (23%) patients with bladder cancer were identified in the D-loop region.[61] Another group reported that up to 76.9% of urothelial carcinoma patients had mtDNA heteroplastic mutations in the D-loop region from urine and peripheral blood samples, which also revealed the importance of mtDNA sequencing in bladder cancer.[65]

The relationship between mtDNA copy number and neoplasm is still being investigated in different studies. The copy number of mtDNA per cell is maintained within a range from 2 to 10,000, which varies constantly depending on the energy demands, oxidative stress, and pathological conditions. The copy number reflects the net result of the energy demand of a cell and is disturbed by imbalanced energy metabolism. The mtDNA copy number decline was reported in a human brain with neurodegenerative disease and patients with hepatocellular carcinoma.[66] Lower mtDNA copy numbers in the neoplasm than in the adjacent tissue was associated with an increased risk of bladder cancer, and a dose-response relationship was also observed ($P < 0.001$).[53] Advanced age, gender, and smoking history were also correlated with low mtDNA copy number.[53] On the other hand, mtDNA copy number increase was also reported in head and neck cancer, breast cancer, and non-Hodgkin's lymphoma.[67-69] This compensatory response may explain that cancer cells could display impaired ATP synthesis and reduced respiratory function.[70] Yoo *et al*. found that in patients with bladder neoplasm, the average mtDNA copy number in their urine samples was nearly three times higher than that in their peripheral blood samples. However, the average mtDNA copy numbers in the urine from patients with low-grade and high-grade tumors did not differ significantly.[65] Novel diagnostic markers, such as circulating mtDNA, in the liquid biopsy may provide additional information for diagnosis. The high copy number, simple organization, and shorter length of mtDNA make it easier to be detected in the serum and plasma containing a low amount of total DNA.[71] A previous study showed that the circulating mtDNA levels of the 140 patients with bladder cancer, renal cancer, and prostate cancer were 14-fold higher than those of healthy individuals. The diagnostic accuracy is best in bladder cancer (area under the curve = 0.961) among these urological malignancies.[72] However, the clinical significance of these circulating cell-free mtDNA needs to be further validated. Table 3 summarizes the updated literatures concerning novel biomarkers in bladder cancer.

Conclusion

Current evidence supports that mitochondrial dysfunction is associated with bladder cancer, erectile dysfunction, impaired spermatogenesis, and infertility in males. Circulating mtDNA could be used as the "liquid biopsy" for the early detection or monitoring of treatment outcomes of urological diseases in future. These findings suggest that mitochondrial dysfunction plays a significant role in some urologic diseases and it could be a potential therapeutic target.

Table 2: Urological diseases associated with defects in the electron transport chain

Respirator chain	Disorders	Features
Complex I	One base-pair alteration in the ND1 gene[8]	Enhanced production of ROS in 73% of patients with bladder cancer
Complex III	A15607G polymorphism in the *Cytb* gene, which is fundamental for the assembly and function of Complex III[9]	Enhanced generation of the ROS and further involved in proliferating signaling pathways associated with tumor progression
Complex IV	Deficiency of cytochrome c oxidase activity[37]	Meiotic arrest and enhanced apoptosis during spermatogenesis: Sperm abnormalities in the middle piece and bending forms
Complex V (ATPase)	The mRNA levels of Atp11 and atp12 genes are decreased by about 50% after partial obstruction in bladder smooth muscle and remained low through 14 days[63]	Bladder outlet obstruction-related decrease of the bladder smooth muscle contractility[64]
	ATPase6 gene mutation[8]	Maintenance of mtDNA
		Cell transformation, elevated ROS production and tumor progression
		Loss of efficient programmed cell death

mtDNA: Mitochondrial DNA, ROS: Reactive oxygen species, Cyt*b*: Cytochrome *b*

Table 3: Summary of updated literatures with novel biomarkers in bladder cancer

Cancer type	Number of patients	Control	Fluids	Novel biomarkers	AUC	Authors
Early NMIBC	92	33	Urine	cfDNA	0.94	Ou *et al*., 2020[73]
Hematuria patients referral for bladder cancer	97	103	Urine	DNA methylation-mutation assay Mutation genes: FGFR3, TERT and HRAS	0.96	van Kessel *et al*., 2017[74]
Bladder cancer	66	48	Urine	microRNA (miR-30a-5p, let-7c-5p and miR-486-5p)	0.70	Pardini *et al*., 2018[75]
Bladder cancer	392	100 healthy subjects 480 other types of cancer	Serum	Combination of seven circulating microRNA (7-miRNA panel: miR-6087, miR-6724-5p, miR-3960, miR-1343-5p, miR-1185-1-3p, miR-6831-5p and miR-4695-5p)	0.97	Usuba *et al*., 2019[76]
Low grade NMIBC	18	10	Serum	Haptoglobin, as a circulating serum protein biomarkers	0.87	Nedjadi *et al*., 2020[77]
NMIBC, MIBC and metastatic bladder cancer	57	48 healthy subjects 15 BPH	Peripheral blood	CTC	0.819	Qi *et al*., 2014[78]

NMIBC: Nonmuscle invasive bladder cancer, MIBC: Muscle invasive bladder cancer, BPH: Benign prostate hyperplasia, CTC: Circulating tumor cells, cfDNA: Cell free DNA, AUC: Area under the curve

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Conflicts of interest

There are no conflicts of interest.

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