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ABOUT COVER

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FIELD OF VISION

Oliver Wendell Holmes' 1836 doctorate dissertation and his journey in medicine

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Abstract

Oliver Wendell Holmes' 1836 hand written doctorate dissertation on acute pericarditis was discovered in the archives of the Boston Medical Library 101 years after it was successfully defended. It was then printed as an unabridged monograph with an explanation of its provenance. The dissertation has received little scrutiny since then. Holmes gathered materials for the scholarly work while he was a third and fourth year student at Ecole de Medecine in Paris. His mentor, Pierre-Charles-Alexandre- Louis insisted on the meticulous gathering and recording of every patient's history and findings. Each category of data was given a weighted numerical value of diagnostic importance and the information was placed in a registry. Holmes became a disciple of Louis in gathering data by direct observation and measuring outcomes in a "statistical" fashion. Holmes dissertation on acute pericarditis describes the state of knowledge about the illness in the 1830s. When Holmes and other students who had studied in Paris returned to the United States, they helped turn American Medicine from opinion and strong personal bias toward scientific objectivity. Oliver Wendell Holmes eventually became both a professor of anatomy/physiology and a dean at Harvard Medical School. He is recognized as a leader in medicine and a popular author in America and beyond. In his late and infirmed years, Holmes questioned the wisdom of his unswerving advocacy for the scientific underpinnings of medicine. In retrospect he had overlooked the importance of also advocating that each patient be approached with comforting compassion.

Key words: Acute pericarditis; Medical statistics; Childbed fever; Harvard medical school; Pierre-Charles-Alexandre- Louis

Core tip: Oliver Wendell Holmes' 1836 Doctorate Dissertation on Acute Pericarditis has

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received little scrutiny since its publication 100 years after it was successfully defended at Harvard Medical School. The state of knowledge about pericarditis in the mid eighteen hundreds was unusually sound considering the inability to study tissue with microscopy. However treatment was a matter of opposing expert opinions and the fashion of the day in Paris was to disparage professors who disagreed. Paris was a mecca for students. Cadavers were plentiful for study. Lectures and clinics were free.

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COMMENTARY ON HOT TOPICS

Medical education and medical practice is constantly changing. Medical schools intermittently modify their curricula to engage their students and have them achieve excellence rather than mere competence. It is said that if one does not have knowledge of history, one does not understand the present. Oliver Wendell Holmes early medical education and observations in Boston was at a time when doctors were guided by opinions derived from their own experiences and those of their highly respected peers. Many of the most respected had studied in London, Scotland or Paris. While a student in Paris, Holmes learned to value his own observations, to record his observations, to give variable weight to symptoms, physical findings and the revelations of autopsy pathology to arrive at a diagnosis. His mentor, Louis, meticulously collected and analyzed such data in an early attempt at a crude scientific method. When Holmes and other American Medical students returned from Paris and gained positions of authority, they instigated and accelerated reforms in medical education and the treatment of patients.

Oliver Wendell Holmes' 1836 Doctorate Dissertation and His Journey in Medicine is about an early attempt to separate fact from fiction, truth from opinion, statistics from mere numbers that ultimately evolved into the scientific method we rely upon today.

AN OVERVIEW OF HOLMES' DOCTORATE DISSERTATION

Oliver Wendell Holmes' 1836 doctorate dissertation at Harvard Medical School has received relatively little scrutiny since it was successfully defended before a board of examiners. At that time, the very last requirement towards a doctor of Medicine degree was a dissertation on a medical subject[1]. The next step was to establish a practice. "In the state of Massachusetts, the Harvard degree alone entitled the student to practice in the state; graduates of other institutions were examined by the state medical society[2]".

Holmes' handwritten, hurriedly completed manuscript lay hidden in the archives of the Boston Medical Library for 101 years. In 1937 it was discovered, preserved, and transformed into an unabridged printed monograph with a brief explanation of its provenance[1].

Materials, methods and personalities known to Holmes while he was a third and fourth year medical student in Paris are included in his dissertation. Holmes selects observations about "acute pericarditis written by authoritative physicians of the French School" and elsewhere. He describes cases of acute pericarditis from his personal experience at the teaching hospitals in Paris, some autopsied cases by faculty anatomist Gabriel Andral and likely other cases from the registry of his Parisian mentor, Pierre-Charles-Alexandre-Louis, who, for practical reasons of brevity, was simply called and answered to "Louis".

The theme of Acute Pericarditis is "…to present the most recent and best established recent ideas upon its history and treatment as they are expounded in the most approved authorities or as they result from a certain number of cases subjected to analysis[1]". Holmes was aware that Louis had published a memoir on pericarditis in 1826, but seldom refers to it. Holmes' contemporaneous-fellow-American student in Paris, James Jackson Jr, in a letter to his father dated January 6, 1832, summarized Louis' four important conclusions about pericarditis. "It is a common disease. Like

pleurisy, it is often latent, being attended neither with pain, nor any of that assemblage of horrid symptoms by which it is generally described. It can be diagnosed by percussion and not divined, as Laennec has said of it. It is by no means so fatal as has been generally supposed[3]".

One might ask—why did Holmes pursue his clinical medical education in Paris and how did that experience influence his journey in medicine?

EARLY LIFE OF OLIVER WENDELL HOLMES

Oliver Wendell Holmes was raised in Cambridge, Massachusetts. His father, Abiel, was a Calvinist and a minister. Holmes' mother, Sarah Wendell, had roots that extended to Scotland. His parents instilled a system of life-long values. They were hard work, discipline and living within one's financial means.

UNDERGRADUATE AND GRADUATE EDUCATION IN THE UNITED STATES

Holmes attended Phillips Academy preparatory school. He then graduated from Harvard College in 1829. Thereafter he briefly attended Harvard Law School. After becoming disenchanted, Holmes changed career goals from the law to medicine. He enrolled in a private proprietary medical school that shared some facilities and faculty with the Massachusetts Medical College of Harvard. There are several sources that specify that Holmes attended a private proprietary medical school rather than Harvard Medical School. The most authoritative is Oliver Wendell Holmes, who in his Farewell Address to the Medical School of Harvard University said, "The Private Medical School that I had joined was one established by Dr James Jackson, Dr Walter Channing, Dr John Ware, Dr Winslow Lewis and Dr George W Otis[4]". Not all of these doctors were on the faculty of the Massachusetts Medical College of Harvard. In addition, three medical historians agree that Holmes was enrolled at a private proprietary medical school. James F Ballard, the director of the Boston Medical Library wrote in the introduction to dissertation on acute pericarditis that Holmes … took two courses at a private medical school in Boston[1]. John T Morse Junior, a relative and biographer of Oliver Wendell Holmes, cites a quotation among Holmes' reminiscences in Life and Letters of Oliver Wendell Holmes Volume I, "The head of the private school at which I studied was Dr James Jackson, a very wise and good man, … Dr Jackson never talked of curing a patient other than in its true etymological sense of taking care of him[5]". Elinor M Tilton, a historian and biographer wrote on several occasions that Doctor Oliver Wendell Holmes attended a private medical school[6].

Early on, Holmes developed an admiration for his mentor, Dr. James Jackson Sr who was on the faculty of both schools. The curricula included two annual courses of lectures for three or four consecutive months each winter to be supplemented by an apprenticeship with a clinician or clinicians for an inclusive time span of approximately three years.

CLINICAL MEDICAL INSTRUCTION IN PARIS, FRANCE

Holmes spent two and a half years in Boston and the remainder of his formal medical studies during 1833 through 1835 at Ecole de Medecine in Paris, considered to be a Mecca of medical expertise and innovative teaching. Holmes sailed for Europe on March 30, 1833 and returned to United States soil on December 14, 1835. Students and fully certified doctors studied in Paris to achieve advanced knowledge, to enhance their skills, to better serve their patients and to gain greater standing within their profession.

Unlike America, Paris had full-time faculty, abundant free lectures, free clinics to all foreign students and 4000 unclaimed corpses each year for dissection[7]. In a letter dated November 14, 1833, Holmes marveled that Ecole de Medecine's enormous anatomy lab could accommodate enough corpses to be dissected by 600 students in a single session[5].

Lectures were by luminaries who encouraged students to accompany them on daily early morning ward rounds. At the bedside, these master-clinicians taught the subtle uses of Renee Laennec's recently invented monaural stethoscope to detect markers of

inner-body illness. Technical modifications and nuances of its use were evolving when Oliver Wendell Holmes was in Paris.

Holmes believed that the worst doctor on the staff of Ecole de Medecine surpassed the best doctor in America, with the exception of his American mentor, James Jackson Sr In a letter home dated August 18, 1833, Holmes wrote that American Medicine is "…where stupidity is tolerated, where mediocrity is applauded and where excellence is deified[5]".

During his exhilarating Paris experience, Holmes quickly became fluent in spoken and written French and bonded with two students from Harvard Medical School who also chose to expand their knowledge at the Mecca. Each had a lineage of medical aristocracy in Boston. Their fathers were founders of the Massachusetts General Hospital. Holmes' comrades were James Jackson Jr, the son of his Boston mentor and Jonathan Mason Warren, the son of John Collins Warren who was the second Professor of Anatomy and Surgery at Harvard Medical School. Mason's grandfather, John Warren, was its first Professor of surgery and founder of the school.

At Ecole de Medecine, Holmes became a disciple of Louis who had developed what might be termed an observation-based numerical method of documenting the symptoms and physical findings of each patient's illness. After a disease caused the death of its host, a post-mortem autopsy was performed and the clinical-pathological data became part of the record. The sum of each patient's findings was organized and placed into a registry. Illness became unified rather than segmented and could be subjected to statistical analytics based on Louis' numerical system. Holmes spent hours at the bedside documenting the symptoms and physical findings of each assigned patient. If death occurred, he personally performed the autopsy and recorded the findings.

Louis specialized in diseases of the chest. He was an expert among experts in the nuances of percussion that was rediscovered by Jean Nicholas Courvisant (1771-1821), an academic French physician who perfected the art of percussion. Louis was also an expert in auscultation of the heart and lungs, especially the treasure of new auscultatory knowledge gained with Renee Laennec's stethoscope.

THE 1836 DOCTORATE DISSERTATION

In Acute Pericarditis, Holmes approaches the topic as he was taught by Louis. The presentation is divided into symptoms, physical findings and patho-physiologic findings. Following the academic custom when he was in Paris, Holmes attacks those who falsely claim priority for having described a pericardial symptom or sign that was first noted by another. The dissertation reflects the status of unenlightened medicine in the 1830s. Yet, it is surprisingly accurate in some areas and not-so-surprisingly inaccurate in other areas —such as treatment.

In Oliver Wendell Holmes' 1836 doctorate dissertation, he describes familiar symptoms and signs that result from pericardial inflammation and an excessive volume of pericardial fluid.

The familiar symptoms are shortness of breath, cough, oppression, anxiety, anguish, precordial chest pain—at times made better or worse during altered bodily position, syncope, chills and perspiration. The familiar signs are fever, rapid heart rate, an enlarged area of precordial dullness, a palpable precordial thrill, distant heart sounds, a "leathery" rub and a pulse that might be irregular or variable in amplitude.

Without the benefit of measurements, Holmes correctly reasoned that cardiac tamponade results from excessive pericardial fluid under pressure that inhibits the ventricles from filling[1].

Autopsied hearts were grossly described. Holmes did not have the benefit of precision tools found in a modern pathology laboratory. The monocular compound microscope was just emerging as a potential diagnostic aid and Holmes wisely procured one in Paris to use upon his return to Boston. The usual pale transparent pericardium of normal hearts, when inflamed from pericarditis, appeared red, possibly thickened and over time calcified. Pericardial fluid was quantified and the color described as clear, turbid, hemorrhagic, yellow, brown, green or thickened with pus. Holmes separates "clear-serous" from "turbid" fluid and comments that the sequelae of the transformed turbid "plastic lymph" might result in fibrous bands that connect the parietal and visceral pericardial layers or actually seal the layers together[1].

There is a section in which Holmes discusses pathological processes associated with acute pericarditis. Germ theory and microbial diseases were not yet known. Undifferentiated "Rheumatism" (that likely included unrecognized acute rheumatic

fever) and diseases of the lungs and pleura were believed to be culpable. Phthisis and consumption (terms for tuberculosis) were endemic. James Jackson Jr wrote that phthisis was the disease that filled Louis' wards. "Over a period of s few months, a student might take part in the examination of as many as fifty cases of tuberculosis[8]". By direct extension from the lungs, tuberculosis rarely causes acute pericarditis, but often results in subacute pericarditis, or late constrictive pericarditis.

Treatment of acute pericarditis was an exercise in unproven opinion. The duration of the disease was three days to three months with a death rate of 15%[1]. Both antisepsis and asepsis were beyond the horizon. It is no wonder that Louis was a therapeutic minimalist and of the opinion that a patient had a better chance of improving with nature's curative powers than with intuitive manipulations to harmonize the body's fluid humors described by Galen as blood, phlegm, black and yellow bile. Standard treatment of the day included purges, emetics, blistering, bloodletting, application of leaches and nostrums. There were a limited number of herbal medications in the Pharmacopeia. Digitalis was often prescribed.

Holmes' Acute Pericarditis Dissertation in 1836 cites a slim therapeutic ray of hope for a better future. Pericardiocentesis is mentioned once without further elaboration.

In his dissertation, Holmes cites a Dr. Fordyce, who, (in contrast to Jean Babtiste Bouillaud's vigorous bloodletting treatment of pericarditis or rheumatism), avoided the practice of bloodletting for 15 years while managing several hundred patients with only 2 or 3 losses. Fordyce concluded that bloodletting hastened death[1]. Yet, at the time, Holmes' contemporaries paid no heed. Many years later, during his retirement Farewell Address, Holmes mentioned that Louis had published an essay titled "Bleeding in Some Inflammatory Diseases" in which using his meticulous observational numerical method, demonstrated to his own satisfaction, that bloodletting failed to improve acute disease such as pneumonia[4]. In 1996, Sylvan Weinberg cited specific data from Louis' experiment in an editorial titled The Quest for Medical Certainty[9].

After returning to Boston, Holmes embraced Louis' numerical-observation-based approach. He believed that a treatment should be abandoned if it was not proven to be beneficial. As he later wrote, "I firmly believe that if the whole materia medica, as now used, could be sank to the bottom of the sea, it would be all the better for mankind, and all the worse for the fishes[10]". Holmes crusaded to eliminate child bed fever. In 1843. He accused doctors who caused puerperal fever and maternal death of criminal behavior because they ignored observational evidence that they were transmitting deadly agents from their clothes and hands to their patients[11].

Acute Pericarditis presents a panoramic view of the illness. Holmes' dissertation is based on his experience in Paris. He cites knowledgeable contributors to the field. Except for Louis, it was common for an esteemed lecturer to skewer a colleague for an inaccurate statement or belief. With distain, Holmes disparages Laennec's successor, M. Bouillaud on several counts. He expropriates Laennec's leather creaking pericardial rub sign to M Collin[1]. Bouillaud "makes an unnecessary parade of what he considers his discovery[1]. He promotes the useless practice of bloodletting and he lectures "…with an egotism and censoriousness of others which imply a profound conviction of truth…[1]", he also undervalues the significance of flatness of precordial percussion[1].

HOLMES' JOURNEY AS A MEDICAL DOCTOR

After Holmes received his doctor of medicine degree from Harvard Medical School and simultaneously qualifying to practice medicine in Massachusetts, it could be said that his journey in medicine was outstanding. He eventually became the Parkman Professor of Anatomy and Physiology and a Dean of Harvard Medical School[12]. He is recognized as a leader in medicine and a popular author in America and beyond. "American Medicals" brought Louis' methods from Paris to Boston and to other medical centers in the United States[13]. It became the Coda for diagnosis and ultimately, with modifications, for research[14]. As medicine was evolving, Holmes was a committed proponent of its scientific underpinnings. Yet in his late and infirmed years, Holmes questioned the wisdom of his unwavering advocacy for Louis' analytical numeric method[10]. In an essay titled Scholastic and Bedside Teaching, he had overlooked the benefits of James Jackson Sr's artful, comforting and compassionate approach to each patient[15].

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OPINION REVIEW

Classic Ehlers-Danlos syndrome and cardiac transplantation - Is there a connection?

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Abstract

Ehlers-Danlos syndrome (EDS) is a heterogeneous group of connective tissue disorders comprised of several types. Classic EDS is an autosomal dominant disorder with stretchable skin, delayed wound healing with poor scarring, joint hypermobility with subluxations or dislocations, easy bruisability, hernias, aneurysms and cardiac abnormalities. Advances in genomics technology using next-generation sequencing has led to the discovery of causative genes for connective tissue disorders, hereditary cardiomyopathies and cardiovascular diseases including several genes for connective tissue disorders. A 55 year-old male exhibited thin stretchable skin, atrophic scars, easy bruising, joint pain and dislocations requiring multiple knee surgeries and a Beighton hyperflexibility score of 6 out of 7. He was found to have a heterozygous missense *COL5A1* gene variant involving exon 3 at nucleotide c:305T>A with an amino acid position change at p.Ile102Asn consistent with classic EDS. He had a heart transplant at 43 years of age due to cardiac failure of unknown cause. This patient with classic EDS is brought to medical attention and should be of interest to cardiologists, heart transplant specialists and surgeons, particularly in individuals with unexplained cardiac failure and then diagnosed prior to surgical intervention to avoid poor wound healing, scarring and other tissue involvement (*e.g.*, vascular anomalies, blood pressure instability, aneurysms) as components of EDS.

Key words: Ehlers-Danlos syndrome; Next-generation sequencing; Surgical complications; Beighton hypermobility scale; Cardiac failure and transplantation

Core tip: Ehlers-Danlos syndrome (EDS) consists of a group of connective tissue disorders involving both autosomal dominant and recessive inheritance patterns often including collagen genes with variants readily detectable using disease-specific gene panels with

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next-generation sequencing. A 55 year-old male is reported with features of a connective tissue disorder. He had a heart transplant at 43 years of age. He was found to have a *COL5A1* gene variant (c:605T7A; p.I1e102Asn) causing classic EDS. He is brought to medical attention for consideration of a genetic cause of cardiac failure including EDS in other patients and complications of surgery which may occur.

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INTRODUCTION

Ehlers-Danlos syndrome (EDS) is a connective tissue disorder comprised of several types due to mutations in genes encoding proteins (*e.g.*, collagen) such as COL5A1 and COL5A2 accounting for 50% of patients with a clinical diagnosis of classic EDS[1]. Classic EDS is an autosomal dominant disorder identified in one in 20000 individuals with stretchable skin, delayed wound healing with poor scarring, joint hypermobility with subluxations or dislocations, pes planus, easy bruisability, hernias, aneurysms, and cardiac abnormalities[2-4]. Historically, EDS is grouped into six categories (classic, hypermobile, vascular, kyphoscoliosis, arthrochalasia and dermatosparaxis) with different genetic causes and inheritance patterns[4,5]. In 2017, EDS was assigned into 13 heritable disorders that affects an estimated 10 million people worldwide. These disorders are: Autosomal dominant classic (cEDS); autosomal recessive classic - like (clEDS); autosomal recessive cardiac-valvular (cvEDS), autosomal dominant vascular (vEDS), autosomal dominant hypermobile (hEDS), autosomal dominant arthrochalasia (aEDS), autosomal recessive dermatosparaxis (dEDS), autosomal recessive kyphoscoliosis (kEDS), autosomal recessive brittle cornea syndrome (BCS), autosomal recessive spondylodysplastic (spEDS), autosomal recessive musculocontractural (mcEDS), autosomal dominant or recessive myopathic (mEDS), and autosomal dominant periodontal[6].

Advances in genomics technology using next-generation sequencing (NGS) has led to the discovery of causative genes along with candidate gene approaches, disease specific panels or whole- exome analysis in patients presenting with features of a connective tissue disorder with newer classifications. Applying genomics to the field of cardiovascular-related disorders has identified over 80 genes causing connective tissue disorders using available comprehensive NGS gene testing panels. Over 150 genes have been identified playing a role in hereditary cardiomyopathies including hypertrophic, dilated or left ventricular non-compaction cardiomyopathy and hereditary arrhythmogenic right ventricular cardiomyopathy but do not include collagen genes. In addition, over 250 genes are found on commercially available comprehensive cardiovascular disease NGS panels with at least three collagen (*i.e.*, *COL3A1*, *COL5A1*, *COL5A2*) genes (*e.g.*, Fulgent Diagnostics, Irvine, CA, United States) and advanced genetic testing should be applied to interrogate gene panels for cardiology services including for heart transplantation[7].

A 13-year-old son with features of a connective tissue disorder was identified previously based on a physical examination with hypermobility assessed using the Beighton scale[8,9]. His numerical rating score was high at 8 out of 9 with scores greater than 5 indicative of a connective tissue disorder. The score comprised passive dorsiflexion of the fifth finger beyond 90° (one point), passive bilateral apposition of both thumbs to the flexor aspects of forearms (two points), hyperextension of the elbows beyond 180° (two points), hyperextension of the knees beyond 180° (two points), and forward flexion of the trunk with palms of hands resting on the floor (one point). He had no heart murmur and a previous echocardiogram showed normal intra-cardiac anatomy and size, but his aortic root was dilated. A comprehensive connective tissue disorder NGS gene panel consisting of 50 genes was ordered and performed at the University of Nebraska Medical Center (Omaha, NE, United States). A heterozygous missense *COL5A1* gene variant was found involving exon 3 at nucleotide c:305T>A with an amino acid position change at p.Ile102Asn. This gene variant was also found in his 55-year-old father exhibiting similar clinical features of thin stretchable skin with poor atrophic scars, hypermobility, joint pain and easy

bruising with increased pigment on the anterior surface of both lower legs. Due to multiple knee surgeries in the past, bilateral knee movement or range could not be assessed, none-the-less his Beighton hyperflexibility score was 6 out of 7 (excluding knee mobility measures) (see Figure 1). Interestingly, his father had a heart transplant at 43 years of age due to cardiac failure with no known cause identified including infections, anatomic defects or metabolic problems. There was also no evidence of a spontaneous dissection or closure of a main coronary vessel causing infarction and subsequent heart failure.

The *COL5A1* gene encodes one of the low abundant fibrillar collagens related to connective tissue abnormalities and when disturbed leads to autosomal dominant classic EDS[6]. The gene variant seen in the father and his son has not been described previously and the amino acid substitution was considered harmful by computer in silica prediction programs impacting protein structure.

The father's cardiac failure was of unknown cause and required a heart transplant, potentially attributable to a connective tissue disorder that should be brought to medical attention as congestive heart failure affects 23 million people worldwide including 7.5 million in North America. It has a prevalence of 2.6% in the United States population in those greater than 20 years of age[10]. It is estimated that about 90000 heart transplants have occurred worldwide since 1983 with a current median survival rate of 50% at 12 years[11,12].

The clinical presentation and autosomal dominant inheritance pattern in this family involving a disturbed connective tissue gene leading to classic Ehlers-Danlos syndrome should be of interest to cardiologists, heart transplant specialists and surgeons with a possible relationship to cardiac involvement and heart failure requiring transplantation. Complications of connective tissue disorders should be recognized early and avoided in those patients due to their poor wound healing, scarring and other tissue involvement (*e.g.*, vascular anomalies, blood pressure instability, aneurysms) and taken into consideration prior to surgical intervention.

CONCLUSION

Patients with unexplained heart failure should be checked for hypermobility (*e.g.*, use of the Beighton scale) and genetically tested for connective tissue disorders using readily available comprehensive NGS gene panels prior to seeking heart transplantation. Evaluating and reporting of other similarly affected patients with genetic testing is encouraged to further elucidate whether connective tissue disorders may play a role in a subset with heart failure requiring transplantation as seen in this patient. In addition, complications of those having connective tissue disorders such as Ehlers-Danlos syndrome may necessitate closer surveillance and monitoring during and after surgical intervention with prolonged recovery and healing, as well as counseling of at-risk family members requiring screening and advanced genetic testing.

Figure 1 The 55-year-old father with classic Ehlers-Danlos syndrome and hypermobility is illustrated by placing palms on the floor.

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REVIEW

Sympathetic nervous system activation and heart failure: Current state of evidence and the pathophysiology in the light of novel biomarkers

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Abstract

Heart failure (HF) is a complex clinical syndrome characterized by the activation of at least several neurohumoral pathways that have a common role in maintaining cardiac output and adequate perfusion pressure of target organs and tissues. The sympathetic nervous system (SNS) is upregulated in HF as evident in dysfunctional baroreceptor and chemoreceptor reflexes, circulating and neuronal catecholamine spillover, attenuated parasympathetic response, and augmented sympathetic outflow to the heart, kidneys and skeletal muscles. When these sympathoexcitatory effects on the cardiovascular system are sustained chronically they initiate the vicious circle of HF progression and become associated with cardiomyocyte apoptosis, maladaptive ventricular and vascular remodeling, arrhythmogenesis, and poor prognosis in patients with HF. These detrimental effects of SNS activity on outcomes in HF warrant adequate diagnostic and treatment modalities. Therefore, this review summarizes basic physiological concepts about the interaction of SNS with the cardiovascular system and highlights key pathophysiological mechanisms of SNS derangement in HF. Finally, special emphasis in this review is placed on the integrative and up-to-date overview of diagnostic modalities such as SNS imaging methods and novel laboratory biomarkers that could aid in the assessment of the degree of SNS activation and provide reliable prognostic information among patients with HF.

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Core tip: Sympathetic nervous system activation is one of the key neurohumoral mechanisms that are operative in heart failure and is robustly associated with adverse myocardial remodeling, arrhythmias, sudden cardiac death, and overall poor prognosis in this population. Therefore, adequate diagnosis and quantification of the degree of upregulated sympathetic nervous system activity must be assessed by the clinician in every heart failure patient. A special emphasis must be put on adequate treatment by neurohumoral antagonists such as beta-blockers that will mitigate these adverse effects and improve outcomes. The adjunct use of advanced imaging methods and novel biomarkers might aid in clinical decision-making.

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INTRODUCTION

Heart failure (HF) is a complex clinical syndrome characterized by the symptoms such as breathlessness, fatigue and ankle edema and signs like elevated jugular venous pressure, lung crepitations during auscultation and peripheral edema[1]. The central hemodynamic consequence of HF is the inability of a heart to support required metabolic demands and perfusion of organs and tissues due to structural and/or functional cardiac abnormalities that predilect to decreased cardiac output (CO) and/or increased intracardiac filling pressures during the rest or exercise[2]. HF nowadays represents a relevant clinical entity and global pandemic that affects more than 26 million adults worldwide while its general prevalence in population accrues to about 2% with yearly incidence of approximately 0.2% in Western countries[3]. Projected burden of HF, assuming the stable incidence of this syndrome in persons ≥ 65 years was already surpassed by the actual burden of the HF in the United States and it is expected that more than 8 million people will have this condition in the United States by 2030[4,5]. This increase in HF prevalence observed worldwide might be attributed not necessarily to increased HF incidence but to phenomena such as advancing age of the population and increased comorbidity burden coupled with improved HF survival due to progress in HF treatment and diagnosis while the decreased incidence of HF according to some data might partially be the consequence of more efficacious treatment of acute coronary syndromes, lower severity of index HF events and improvements in HF primary prevention programs[5,6].

Despite the advancements in therapeutic management, HF is still characterized by the rather high morbidity and mortality rates and considerable healthcare expenditures while these outcomes appear to be strongly dependent on the region of the world, healthcare infrastructure and the level of quality/access to specialized HF care[5,7-10]. Of note, HF is at least as deadly or even deadlier than some of the common malignancies in both men and women. Among men, patients with HF had worse mortality outcomes than those with prostate and bladder cancer while among women those with HF had worse mortality outcomes than female patients suffering from breast cancer[11]. Survival after a diagnosis of HF has shown only modest improvement in the 21st century, with an increase in average survival rates between 6.4% to 7.2% during nearly two decades thus clearly indicating that our clinical efforts to improve outcomes in HF substantially lag behind advancements in other severe conditions such as cancer[12]. In support to this notion, a recent data from the United States nationwide temporal analysis showed that age-adjusted death rates for HF did not change significantly, in fact there even emerges a trend of the slight rise of HF-related mortality recently, after nearly 15 years of modest but gradual decline in HF-related

mortality since the late nineties[13]. Similarly, recent longitudinal data acquired from the Framingham Heart Study and Cardiovascular Health Study showed that HF incidence was relatively stable for almost two decades and this was true for mortality outcomes as well (including cardiac death, non-cardiac death, and all-cause mortality)[14]. This study also showed that the incidence of heart failure with reduced ejection fraction (HFrEF) significantly declined whereas the incidence of heart failure with preserved ejection fraction (HFpEF) significantly increased over time in both sexes. Approximately 50% of community patients with HF nowadays have an HFpEF clinical phenotype while multimorbidity seems to be a stronger driver for HFpEF onset although it is a highly prevalent phenomenon in both HFrEF and HFpEF. Likewise, both phenotypes portend a comparable 5-year mortality[15-17]. Finally, the proportion of those dying of non-cardiovascular causes seems to be higher in HFpEF than HFrEF and this holds for non-cardiovascular-related 30-d readmissions that are more common among HFpEF compared with HFrEF patients[15,18].

Taken together, these recent trends strongly suggest that HF is a clinical entity that will continue to impose a significant burden on modern societies, urging for the advances in our understanding of its complex pathophysiology and development of new treatments. Equally important, the discovery and implementation of biomarkers that might aid in the diagnosis, prognosis, and risk stratification of patients with HF is required in a contemporary clinical practice[19]. For these reasons, aims of the present review are to provide recent updates regarding the HF pathophysiology with the special emphasis on novel biomarkers that might reflect the sympathetic nervous system (SNS) activation as one of the constituent neurohumoral pathways that are upregulated to preserve CO in the setting of a failing heart.

PATHOPHYSIOLOGY AND COMPENSATORY MECHANISMS IN HEART FAILURE

Any abnormality or combination of abnormalities that cause structural, mechanical, or electrical dysfunction of the heart carry the potential to induce HF. Most commonly HF is the consequence of the myocyte injury caused by coronary artery disease, uncontrolled arterial hypertension and diabetes mellitus, however, adverse myocardial remodeling can be triggered and sustained by valvular dysfunction, tachyarrhythmias (especially atrial fibrillation/flutter), interatrial and interventricular conduction abnormalities or pulmonary disorders such as chronic obstructive pulmonary disease or pulmonary arterial hypertension[20]. Less common etiologies include cardiomyopathies, myocarditis, infections, systemic toxins, and cardiotoxic drugs that are nowadays increasingly used in various chemotherapeutic regimens[21,22]. At least several pathophysiological mechanisms are at play in the setting of failing myocardium such as increased hemodynamic overload, ventricular dysfunction due to subclinical or overt ischemia, pathologic ventricular remodeling, upregulated neurohumoral activation, impaired intracellular calcium cycling and accelerated apoptosis of cardiac myocytes, imbalance in the formation and breakdown of the extracellular matrix, and various genetic predilections[2].

Clinically, a majority of patients with HF have both systolic and the diastolic dysfunction occurring at the same time and these two pathophysiological mechanisms often overlap but even in the isolation of each other, they cause a similar degree of HF signs and symptoms[23,24]. For the didactic purposes, in the systolic dysfunction, the primary pathomorphological substrate is the loss of functional myocardium (primary myocyte injury) most commonly due to ischemic disease and myocardial infarction causing impaired contractility and insufficient emptying of the ventricles consequently leading to increased left ventricular (LV) end-diastolic and end-systolic volumes and rise in end-diastolic pressure (LVEDP) within the left ventricle further decreasing stroke volume and left ventricular ejection fraction (LVEF)[25,26]. An increase in LVEDP might retrogradely increase left atrial (LA) pressure which consequently increases pressure in the pulmonary circulation, and if this cascade progresses even further can induce right heart failure, congestive hepatopathy and affect portal and peripheral circulation thus altogether precipitating fluid extravasation leading to pulmonary and/or splanchnic and peripheral congestion.

On the other hand, in diastolic dysfunction, the contractile ability of the heart might be preserved, however, functional mechanisms that are responsible for the adequate filling of the heart are impaired. It is estimated that up to 50% of patients presenting with signs and symptoms of HF have normal or near-normal LVEF but exhibit abnormalities predominantly in diastolic function[27,28]. Even more, those with normal

LVEF by conventional transthoracic echocardiography and verified diastolic dysfunction can often have subclinical contractile dysfunction that is captured only by the means of myocardial deformation studies such as LV global longitudinal strain and speckle tracking techniques or advanced cardiac imaging methods such as cardiac magnetic resonance[29-31]. These filling abnormalities may occur due to impairments in early diastolic relaxation of the LV (an active energy-consuming process) and/or increased stiffness and rigidity of the LA and LV (a passive process independent of energy) coupled with reduced arterial compliance in both major vessels such as the aorta and peripheral arteries[32,33]. Among patients with HFpEF both processes of active relaxation and increased passive stiffness are impaired and are predominant pathophysiological mechanisms leading to diastolic dysfunction[34,35]. These abnormalities altogether act synergistically to produce a rise in the LVEDP thus causing significant venous congestion in HFpEF patients that is as severe as among those with HFrEF[36]. Of note, significant increase in passive LV stiffness is propagated by aberancies in collagen-dependent and titin-dependent deposition cellular pathways[35]. Similarly, longstanding elevated ventricular pressures further perpetuate LA dilation that is clinically detected as an increased LA volume at rest and reduced LA filling during submaximal exercise[37]. Also, peripheral oxygen extraction is blunted in HFpEF resulting in exercise intolerance while reduced peak oxygen uptake and increased perfusion/ventilation mismatch carry important prognostic information and assist in the selection of patients that might require advanced HF interventions such as heart transplantation or deployment of ventricular assist devices[38-41].

A complex interaction of highly prevalent comorbidities such as salt-sensitive hypertension, obesity, diabetes mellitus, metabolic syndrome, iron deficiency, chronic obstructive pulmonary disease and, atrial fibrillation (AF), combined with natural pathophysiological effects of aging can give rise to systemic proinflammatory state that affects coronary microvasculature and endothelium by upregulating cytokine-mediated inflammation pathways[42,43]. In this proposed pathophysiologic scheme, pioneered by Paulus and Tschöpe[44] in 2013, endothelial inflammation of coronary microvasculature acts as a central transitioning mechanism by which synergistic effects of comorbidities are translated onto heart thus causing secondary myocyte injury that ultimately leads to structural and functional alterations of the myocardium in HFpEF[44]. According to the postulated model, coronary microvascular endothelial inflammation reduces nitric oxide (NO) bioavailability and cyclic guanosine monophosphate (cGMP) content and reduces protein kinase G activity in adjacent cardiomyocytes thus highlighting NO-cGMP-PKG signaling pathway disruption as the key culprit in HFpEF pathophysiology. This disruption leads to the onset of cardiac hypertrophy and increased resting tension ($F_{passive}$) of cardiomyocytes due to hypophosphorylation of titin and increased myocardial nitrosative/oxidative stress[45-47]. Furthermore, hypophosphorylation of constitutive myofilament proteins and increased calcium sensitivity of sarcomeres causes increased LV stiffness and abnormal relaxation contributing to HFpEF onset while these derangements are not present in normal myocardium[48]. Graziani *et al*[49] also proposed that microvascular dysfunction is the common pathophysiological pathway contributing to both microvascular angina and HFpEF[49]. Of note, endothelial dysfunction represents a pathological vascular phenotype of all systemic arteries that encompasses damaging effects of vasoconstrictive, prothrombotic and proinflammatory substances and mediators on the endothelial vascular lining and diminished repairability of endothelium thus further acting as an independent pathobiological driver of atherosclerosis and overt cardiovascular disease[50-52].

Furthermore, a novel pathophysiological concept of endothelial-to-mesenchymal transition has been recently proposed describing a process by which endothelial cells undergo a series of molecular events that lead to a loss of their endothelial properties and a consequent shift in phenotype toward mesenchymal cells such as myofibroblasts, smooth muscle cells, and osteoblasts[53]. Accumulation of these cells promotes plaque formation and atherosclerosis by secreting proinflammatory cytokines and metalloproteinases and increasing extracellular matrix and collagen deposition thereby affecting the structure and function of cardiac valves, native vein grafts that are used in coronary artery bypass graft surgery and inducing interstitial cardiac fibrosis, diastolic dysfunction, endocardial fibroelastosis and contributing even to the development of pulmonary arterial hypertension[54-59]. From the molecular perspective, it seems that activation of transforming growth factor-beta plays a key role in the initiation of endothelial-to-mesenchymal transition cascade and tissue fibrosis through its interaction with SMAD-2/3/4 and SLUG signaling pathways[57,60,61]. Furthermore, endothelial cells in which the EndoMT pathway was experimentally activated had significantly elevated secretion of proinflammatory

cytokines such as interleukin-6, interleukin-8 and tumor necrosis factor-alpha thus likely representing an integrative pathophysiological cross-talk between fibrosis and inflammation[59,62]. In summary, EndoMT might be the key link in interaction between inflammation, endothelial dysfunction, and chronic cardiac fibrosis, and thus might become a viable target for novel therapeutic solutions for cardiovascular disease[63,64]. Altogether, these converging and mutually complementary pathophysiological mechanisms may contribute to a net effect of stiffening of cardiac myocytes and overt interstitial fibrosis thus directly inducing myocardial dysfunction during diastole and subsequent HF development.

In order to maintain adequate tissue perfusion in the setting of the failing heart, several compensatory mechanisms are activated to increase CO *via* the Frank-Starling mechanism, increased ventricular volume and wall thickness through the process of ventricular remodeling and augmenting mean arterial pressure (MAP) by activating several neurohormonal pathways and cytokine systems[21]. These compensatory mechanisms are initially able to compensate for impaired myocardial function, however, they inflict deleterious effects on cardiac structure and function if chronically activated leading to further worsening of HF and progressive clinical deterioration of a patient. Neurohumoral systems that are upregulated act to promote beneficial short-term changes in heart, kidneys, and vasculature to maintain cardiovascular homeostasis[65]. They encompass the activation of the renin-angiotensin-aldosterone system (RAAS), arginine-vasopressin (antidiuretic) system, kallikrein-kininogen-kinin system, activation of natriuretic peptides system, neprilysin signaling pathway, endothelin pathway, and cytokine systems[66-70]. Finally, the upregulation of adrenergic/SNS pathways and blunted responsiveness of the parasympathetic nervous system (PNS), also collectively known as autonomic nervous system (ANS) imbalance, is one of the hallmark neurohumoral disturbances that are operative in HF and is of central interest in this review[71,72]. The summary of the most common etiologies, pathophysiological effects, and compensatory mechanisms in HF is shown in Figure 1.

It is worth of brief mentioning that evidence-based treatments such as angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers, beta-blockers, mineralocorticoid receptor antagonists, angiotensin receptor neprilysin inhibitors, ivabradine, sodium/glucose cotransporter 2 inhibitors, digoxin, and cardiac resynchronization therapy (CRT) devices are developed around our understanding of compensatory and maladaptive mechanisms in HF[1,73]. Importantly, these treatment modalities were found successful in reducing mortality rates and hospitalizations in patients with HFrEF, however, no evidence-based pharmacologic treatments with clear beneficial effects on these endpoints were observed in patients with HFpEF while current guidelines stipulate symptom control with diuretics and efficacious management of comorbidities such as arterial hypertension, AF, obesity, and diabetes in this population[1,74].

Recent European Society of Cardiology and Heart Failure Association expert panel issued a scientific position statement in which ANS imbalance is recognized as an important contributor to cardiac disease progression and is designated as a prognostic parameter and a therapeutic target in HF by the means of novel pharmacologic and/or device therapies[75]. Furthermore, heart and brain are in bidirectional interaction meaning that depressed cardiac function affects cerebral structures and functional capacity while dysregulation of neuro-cardiac reflexes significantly affects the cardiovascular system thus aggravating and further sustaining the progression of HF[76].

PHYSIOLOGY OF SYMPATHETIC NERVOUS SYSTEM AND ITS MEDIATORS

In the advent of our understanding of HF, this syndrome was largely perceived as a hemodynamic disorder thus all treatment strategies were primarily directed toward the correction of hemodynamic abnormalities. However, since hemodynamic derangements could not fully explain the progression and long-term effects of the disease, a neurohormonal hypothesis was developed in which neurohumoral mechanisms encompassing RAAS and SNS activation were emphasized as independent drivers of cardiac dysfunction and progression of HF[77].

SNS activation is a fundamental physiological response to stress conditions (also known as the fight-or-flight response) such as hypovolemia, hypoglycemia, hypoxia or cardiovascular dysfunction[78]. SNS activity can modify and induce a wide spectrum of

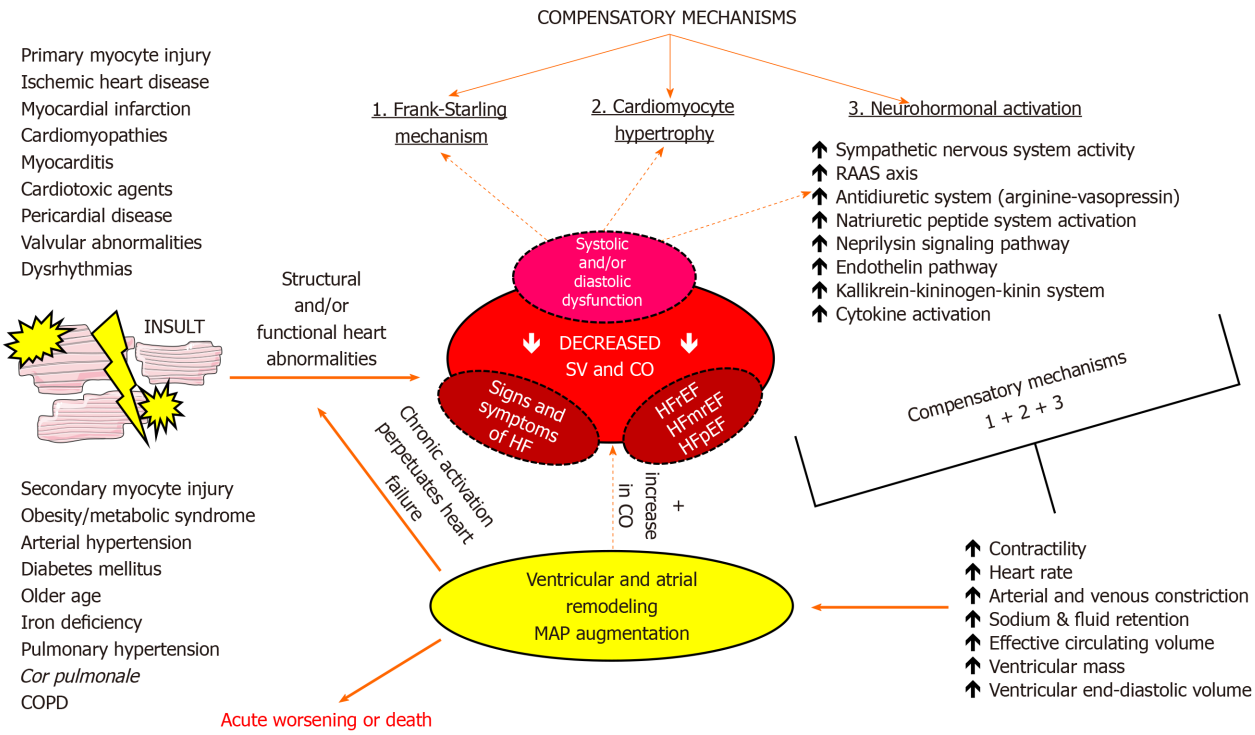

Figure 1 A diagram showing basic etiology, pathophysiology and compensatory mechanisms that are activated in heart failure. CO: Cardiac output; COPD: Chronic obstructive pulmonary disease; HF: Heart failure; HFmrEF: Heart failure with midrange ejection fraction; HFpEF: Heart failure with preserved ejection fraction; HFrEF: Heart failure with reduced ejection fraction; MAP: Mean arterial pressure; RAAS: Renin-angiotensin-aldosterone system; SV: Stroke volume.

potent hemodynamic effects such as an increase in heart rate (positive chronotropic effect), augmentation of cardiac contractility (positive inotropic effect), accelerated cardiac relaxation (positive lusitropy), enhanced (shortened) atrioventricular conduction (positive dromotropy), reduced venous capacitance and peripheral vasoconstriction of resistance and cutaneous vessels[71,79]. The actions of SNS are dominantly mediated by secreted neurotransmitters such as norepinephrine (NE) that is released by sympathetic nerve terminals and, to a lesser degree, by the adrenal medulla and by epinephrine (EPI) that is chiefly released into peripheral circulation by the adrenal medulla.

Peripheral target organs are regulated by the two major sets of neurons serially connected to control the motor outflow of the SNS: (1) Preganglionic neurons that originate in the brainstem or the spinal cord; and (2) Postganglionic neurons that are part of sympathetic ganglia that are located outside of the central nervous system (CNS). Intrathoracic and extracardiac ganglia including stellate ganglia, middle cervical ganglia, and T2-T4 thoracic ganglia modulate the sympathetic outflow to the heart while sympathetic afferent impulses are carried through the dorsal root ganglia and reach the spinal cord, brain stem, and higher CNS centers. Cardiac sympathetic nerve fibers innervate myocardium at the subepicardial level, follow the path of major coronary arteries and are a predominant autonomic component in the ventricular tissue while parasympathetic nerve fibers, along with vagus nerve, run through subendocardium crossing the atrioventricular groove and are significantly more abundant in the atrial than ventricular myocardium thus exerting negative chronotropic effect with minimal effects on cardiac contractility[79]. Furthermore, sympathetic innervation has a relatively higher density in the anatomical areas around sinoatrial node and coronary sinus while its density gradually increases from the base of the ventricle to the apex (positive base-to-apex gradient)[80,81]. Intrinsic cardiac ganglia are located epicardially and receive innervation from post-ganglionic sympathetic and pre-ganglionic parasympathetic connections while most of sympathetic efferent and parasympathetic preganglionic fibers exhibit a large degree of intermixing thus most of the nerves reaching the heart in the mediastinum have mixed fibers (both sympathetic and parasympathetic components)[82].

The degree of SNS activation and sympathetic outflow to the heart and peripheral circulation, under physiological conditions, is regulated by a complex integration of

autonomic cardiovascular reflexes. These reflexes include arterial baroreflexes, cardiopulmonary mechanosensitive reflexes, cardiac chemoreflexes, peripheral and central chemoreceptor reflexes, pulmonary stretch reflexes, cardio-cardiac reflexes and reflexes that are afferently projected from skeletal muscles[72]. All of these reflexes have a common role in fine-tuning and maintaining adequate heart rate, mean arterial blood pressure, vascular tone, ventilation, and respiratory drive in response to various hemodynamic changes[83]. These reflexes are listed with a summary of their function and potential impairment in HF (Table 1). According to modern pathophysiological findings, any depression of ventricular systolic function (irrespective of the underlying etiology) is augmenting cardiac reflex sympathoexcitation in chronic HF but might also be a leading culprit for the acute HF onset[72]. For example, a physiological response to the sudden increase in the cardiac filling pressures should act to vasodilate venous capacitance vessels to accommodate for excessive fluid, however, paradoxical sympathetic discharge in HF instead causes vasoconstriction of venous pool (mainly splanchnic circulation) and redistributes fluid to cardiopulmonary pool thus precipitating congestion and causing dyspnea. For this reason, it could be that rapid increase in the effective circulating volume from the mobilization of fluid from the splanchnic bed is the dominant driving force behind increased central venous pressure and congestion encountered during HF decompensation episode and might depend on an external fluid gain to a lesser degree[84,85]. Finally, cardiovascular-low threshold polymodal receptors are sensory endings localized in all cardiac chambers and large thoracic vessels that detect both mechanical and chemical stimuli and act in positive-feedback fashion with stimulatory effects on SNS[86].

In terms of prinicipal neurotransmitters that propagate the effects of SNS, NE is ejected in the synaptic cleft upon the stimulation from stellate ganglions *via* post-ganglionic fibers thus activating adrenergic receptors (ARs) in the heart and physiologically augmenting contractile strength, chronotropy, dromotropy and increasing mean arterial perfusion pressure. About 80% to 90% of released NE is reuptaken by the noradrenaline transporter 1 which is a monoamine transporter that clears NE from sympathetic nerve terminals/chromaffin cells while about 10% to 20% of remaining NE content is spilled into circulation[87,88]. This NE turnover and metabolism can be evaluated with imaging methods such as scintigraphy by using radiolabelled guanethidine analogs of NE[89,90]. Similarly, sympathetic fibers that innervate the adrenal gland stimulate chromaffin cells in the adrenal medulla that act as modified post-ganglionic fibers to release catecholamines in response to stressors or exercise. This efflux of catecholamines from adrenal medulla is predominantly comprised of EPI (about 80%) while NE makes up the remaining 20% with small amounts of dopamine being released into peripheral circulation as well[91]. EPI and NE bind to specific ARs that are proteins embedded within the cell membrane with 7 transmembrane structures coupled to heterotrimeric G proteins. A total of two classes of ARs (alpha- and beta-adrenergic receptors) with 9 subtypes have been identified thus far: three α_1 receptors, three α_2 receptors and three β receptors (β_1, β_2, and β_3)[92]. A healthy human heart mostly consists of β_1 (75%-80%) and β_2 (20%-25%) adrenergic receptors and they represent the key effectors behind positive chronotropic and inotropic effects of catecholamines while β_3 adrenergic receptors (comprising less than 5% of total beta-receptor density) have been postulated to exert negative inotropic effects through upregulation of nitric oxide synthase pathway in human ventricle[93-95]. It has been recently confirmed that β_1 and α_{1B} receptors are present in all ventricular cardiomyocytes[96]. Alpha-1 adrenergic receptors (α_1) and alpha-2 adrenergic receptors (α2) are chiefly expressed in vascular smooth muscle cells proximal and distal to sympathetic nerve terminals, respectively, and their activation elicits vasoconstriction of peripheral arteriolar and venous vessels while in the brain stem they modulate sympathetic outflow[97]. A recent study by Becker *et al*[98] showed that activation of neuronal endothelin B receptors can increase arterial blood pressure mediated through α_1-adrenergic receptor signaling showing that abnormalities of endothelin system have a cross-talk with adrenergic systems in hypertension and HF[98].

Beta-adrenergic receptors act as powerful regulators of cardiac output and upon acute stimulation by catecholamines they facilitate fight-or-flight response while their chronic stimulation results in maladaptive and pathologic cardiac remodeling[99-101]. Activation of β adrenergic receptors induces the activation of the stimulatory G protein (Gs) which further activates adenylyl cyclase leading to an increase in levels of intracellular cyclic adenosine monophosphate and activation of protein kinase A that phosphorylates several target proteins within the cardiomyocyte such as phospholamban, L-type calcium channels, troponin I, contractile proteins, and the cardiac ryanodine receptor and this mainly is the mechanism by which β_1 receptors regulate cardiac contractility/relaxation and heart rate[99,102]. Furthermore, activation of

Table 1 Cardiovascular reflexes and their pathophysiological implications in heart failure

Type of neurally-mediated cardiovascular reflex	Proposed mechanism of action	Pathophysiological consequence in heart failure
Arterial baroreceptor reflexes	In HF acts as a response to perceived reduction in stroke volume or diastolic blood pressure; It is implicated that reduced carotid sinus and aortic arch afferent nerve firing as a response to systolic stretch disinhibits efferent sympathetic discharge; This reflex is impaired in terms of heart rate control, however, efferent sympathetic nerve activity might be preserved in human HF, even in advance stage	↓ Reduced reflex vagal response; ↓ reduced heart rate variability; ↑ increased cardiac NE spillover; ←→no change in renal NE spillover; ↑mean sympathetic discharge to peripheral muscles is increased
Cardiac chemosensitive reflexes	Myocardial ischemia and reperfusion elicits increased sympathoexcitatory response by chemically (reactive oxygen species) stimulating sympathetic afferent fibers in both anterior and infero-posterior regions of the left ventricle; Platelet activation and local release of serotonin (5-HT) through a 5HT3 receptor mechanism and regional changes in pH from lactic acid stimulate sympathetic afferents in myocardium; Cardiac sympathetic afferent reflex is enhanced in HF and acts in the positive-feedback fashion	↑ Increased shift and predominance of sympathetic efferent discharge; ↓ parasympathetic depletion; ↑ sympathetic activation; ↑ increased blood pressure; ↑ adverse left-ventricular remodeling; ↑ increased propensity for malignant arrhythmias and sudden cardiac death
Cardiopulmonary mechanosensitive reflexes	Normally elicited by the stretch of unmyelinated afferents sensitive to mechanical input, located intracardially and within pulmonary veins; It is implicated that impairement of this reflex decreases efferent sympathoinhibition to periphery; Cardiac-specific myelinated afferent are responsible for observed sympathoexcitatory effects characterized by the increased local cardiac NE spillover due to increased filling pressures (*e.g.* ↑ high LA pressure); Bezold-Jarisch reflex – mediated by nonmyelinated vagal afferent pathways – acts in sympathoinhibitory fashion and promotes reflex bradycardia, vasodilation and hypotension	↓ Reduced cardiopulmonary reflex regulation of central sympathetic outflow to peripheral tissues (dominantly skeletal muscles); ↑ paradoxical excitation and increase in sympathetic outflow in the setting of high LA pressure
Cardio-cardiac reflexes	Coronary occlusion elicits the activity of preganglionic fibers in left thoracic sympathetic ramus communicans (T_3) and increases discharge towards heart via efferent sympathetic innervation	↑ Increased myocardial oxygen consumption; ↑ facilitation of malignant arrhythmias; ←→ might also have a protective effect in sense that they augment contractility, therefore, opposing ventricular dilatation and/or impending cardiogenic shock
Peripheral and central chemoreceptor reflexes	These receptors monitor partial pressures of oxygen and CO_2 within arterial vessels and close to heart and escalate afferent sensory discharge according to changes; Peripheral chemoreceptors – dominantly respond to hypoxia; Central chemoreceptors – dominantly respond to hypercapnia; Peripheral and central receptor chemosensitivity is significantly increased in HF and is linked to augmented MSNA	↑ Increased ventilation; ↑ increased sympathetic outflow; ↑ increased heart rate and systolic blood pressure; ↓ suppressed inhibition of sympathetic outflow that is mediated by arterial baroreflexes; ↑ increased peripheral and central chemoreflex-mediated sympathoexcitation is linked to poor 4-yr survival in HF patients
Pulmonary stretch receptor reflex	Fast and shallow breathing (high respiratory rate and low tidal volume) decreases stimulation of sympathoinhibitory reflex that is initated with lung stretch; HF patients with such breathing had increased MSNA burst frequency or amplitude; There is a correlation between decrease in resting tidal volume and attenuated sympathoinhibitory effect of lung inflation reflex with increased sympathoexcitation	↓ Decreased the resting tidal volume; ↓ attenuated sympathoinhibitory effect of lung inflation reflex
Reflexes originating from skeletal muscles	Autonomic responses of skeletal muscles during exercise are modulated by skeletal ergo-receptors in order to optimize muscle work; HF patients had augmented afferent reflexes originating from skeletal muscles	↑ Increase in the efferent ventilatory and sympathoneural responses to exercise

HF: Heart failure; LA: Left atrium; MSNA: Muscle sympathetic nerve activity; NE: Norepinephrine.

β_1 receptors results in apoptotic and maladaptive remodeling signaling in the heart *via* protein kinase A-independent pathway mediated by Ca^{2+}/calmodulin-dependent protein kinase II[99]. On the other hand, β_2 receptors are distributed widely in the lungs, kidneys and blood vessels and possess a distinct function from the β_1 subtype as they are coupled both to G_s and inhibitory G protein (G_i) in cardiomyocytes and their activation enhances cardiac function and myocyte viability[103].

Finally, β_3 adrenergic receptors have a relatively minimal expression in the heart

and they mediate unique downstream cellular effects once activated by catecholamines as they are mostly expressed in white adipose tissue where they mobilize stored fatty acids and regulate the release of adipokines while in brown adipose tissue they stimulate adaptive nonshivering thermogenesis[104]. A study by Napp *et al*[105] showed that β_3 adrenergic receptors were mostly expressed in the endothelium of failing myocardium thus negative inotropic effect was most likely elicited by the NO liberation from the cardiac endothelial cells while β_3 stimulation itself seemed to deactivate rather than activate endothelial NOS[105]. A study by Dessy *et al*[106] showed that β_3 receptors are abundantly expressed in the microvasculature of human coronary arteries in which their activation caused vasodilatation through NO-dependent pathway and vessel hyperpolarization[106]. Of note, the expression of β_3 adrenergic receptors in diverse cardiovascular pathologies seems to be upregulated and resistant to desensitization while in normal heart their activation resulted with a moderate negative inotropic effect[107,108]. Similarly, in septic cardiomyopathy, functional β_3 receptors were upregulated and they increased negative inotropic response to β_3 agonists[109]. Furthermore, in the setting of HF, activation of these receptors conferred beneficial effects with respect to excitation-contraction coupling and electrophysiological and mechanical remodeling of cardiomyocytes while also mediating vasodilatative pathways when β_1 and/or β_2 receptors are inoperative[110]. In the clinical and translational realm, relevant studies confirmed these initial findings as they showed that the third-generation beta-blocker, nebivolol, exhibited agonistic action on β_3 adrenergic receptors in human ventricle thus providing evidence that highly selective blockade of β_1 receptors coupled with NO-dependent endothelial vasodilatation and neoangiogenesis in coronary microcirculation could improve cardiac energetics[111,112]. Taken together, in the HF context, β_3 adrenergic stimulation might confer cardioprotection by attenuating excessive catecholaminergic stimulation mediated by β_1 adrenoceptors thereby presenting an attractive therapeutic target. The physiological effects of beta-adrenergic receptors are summarized and shown in Figure 2.

Finally, the expression of β-adrenergic receptors is physiologically modulated through G protein-coupled receptor kinases (GRKs), β-arrestins and complex intracellular signalosome[113]. GRK family consists of seven different protein kinases that canonically recognize and phosphorylate agonist-activated G protein-coupled receptor signaling and initiate downstream β-arrestin-mediated cellular pathways[114]. β-arrestins have a crucial role in the desensitization of activated seven transmembrane receptors such as β-adrenergic receptors, and they are key mediators of receptor endocytosis, ubiquitylation, and G-protein-independent cellular signaling[115]. Therefore, it becomes obvious that the normal expression of GRKs is a cellular prerequisite to maintain physiological homeostasis regarding β-adrenergic receptor turnover by phosphorylation, degradation or clathrin-mediated receptor downregulation and internalization[116].

SYMPATHETIC NERVOUS SYSTEM PATHOPHYSIOLOGY AND ADRENERGIC DYSREGULATION IN HEART FAILURE

A chronic SNS overactivity is one of the key pathophysiological mechanisms that are operative in HF. In the acute phase, this upregulated SNS activity is an essential compensatory response initiated in order to counteract reduced contractility, however, in the long-term, it becomes a major contributor to cardiac dysfunction as it promotes maladaptive cardiac hypertrophy and cell death.

In a seminal study performed more than three decades ago, Swedberg *et al*[117] showed that patients with chronic HF (CHF) had significantly higher arterial and coronary sinus venous NE concentrations compared to patients without HF while the net myocardial NE release in patients with CHF was about 20 times higher than that in patients without CHF[117]. This was subsequently confirmed by Viquerat *et al*[118] demonstrating that endogenous plasma levels of NE and dopamine were significantly higher among patients with CHF compared to patients without CHF thus reflecting enhanced sympathetic activity in response to failing heart[118]. Such overt sympathetic activity in HF closely paralleled increases in pulmonary artery pressures while activation of noradrenergic neurons in the brain might also be an underlying CNS mechanism of generalized sympathoexcitatory response observed in HF[119,120]. In fact, it has been shown that the RAAS axis is the major regulator of the SNS activity in the brain *via* angiotensin II type 1 receptors[121]. This likely occurs due to the upregulated expression of angiotensin II type 1 receptors (promoting sympathoexcitation) and

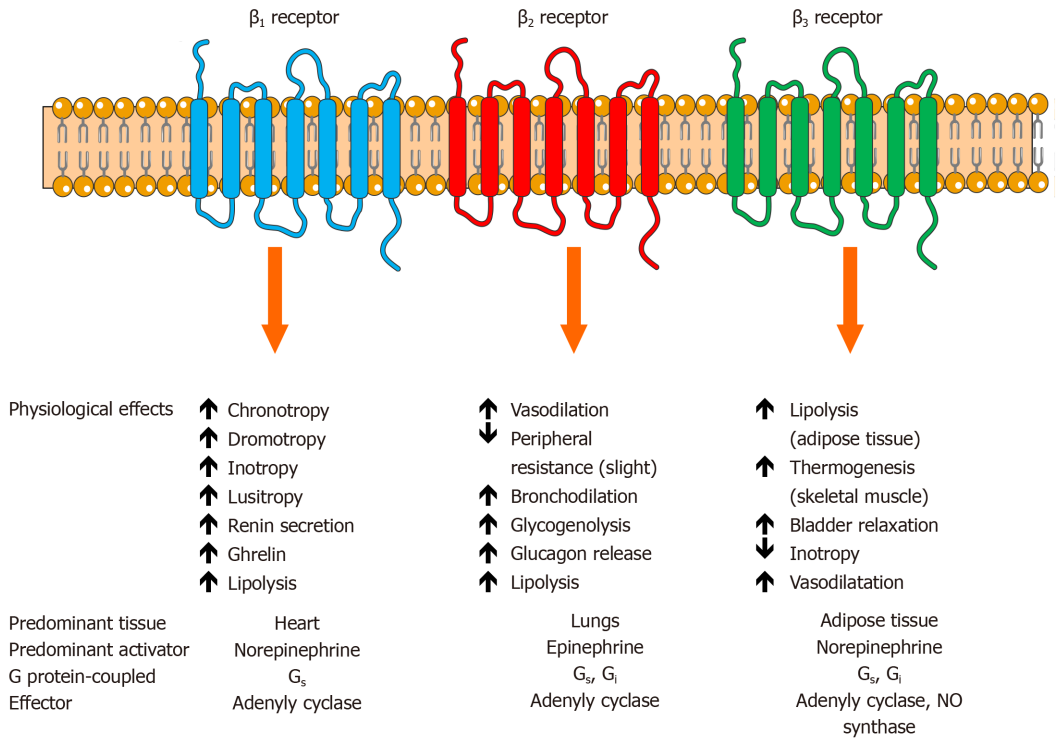

Figure 2 The function and physiological actions of beta-adrenergic receptors and adrenergic signaling. β: Beta; G_i: Inhibitory alpha subunit of G protein; G_s: Stimulatory alpha subunit of G protein; NO: Nitric oxide.

decreased expression of angiotensin II type 2 receptors (promoting sympathoinhibition) in the rostral ventrolateral medulla[122]. Likewise, historical studies showed that 24-h urinary excretion of NE, EPI, and their O-methylated metabolites – normetanephrine and metanephrine was significantly higher in patients with congestive HF and reflected functional disease severity as assessed by the New York Heart Association (NYHA) class[123,124]. Furthermore, there is not only a significant increase in circulating catecholamines but there is also an augmented neuronal NE spillover due to increased cardiac sympathetic nerve activity (SNA) while renal SNA nearly reached its maximum in the state of HF and showed to be an independent predictor of mortality in HF[125-127]. Recent research efforts demonstrated that NE spillover does not only depend on increases in SNA but it also partially occurs due to mechanisms controlling NE release and reuptake in the synapse and these mechanisms seem to be deranged in HF[128]. Increased NE spillover is in most cases paralleled by the reduced neuronal NE reuptake thus higher net concentrations of NE are present in the sympathetic synaptic cleft which further desensitizes myocardial β-adrenergic receptors[129,130]. A study by Hasking *et al*[131] further showed that cardiac and renal NE spillover in subjects with congestive HF was increased by 540% and 206%, respectively, compared to patients without HF while adrenomedullary-mediated EPI spillover was also markedly increased among these patients[131].

As previously mentioned, in a failing human heart, an important pathophysiological characteristic is a decreased sensitivity of β-adrenergic receptors to catecholamines while β-receptors are downregulated and decreased in their density and quantity[132]. For example, β_1 adrenergic receptors are reduced up to 50% in HF and there is a 200% increase in G_i-mediated cellular pathways with concomitant significant upregulation of GRK2 activity (also known as β-adrenergic receptor kinase 1 or βARK1) that further promotes adrenergic receptor internalization[133]. Myocardial GRK2 activity and expression have been increased in the failing heart as shown in several studies[134]. Conversely, experimental inhibition of βARK1 resulted in a marked reversal of ventricular dysfunction[135]. Finally, a wide variability of HF phenotypes and different response to HF treatment might suggest variants and functional polymorphisms of beta and alpha-adrenergic receptor genes[79]. Some pharmacogenomic studies suggested that polymorphisms in β_1-adrenergic receptors might affect susceptibility to HF such as Gly389 allele and Gly389 homozygotes; improved response to β-blocker treatment among Arg389 homozygotes while none of the candidate polymorphisms was an independent predictor of prognosis in HF[136,137].

Likewise, specific β_2-adrenergic receptor polymorphisms were linked with lower myocardial infarction rate and improved reverse left ventricular remodeling among patients with HF[138,139].

From the structural perspective, catecholamine spillover is cardiotoxic and its overexpression promotes senescence and inflammation of cardiomyocytes, upregulates tumor suppressor p53 pathway, and production of adhesion molecules by endothelial cells and macrophages and mediates cardiac dysfunction[140]. Chronic and persistent stimulation by catecholamines in HF causes interstitial fibrosis, myocyte hypertrophy, oxidative stress, and impairs the responsiveness and function of cardiac β-adrenergic receptors[141]. Engelhardt and colleagues experimentally demonstrated that increased chronic stimulation of β_1 adrenergic signaling resulted in a significant cardiomyocyte hypertrophy and apoptosis resulting in a marked loss of contractility and progressive reduction of LVEF with histological and functional deficits typical of HF[142]. Catecholamine toxicity and generalized autonomic storm also have an important pathophysiological role in causing acute stress-related cardiomyopathies such as Takotsubo cardiomyopathy, acute LV dysfunction associated with subarachnoid hemorrhage, pheochromocytoma, and exogenous catecholamine administration as well as acute LV dysfunction in critically ill[143]. Contrary to this, activation of β_2 adrenergic receptors delivered an antiapoptotic signal to cardiac myocytes through G_i-dependent coupling to phosphoinositol 3-kinase[144]. Furthermore, it seems that the number of β_2-adrenergic receptors does not change significantly in HF[145]. These findings suggest that a fine balance between proapoptotic and antiapoptotic pathways initiated by differential adrenergic signaling is of fundamental importance for physiological cardiomyocyte function[146].

Importantly, studies have shown that the activation of SNS in the course of heart failure exhibits specific temporal dynamics and regional sympathetic profile. Rundqvist *et al*[147] showed that a selective increase in cardiac NE spillover (defined as increased amounts of NE at neuroeffector junctions) in patients with mild-to-moderate CHF was higher for more than a three-fold compared to healthy subjects while total body and renal NE spillover, as well as sympathetic outflow to skeletal muscles, were not different in HF patients compared to healthy controls[147]. This study clearly showed that in the early stages of HF, selective increase in cardiac adrenergic drive precedes generalized sympathetic hyperactivity and outflow towards the periphery (skeletal muscles and kidneys) which is characteristic of advanced HF. In the early stages of HF, such cardiac sympathoexcitation might trigger ventricular arrhythmias and is associated with poor prognosis[126,148]. Furthermore, local cardiac NE spillover might be the first component required for further β-receptor downregulation and depletion, adverse myocardial remodeling, depletion of NE stores, and impairment in G-protein signaling pathways, as discussed earlier. This might further drive hemodynamic deterioration and progressive LV dysfunction. Even more, blunted response and withdrawal of parasympathetic cardiac control seem to precede sympathetic activation during the development of HF. In support of this claim, in the tachycardia-induced model of HF, Ishise *et al*[149] showed that parasympathetic withdrawal occurs rapidly and correlates with the decline in LV contractility and plasma NE increased gradually as LV diastolic function worsened while all of these changes recovered toward baseline values once pacing was ceased[149]. The proposed mechanism was that depressed contractility resulted in the attenuated stimulation to the carotid sinus baroreceptor which diminished vagal efferent activity towards the heart thus demonstrating parasympathetic tonic withdrawal. Together, these findings suggest that in the course of SNS dysfunction in HF, sympathovagal imbalance might occur earliest as evidenced in parasympathetic withdrawal while sympathetic hyperactivity likely first occurs at the cardiac level before it is propagated to peripheral tissues and organs as observed in the advanced stages in HF.

Furthermore, dysfunction of cardiac reflexes is a hallmark of SNS hyperactivity in HF and it occurs to a similar degree regardless of HF etiology (ischemic or nonischemic)[150,151]. There is a diminished baroreflex sensitivity in HF characterized by the marked suppression of inhibitory SNS reflexes such as arterial baroreceptor reflex while excitatory SNS reflexes such as those fired from peripheral chemoreceptors are enhanced[152]. Floras *et al*[153] showed that a failing heart reacts to increased cardiopulmonary filling pressures through responsive and sensitive arterial baroreflex that elicits potent sympathoexcitatory hemodynamic actions[153]. Furthermore, even among patients with mild CHF, an SNS-inhibiting baroreceptor function is already significantly impaired thus implying that baroreflex dysfunction might be one of the earliest constitutive phases in SNS activation during the natural course of CHF[154]. Reduction in baroreflex sensitivity is even more severe if obesity and arterial hypertension are present among HF patients[155]. Conversely, baroreflex activation

therapy in HF, encompassing the deployment of a device electrically stimulating carotid sinus, succeeded in improving muscle sympathetic nervous activity and relevant clinical indices thus showing that modulation of autonomic balance in HF might improve relevant outcomes[156,157].

Collectively, these findings are of clinical relevance because ANS imbalance and predominance of sympathetic excitation cause electrophysiological perturbations in the vulnerable cardiac syncytium and can initiate arrhythmogenesis[158]. For example, simultaneous stimulation of both sympathetic and parasympathetic systems can trigger AF while increased sympathetic stimulation is a contributing culprit to initiation of ventricular fibrillation (VF) or ventricular tachycardias (VT) or sudden cardiac death (SCD)[159]. Beat-to-beat variability of ventricular action potential duration is increased with elevated sympathetic activity in HF patients and might precipitate ventricular arrhythmias while beta-blocker, bisoprolol, attenuated these effects[160]. It was previously shown by Brunner-La Rocca *et al*[161] that high cardiac sympathetic activity in HF was an independent risk factor for sudden death, especially if sympathetic innervation was intact[161]. Sympathetic denervation in the heart combined with the presence of high NE levels is tightly correlated to progression of HF and SCD[162]. From the other way around, stellate ganglion blockade was effective in the acute reduction of ventricular arrhythmia burden and suppression of electrical storm thus clinically validating the concept that attenuation of sympathetic outflow to the heart from sympathetic ganglia can indeed mitigate the risk of future arrhythmic events[163-165]. These clinical observations were inspired by the previous animal study demonstrating that spontaneous high-amplitude discharge activity from left stellate ganglion was strongly associated with the induction of malignant ventricular arrhythmias[166]. Modern state-of-the-art neuromodulation strategies that are capable of mitigating VT/VF and atrial arrhythmias are, therefore, focused on increasing parasympathetic drive and inhibiting sympathetic neurotransmission[167,168].

Finally, it should also be noted that the widespread SNS activation also affects the function of skeletal muscles and promotes exercise intolerance in HF. Of note, diminished exercise capacity in terms of reduced peak oxygen uptake is present among HF subjects and is related to increased efferent sympathetic traffic to skeletal muscles, compared to control subjects[169]. This study also showed that resting muscle SNA is inversely related to peak oxygen uptake thus suggesting that there is a peripheral neurogenic limit to exercise in HF. As later validated, this reduced exercise capacity in HF is more dependent on sympathetic outflow to skeletal muscles than to cardiac sympathetic outflow, as assessed by NE spillover[170]. Furthermore, a subsequent study showed that muscle SNA was significantly higher while peak oxygen uptake was significantly lower in patients with ischemic *vs* nonischemic cardiomyopathy[171]. The most recent clinical study also demonstrated that the α-adrenergic-mediated vasoconstriction in HFrEF patients elicited a marked decrease in exercising skeletal muscle blood flow thus contributing to reduced exercise capacity in this population[172]. Finally, HF patients present with a high degree of chronotropic incompetence and attenuated heart rate response to exercise which is partially due to postsynaptic desensitization of the β-adrenergic receptor pathways[173].

CARDIAC IMAGING AND SYMPATHETIC ACTIVATION IN HEART FAILURE

A noninvasive *in vivo* imaging modalities can assess sympathetic innervation of the heart and for these purposes single-photon emission computed tomography and positron emission tomography (PET) are used by employing radiolabeled analogs of NE. The myocardial uptake of these radioanalogs dominantly represents presynaptic nerve function and their density in the heart. The most commonly used single-photon emission computed tomography tracer is ^{123}I-metaiodobenzylguanadine (^{123}I-mIBG) while most common PET tracer in clinical use is ^{11}C-hydroxyephedrine (^{11}C-HED)[174,175].

Recent studies demonstrated that impaired myocardial sympathetic innervation and regional sympathetic denervation, as detected by the presence of ^{11}C-HED by PET imaging, were independently associated with grade 2-3 diastolic dysfunction and contractile dysfunction and fibrotic burden among patients with HFpEF, respectively[176,177]. Similarly, data from prospective HF cohort studies demonstrated that diminished ^{123}I-mIBG uptake quantified as the reduced heart-to-mediastinum uptake ratio (H/M, indicating neuronal function including uptake and release of ^{123}I-mIBG) or increased myocardial ^{123}I-mIBG washout rate (indicating higher adrenergic drive) were strong markers of abnormal myocardial sympathetic innervation and consistent predictors of poor prognosis among patients with HF[90,178-180]. Furthermore,

the ADMIRE-HFX study confirmed that H/M remained as a significant and independent predictor of all-cause mortality and the composite endpoint of death or death-equivalent events among nearly thousand NYHA II-III HF subjects during the median of 24 mo follow-up[181].

An elegant study by Wakabayashi *et al*[182] exploring ^{123}I-mIBG kinetics in terms of underlying HF etiology showed that ^{123}I-mIBG activity provided independent long-term prognostic information for both ischemic and non-ischemic etiologies of HF with lower H/M values having a greater impact on cardiac death among patients with ischemic compared to non-ischemic cardiomyopathy[182]. In concordance with such findings among HF patients with ischemic cardiomyopathy, ^{11}C-HED PET-based studies revealed that regional myocardial sympathetic denervation and volume of denervated myocardium accurately predicted the risk of sudden cardiac arrest thus clearly correlating SNS innervation abnormalities with future arrhythmogenic events[183,184]. Similar findings were confirmed by another research group showing that denervated myocardium quantified using PET strongly predicted the risk of sudden cardiac arrest, independent of LVEF, infarct volume and other clinical variables among HF patients with ischemic cardiomyopathy and with LVEF < 35% that were eligible for implantable cardioverter-defibrillator device for primary prevention[185]. Finally, the most recent study conducted among patients admitted for acute decompensated heart failure and prospectively enrolled in the OPAR registry demonstrated that patients with cardiac sympathetic nerve dysfunction, defined as low late H/M, had a significantly greater risk of future adverse cardiac events, irrespective of clinical phenotype based on the LVEF values[186]. This study also showed that even a mild impairment in cardiac contractility (as shown in borderline LVEF values represented in HFmrEF cohort) was associated with sympathetic nerve dysfunction and was independently linked to poor outcomes thus suggesting that use of beta-blocker therapy in patients with HFmrEF phenotype is a viable pharmacotherapeutic option, as also supported by expert consensus statement and data from a large meta-analysis[73,187].

Taken together, these studies suggest that non-invasive cardiac imaging with norepinephrine analogs provides a reliable estimation of cardiac sympathetic nerve activity and this activity is strongly associated with clinical outcomes, regardless of clinical phenotypes or if HF is of chronic or acute onset. Such findings validate the concept that SNS overactivity is an important pathophysiological target in HF that must be efficaciously treated to improve outcomes and prevent sudden cardiac death.

HEART RATE VARIABILITY

Heart rate variability (HRV) is an established and widely used noninvasive method for the assessment of autonomic modulation of heart rate. It uses electrocardiographic (ECG) signal to measure subtle variations in the beat-to-beat heart intervals and is considered as a surrogate parameter of the complex interaction between CNS and cardiovascular system[188,189]. These periodic oscillations in heart rate signals are transformed into different frequency areas and their relative intensity is reported as a numerical value[190]. Briefly, low-frequency power (LF) and high-frequency power (HF), as well as the LF/HF ratio, are the most commonly used parameters in HRV analysis[189,191]. In most of the studies, HF power is regarded as a surrogate of PNS activity while LF power is modulated by both SNS and PNS. Likewise, high LF power values are associated with increased sympathetic activity while the LF/HF ratio reflects global sympathetic/vagal balance[191]. Generally, decreased HRV is associated with various pathologies and decreased life expectancy in several studies[188].

Regarding cardiovascular diseases, depressed HRV has been associated with autonomic neuropathy, heart transplantation, congestive HF, MI, and other incident cardiac conditions[192,193]. Most data for low HRV and increased mortality have been corroborated from studies investigating populations with cardiovascular diseases such as post-MI patients, patients with HF and those experiencing SCD, and in contrast to this, such associations of HRV were historically more diluted when it comes to risk stratification among the general asymptomatic population[194]. However, a recent study by Hillebrand *et al*[195] showed that low HRV was associated with a 32%-45% increased risk of a first cardiovascular event in populations without known cardiovascular disease[195]. In a similar fashion, abnormal HRV parameters were independently associated with incident CHF in asymptomatic older adults[196].

In the setting of a failing heart, HRV is significantly reduced in most patients and associated with the high risk of death due to progressive HF, SCD and syndrome

severity[197-199]. Ponikowski *et al*[200,201] demonstrated that depressed HRV on 24-h ambulatory ECG monitoring was an independent risk factor for poor prognosis in patients with CHF and was related to a higher risk of ventricular tachycardia[200,201]. Similar findings were also confirmed in patients hospitalized for decompensated HF[202]. An important study by Pousset *et al*[203] showed that a beta-blocker, bisoprolol, administered in a single dose of 5 mg per day managed to reduce heart rate and significantly increase HRV as per 24-h Holter ECG monitoring among patients with HF[203]. This effect was attributed to the increased parameters of parasympathetic activity in HF thus showing that increased vagal tone may be responsible for the protective effect of beta blockers and may provide prognostic implications in HF. Similarly, beta-blockers improved cardiac autonomic regulation during high sympathetic stress of decompensated HF[204].

However, the foundational framework that links low-frequency and high-frequency components of HRV with sympathetic and parasympathetic nervous system division was developed decades ago and this algorithm does not integrate findings and data on HRV that were gathered in the past 30 years thus might have certain limitations in clinical practice[205]. Another potential limit for the use of HRV in risk stratification of HF patients might lie in the fact that these parameters tend to be very low in most HF subjects, therefore, data dispersion might be small thus limiting survival regression models while many confounding non-neural factors might affect HRV values in HF[206]. The future of risk stratification of events in HF likely lies in the improvement of HRV spectral analyses algorithms and integration of HRV data with other biosignals acquired from novel HF devices, imaging methods, and laboratory biomarkers.

LABORATORY BIOMARKERS OF SYMPATHETIC NERVOUS SYSTEM ACTIVATION IN HEART FAILURE

Laboratory biomarkers that can be measured in the peripheral circulation of HF patients can give us insight on underlying pathophysiological mechanisms that are occurring in patients with both acute and chronic HF. Since HF is a complex syndrome characterized by the high prevalence of comorbidities an integrated approach using multiple biomarkers could aid in the diagnosis, accurate risk stratification regarding mortality and future hospitalizations and perhaps enable optimal tailoring of pharmacotherapeutic and/or device therapies for the individual HF patient[207,208]. A wide array of novel biomarkers reflecting pathophysiological processes of myocardial stretch, matrix remodeling, myocyte injury, oxidative stress, inflammation, neurohumoral activation, and renal dysfunction are becoming increasingly studied and integrated into the process of care for HF patient and clinical decision-making[19]. The early adoption of these novel biomarkers in modern clinical practice has a great potential to complement traditional biomarkers that are regularly used in the workup of HF patients such as N-terminal prohormone of brain natriuretic peptide (NT-proBNP), brain natriuretic peptide (BNP), high sensitivity cardiac troponin (hs-cTn), soluble suppression of tumorigenicity 2 or C-reactive protein[19].

In this last section of the review, we will focus on both established and novel laboratory biomarkers that are implicated in the pathophysiology of SNS activation in HF and as such might be potentially used in clinical practice. The summary of pathophysiological effects, cellular mechanisms of action, circulating levels, and association of selected biomarkers with outcomes in HF is presented in Table 2.

Norepinephrine

As previously discussed, circulating plasma levels and urinary excretion of norepinephrine (NE) are significantly higher among patients with congestive HF compared to those without, reflecting elevated sympathetic drive[117,118,124]. A recent study by Matsushita *et al*[209] showed that the endogenous catecholamine surge might be the cause of urgently presenting acute HF by eliciting an abrupt and excessive rise in blood pressure leading to increased after-overload and volume-shift lung congestion[209]. A few decades ago, Cohn and colleagues showed that plasma NE has been independently related to subsequent risk of mortality among patients with chronic congestive HF and was also higher among those that had progressive HF compared to patients that died suddenly[129]. This was later confirmed in the V-HEFT II study that enrolled patients with congestive HF showing that plasma NE was an independent predictor of prognosis and plasma NE values > 900 pg/mL were associated with significantly greater mortality risk compared to lower NE tertiles[210]. In the longitudinal follow-up of patients with HF from the Val-HeFT trial, changes of

Table 2 Selected biomarkers in respect to their pathophysiological effects, cellular mechanisms, circulating levels and outcomes in heart failure

	NE	NPY	GAL	ET-1	CST
Pathophysiological effects in heart failure or cardiovascular diseases	↑ Promotes cardiac hypertrophy; ↑ promotes induction of fetal genes in myocardial remodeling; ↑ mediates and enhances apoptosis of cardiac myocytes *in vitro*; ↑ promotes arterial vasoconstriction; ↑ promotes tachyphylaxis; ↑ increased cardiac and renal spillover in HF; ↓ impaired oxygen utilization and exercise efficiency in patients with stable HF; ↑ increased sympathetic nerve activity and reduced clearance of norepinephrine	↑ Vasoconstriction; ↑ promotes adverse cardiac remodeling; ↑ increased cardiac spillover; ↑ promotes angiogenesis; ↑ associated with increased platelet aggregation and adhesion following thrombosis; ↑ stimulates atherosclerosis; ↑ promotes vasoconstriction of coronary microvasculature; ↑ enhancing the NE-mediated effect of sympathetic discharge, associated with the increased incidence of ventricular arrhythmia; ↑ enhances inhibition of vagally-mediated ;bradycardia through Y2 receptors; ↑ potentiates arrhyhtmias following STEMI, despite beta-blocker therapy	↓ Reduces cardiac cholinergic neurotransmission; ↓ reduces acetylcholine biovailability in the synapse junctions; ↓ reduces vagally-mediated bradycardia; ↑promotes antithrombotic phenotype on endocardial endothelial cells; ↑ increased cardioprotective activity against ischemia-reperfusion injury in H9C2 cardiomyoblasts *in vitro*	↑ Promotes vasoconstriction (most potent vasoconstrictor in humans); ↑ promotes vascular and cardiac hypertrophy; ↓ decreases NE reuptake thus propagating adrenergic effects; ↓ reduces coronary flow; ↑ promotes inotropic and chronotropic responses in cardiomyocytes; ↑ promotes mitogenic actions; ↑ activation of endothelin-dependent pathways is observed in HF; ↑ correlates with hemodynamic impairment and severity of pulmonary hypertension in HF; ↑ promotes angiogenesis	↓ Decreases arterial blood pressure (direct and indirect vasodilation); ↓ inhibits catecholamine release; ↓ decreases NPY and ATP release; ↓ attenuates cardiac inotropy and chronotropy; ↑ promotes angiogenesis; ↓ blunts atherosclerosis; ↓ reduces inflammation; ↓ reduces thrombogenicity; ↑ promotes VSMC proliferation; ↓ decreases arrhytmogenic events; ↓ decreases ventricular remodeling
Cellular mechanism	Activation of α and β adrenergic receptors (G protein-coupled)	Activation of G protein-coupled post-synaptic Y1-Y6 receptors (Y2 is also pre-synaptic) on sympathetic nerve endings	Activation of G protein-coupled receptors – GAL1R, GAL2R, GAL3R	Activation of endothelin A (ET_A) and B (ET_B) receptors(both G protein-coupled)	Acts on neuronal nicotinic acetylcholine receptor (nAchR)
Circulating levels in HF *vs* controls	↑ Circulating plasma levels;↑ urinary excreted levels	↑ Circulating plasma levels	←→ Not significantly different plasma levels	↑ Plasma levels;↑ renal tissue levels	↑ Circulating plasma levels
Association with mortality and morbidity in HF	↑ High NE levels were associated with significantly increased mortality and morbidity in patients with congestive HF; ↑ circulating NE levels positively correlate with HF syndrome severity	↑ Elevated levels in coronary sinus were associated with composite endpoint of VAD implantation, death, and cardiac transplant among patients with stable chronic HF undergoing CRT implantation	Not established (no studies available)	↑ Increased ET-1 levels associated with higher HF syndrome severity;↑ increased ET-1 levels associated with mortality in HF	↑ Increased CST levels were independently associated with all-cause and cardiac mortality in patients with chronic HF; ↑ correlates with NYHA functional class

NE: Norepinephrine; NPY: Neuropeptide Y; GAL: Galanin; ET-1: Endothelin; CST: Catestatin; CRT: Cardiac resynchronization therapy; FU: Follow-up; HF: Heart failure; NYHA: New York Heart Association; STEMI: ST-elevation myocardial infarction; VAD: Ventricular assist device; VSMC: Vascular smooth muscle cell.

BNP and NE from baseline to 4 and 12 mo post-discharge significantly correlated to changes in morbidity and mortality[211]. However, the administration of neurohormonal antagonists such as ACE inhibitors and beta-blockers in HF patients had variable and heterogeneous effects on circulating NE levels and there was a significant incongruency of these levels with endpoints such as mortality and reverse ventricular remodeling in a handful of relevant trials[212]. These data suggested that reducing NE levels might not be the appropriate goal of neurohumoral antagonists and that NE is not a feasible laboratory biomarker of choice when it comes to measuring response to HF-directed pharmacotherapy. Finally, the fact that circulating NE measurements require high-performance liquid chromatography is a significant limitation to its wide use in clinical practice and imposes several analytical challenges and physiological limitations thus making it likely impractical as a routine biomarker in HF[213].

Neuropeptide Y

Neuropeptide Y (NPY) is a sympathetic co-transmitter with a longer half-life than NE and is widely distributed in the CNS and peripheral nervous system with pleiotropic physiological actions. In the cardiovascular system, NPY is co-released from cardiac sympathetic nerve terminals along with catecholamines (predominantly NE) and galanin[214]. These sympathetic nerves supply vasculature, cardiomyocytes and endocardial endothelial cells in the ventricle while NPY physiologically modulates cardiovascular function, potentiates pressor effects of angiotensin II, elicits arterial and venous constriction, blunts parasympathetic activity, augments cardiomyocyte calcium loading, participates in cardiomyocyte remodeling and promotes angiogenesis[215-223]. NPY and galanin have a direct ability to modulate vagus nerve to release acetylcholine and control heart rate while NPY plasma levels had a strong correlation with coronary microvascular function among patients with ST-elevation myocardial infarction[224]. Maisel and colleagues were the first to report on elevated levels of plasma NPY in patients with congestive HF and this was later confirmed in several subsequent studies[225-227].

In the recent clinical study by Ajijola *et al*[228], NPY was sampled from the coronary sinus (CS) among patients with stable CHF during the elective CRT device implantation[228]. Researchers sought to answer if NPY as a peptide involved in adrenergic signaling is associated with outcomes among patients with stable CHF. They found that patients with NPY CS levels > 130 pg/mL had significantly worse outcomes compared to those with lower NPY CS levels, even after adjusting for age, estimated glomerular filtration rate (eGFR), and LVEF (HR: 9.5, 95%CI: 2.92-30.5, P < 0.001) during the median follow-up of 28.8 mo while the composite endpoint consisted of death, ventricular assist device placement and cardiac transplant. Most of the signal from the composite endpoint was driven by death events and interestingly, CRT data at 6-mo follow-up showed that CS NPY levels did not significantly differ between CRT responders and non-responders (P = 0.76). Finally, immunohistochemical analyses revealed that sympathetic ganglia (stellate and middle cervical ganglion) of CHF patients contained less NPY compared to ganglia tissue obtained from healthy donors while no significant difference was observed in the NPY production between both groups as examined by the measured NPY mRNA levels. This study showed that CS NPY levels were elevated in stable CHF patients and associated with adverse outcomes and relevant clinical and laboratory characteristics while increased stellate ganglia sympathetic discharge was likely the culprit for these elevated levels.

Although CS NPY levels provided robust prognostic information among stable CHF patients, a problem in clinical practice arises in the peripheral venous sampling of NPY since those levels are not cardiac-specific and are mostly of hepatomesenteric origin since NPY has been identified as a stimulator to food intake[229]. In cardiac failure, there is an increase in resting NPY spillover within the myocardium, however, the net overflow of NPY to plasma was dominantly from hepatic circulation, but not the cardiac, forearm or cerebral circulations showing a marked difference in regional distribution of NPY content[230]. It has also been shown that sympathetic activation by exercise produced only a modest increase in cardiac NPY overflow without the concomitant change in arterial NPY concentrations finally concluding that plasma NPY concentrations are less sensitive than those of plasma NE in terms of quantifying SNS responses regulating the systemic circulation and cardiac hemodynamics in HF, as implied in some previous studies[225,230,231]. Finally, a recent preclinical study showed that NPY blockade by experimental Nur77 agent protected against adverse cardiac remodeling by limiting NPY-mediated signaling (NPY-NPY_{1R}) in the cardiomyocytes[232]. Most important characteristics and effects of NPY are depicted in the Figure 3. In the future, antagonists of NPY receptors Y1 and Y2 might be a feasible therapeutic option in acute myocardial infarction but also during chronic HF and hypertension[224]. These pharmacotherapeutic options would complement beta-blockers and implantable vagus nerve stimulators to improve outcomes in patients with cardiovascular diseases[224].

Galanin

Similarly to NE and NPY, galanin is an adrenergic co-transmitter with a short half-life (about 5 min) released from peripheral postganglionic neurons and is implicated in attenuation of cardiac cholinergic tonus after burst sympathetic activity thus contributing to autonomic imbalance and the pathophysiological phenomenon known as "sympathovagal crosstalk"[224,233]. This phenomenon can remain chronically activated and sustained even in the presence of beta-adrenergic blockade thereby it could be a valid therapeutic target in the spectrum of neurohumoral activation in

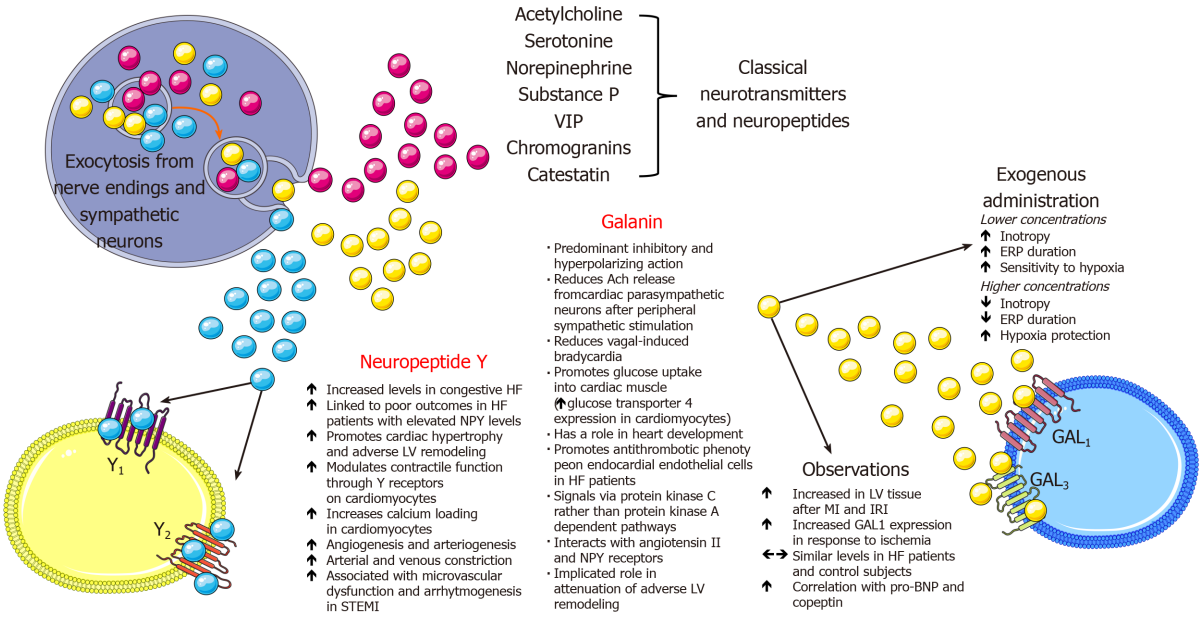

Figure 3 Physiological and pathophysiological implications of neuropeptide Y and galanin with respect to cardiovascular system. Ach: Acetylcholine; ERP: Effective refractory period; HF: Heart failure; IRI: Ischemia-reperfusion injury; LV: Left ventricular; MI: Myocardial infarction; NPY: Neuropeptide Y; STEMI: ST-elevation myocardial infarction; VIP: Vasoactive intestinal peptide.

HF[224]. Furthermore, nerve terminals of parasympathetic neurons in the heart express both galanin receptors and NPY receptors (NPY Y2) which, upon activation, reduce acetylcholine release[233]. During the prolonged sympathetic activation there is a release of a slowly diffusing co-transmitter galanin, together with NPY, that bind to these receptors and reduce cholinergic neurotransmission in the heart[234]. Furthermore, galanin through its receptors interacts with other neuropeptides such as NPY and angiotensin II and their receptors, namely Y_1 and AT_1 thus having a potential role in neurochemical modulation of central cardiovascular control[235].

A recent prospective case-control study in the clinical realm showed that unlike pro-BNP, copeptin and NPY, galanin levels were similar among patients with HF patients and control subjects while pro-BNP was the only significant determinant of galanin levels in HF patients[236]. Authors postulated that galanin most likely has a predominant paracrine modulatory function at the level of peripheral cardiac sympathetic nerves, therefore, its circulating levels in plasma might not reflect the degree of its local involvement in sympathovagal crosstalk. Finally, since natriuretic peptides promote catecholamine release from cardiac sympathetic neurons, authors suggested biological plausibility of their finding that galanin positively correlated with BNP[237]. On the other hand, galanin promoted anti-thrombotic phenotype on cultured endocardial endothelial cells from HF patients through attenuation of von Willebrand factor extrusion and multimer expression while this effect was not elicited by the NPY[238]. One preclinical study in the animal model of HF showed that galanin receptor type 1 agonist improved cardiac function and attenuated ventricular remodeling[239]. Most important characteristics and effects of galanin are depicted in Figure 3. Due to the scarcity of studies examining the role of galanin in HF, future preclinical and clinical studies are warranted to further elucidate its biological functions and its potential as a biomarker in HF.

Endothelin

Endothelins represent a family of three similar 21 amino acid length peptides – endothelin 1 (ET-1), 2 (ET-2) and 3 (ET-3) of which ET-1 and ET-2 bind to G-protein coupled endothelin receptors A (ET_A) and B (ET_B) on vascular smooth muscle cells with equal affinity to both while ET-3 exhibits lower affinity for ET_A relative to ET_B receptor[240]. Of all endothelins, ET-1 is predominantly produced by vascular tissue, has inotropic, chemotactic and mitogenic properties, induces collagen synthesis by cardiac fibroblasts, and is biologically the most potent vasoconstrictor in the human cardiovascular system[241]. Furthemore, autocrine binding of ET-1 to ET_B receptors promotes NO and prostaglandin release and consequent relaxation of vascular smooth muscle cells. ET-1 plays a role in neuronal development, growth, and function while

biologically promoting vascular and cardiac hypertrophy, inflammatory responses and is an independent factor contributing to exacerbation of the cardiovascular disease[242-244]. The main source of ET-1 and its precursor, big endothelin-1 (BigET-1) are pulmonary vascular endothelial cells, therefore, elevated plasma levels of ET-1 or bigET-1 might closely reflect the degree of pulmonary endothelial dysfunction in HF while ET-1 was significantly overexpressed in the lungs of patients with pulmonary hypertension[245-247]. Stangl *et al*[246] demonstrated that in severe congestive HF lungs act as a producer while coronary and peripheral circulation act as consumers of BigET-1 and ET-1 while short-term vasodilator therapy decreased endothelins and restored pulmonary, coronary, and peripheral balance[246]. Endothelin receptors are also expressed in the CNS and central administration of endothelin modulated endocrine and cardiovascular regulation, behavior and MAP[248]. In the preclinical experiment, an injection of ET-1 in different regions of the brainstem of normotensive rats resulted in a differential response in heart rate, arterial blood pressure, and respiratory frequency indicating that endothelin has a modulatory role in cardiovascular function[249].

Previous studies showed that HF is associated with high levels of ET-1 in plasma and renal tissue and these levels correlated with syndrome severity, especially with the extent of pulmonary hypertension, and overall contributed to the progression of chronic HF[250-254]. In a preclinical study, infusion of tezosentan (ET-1 antagonist) significantly decreased MAP in both normal and HF animals and reduced cardiac sympathetic nerve activity (CSNA) in normal animals, however, no decrease was observed in HF animals[255]. Therefore, this study showed that endogenous levels of ET-1 contribute to the baseline levels of CSNA in healthy animals, however, this correlation was absent in experimentally induced HF. Contrary to this, a non-selective experimental ET_A and ET_B antagonist (TAK-044) suppressed sympathetic activity and improved arterial baroreflex function in rats with HF[256]. Similarly, the addition of ACE inhibitor to ET_A receptor antagonist significantly improved cardiac failure after extensive MI in a rat model of congestive HF, compared with ACE inhibition monotherapy[257]. A cross-talk between the endothelin system and the adrenergic system has been demonstrated as activation of ET_B receptors on sympathetic neurons caused an increase in arterial blood pressure through vasoconstriction mediated by α_1-adrenergic receptors[98]. Sympathoexcitatory effects are also promoted through the interaction of ET-1 with ET_A receptors as this resulted in cardiomyocyte hypertrophy through adrenergic signaling pathways and massive NE release while it also contributed to impaired responsiveness of renal mechanosensory nerves in congestive HF[258,259]. In the rat model of HF, endogenous ET-1 impaired NE reuptake through activation of ET_A receptors while in a healthy heart ET_A-mediated inhibition of NE reuptake was countered, but to a lesser degree, by the ET_B-mediated silencing of NE release resulting in a net increase in left ventricular contractility suggesting that fine balance between NE reuptake and exocytotic release is modulated by endothelin signaling as it was also suggested in previous studies[260,261].

However, while endothelin pathway inhibition seemed promising in animal and preclinical models of HF, these observations did not translate to human clinical studies as ET-1 antagonist tezosentan did not improve symptoms or clinical outcomes in patients with acute HF although ET-1 levels were independently associated with short term in-hospital outcomes and 180-d mortality in patients hospitalized for acute HF, as demonstrated in ASCEND-HF substudy[262-264]. A predictive value of BigET-1 in patients with left ventricular dysfunction after AMI on the composite endpoint of cardiovascular death or hospitalization for worsening HF has been demonstrated in the subanalysis from EPHESUS study, however, neurohumoral antagonist – eplerenone seemed to have no significant effect in modifying BigET-1 levels at follow-up[265]. Authors proposed that levels of BigET-1 (as a precursor of ET-1) likely reflect the degree of ET-1 synthesis while BigET-1 is also a more feasible laboratory biomarker due to its longer half-life than that of ET-1[266]. This notion has been confirmed in a previous study that established how elevated plasma ET-1 levels in human CHF dominantly represent the elevation of Big-ET-1 while ET activity was not changed in CHF compared to a healthy state[267]. Furthermore, increased ET-1 levels were detected only in moderate or severe CHF and not among asymptomatic patients or those with mild CHF while plasma concentrations in range 5-40 pmol/L seemed to exhibit vasoactive effects[267,268]. Previous studies confirmed that BigET-1 provided prognostic information regarding the cardiovascular mortality during the 12-mo follow-up (HR: 1.42, 95%CI: 1.04-1.95, $P = 0.03$), all-cause mortality during the 23-mo follow-up (HR: 1.49, 95%CI: 1.20-1.84, $P = 0.0003$) and the composite endpoint of mortality and morbidity (HR: 1.43, 95%CI: 1.20-1.69, $P < 0.001$) at 23 mo, however, in the latter study BNP remained the strongest neurohormonal prognostic factor[269,270]. In the small study that enrolled patients with severe CHF, Big-ET-1 and ET-1 levels were

higher at baseline than in patients with mild to moderate CHF or healthy subjects and were found as robust independent predictors of survival, even beyond natriuretic peptide levels[271].

When 32 studies with 18497 HF patients were summarized in the meta-analysis, it was shown that plasma ET-1 and its related peptides were associated with poor prognosis and mortality in diverse spectrum of HF populations[272]. On the other hand, a meta-analysis of randomized clinical trials showed that neurohumoral antagonism of ET receptors in HF patients improved cardiac output, pulmonary and systemic hemodynamics but had a modest effect on clinical outcomes[273]. Therefore, these data suggest that there is a significant discrepancy between these observations – on one hand, ET signaling has been consistently associated with poor outcomes and prognosis in HF and on the other hand, pharmacological targeting of these adverse pathways seems less impressive in improving outcomes.

Perhaps there is a need to fine-tune and identify which subgroups of HF patients would have the greatest benefit from drugs interfering with ET pathways. In that regard, ET-1 and its fragments have shown some potential as valuable biomarkers among HFpEF patients with pulmonary hypertension or pulmonary dysfunction as its levels were associated with the degree of pulmonary hemodynamic derangements, reduced functional reserve of the right ventricle, diminished cardiac output and impaired cardiac response to exercise and peak oxygen consumption[274,275]. Even in the general population, elevated plasma ET-1 levels were in strong relation with elevated pulmonary artery systolic pressures on the echocardiogram and correlated with mortality and incident HF[276].

Therefore, current data suggest that activation of the endothelin system may play an important role in the pathophysiology of pulmonary hypertension in HFpEF and that it might present a viable target and a step towards precision medicine approach in HFpEF[277]. Regarding the potential ET pathway inhibition in HFpEF, thus far there are limited but encouraging preliminary reports. In the preclinical murine model of HFpEF, dual ET_A/ET_B blockade by macitentan improved HFpEF by abrogating aldosterone-induced cardiomyocyte hypertrophy and reducing stiffness through decreased expression of type I collagen and titin n2B in the left ventricle[278]. In the clinical domain, in patients with HFpEF, ET_A receptor antagonist sitaxsentan improved exercise tolerance, however, failed to decrease left ventricular mass or improve diastolic function while the study was not powered for mortality and rehospitalization analyses[279]. A small and prematurely stopped study showed that ET receptor blocker bosentan did not improve outcomes in HFpEF patients with pulmonary hypertension[280]. Therefore, due to the size and scarcity of available studies, a question whether ET-1 antagonists would improve outcomes in HFpEF yet remains to be answered by future and adequately powered randomized controlled trials. It is possible that neurohumoral biomarkers such as endothelin and its derivatives will enable us a more precise phenotyping of HFpEF patients to identify those that have a significantly impaired pulmonary function and that would receive the greatest benefit from ET pathway-oriented therapeutic interventions. The summary of synthesis, cellular effects, and pathophysiological implications of ET-1 are presented in Figure 4.

Catestatin

Catestatin (CST) is a product of precursor hormone chromogranin A (ChgA) and was isolated in 1997 by Mahata *et al*[281]. Its principal physiological action is the negative regulation of catecholamine release into circulation through the mechanism of non-competitive and reversible antagonism of neuronal nicotinic cholinergic receptors (nAChR)[281,282]. Its precursor molecule, ChgA, and other soluble secretory proteins are co-stored and co-released with catecholamines from vesicles in the neuroendocrine, endocrine and immune cells and sympathetic neurons thus have an important modulatory role of the adrenergic system[283]. Upon stimulation of chromaffin cells or sympathetic axons, a marked elevation of ChgA levels was detected[284]. Levels of ChgA are elevated in peripheral blood of patients with chronic HF and AMI and correlate with mortality and poor outcomes[285-287]. Even more, an intramyocardial production of ChgA is established in humans and was associated with negative inotropic and lusitropic effects on the mammalian heart thus providing evidence for neuroendocrine regulation of cardiac function by ChgA[288]. Furthermore, immunohistochemical biopsy studies showed that ChgA is co-localized with BNP in the dilated and hypertrophic left ventricle while ChgA levels correlated with end-diastolic left ventricular pressures[288].

CST is a 21 amino acid fragment derived from ChgA ($ChgA_{352-372}$) and is secreted by neuroendocrine tissues and nerve endings while it is widely distributed in the secretory granules of skin, sensory organs, and myocardium[289]. Its most important

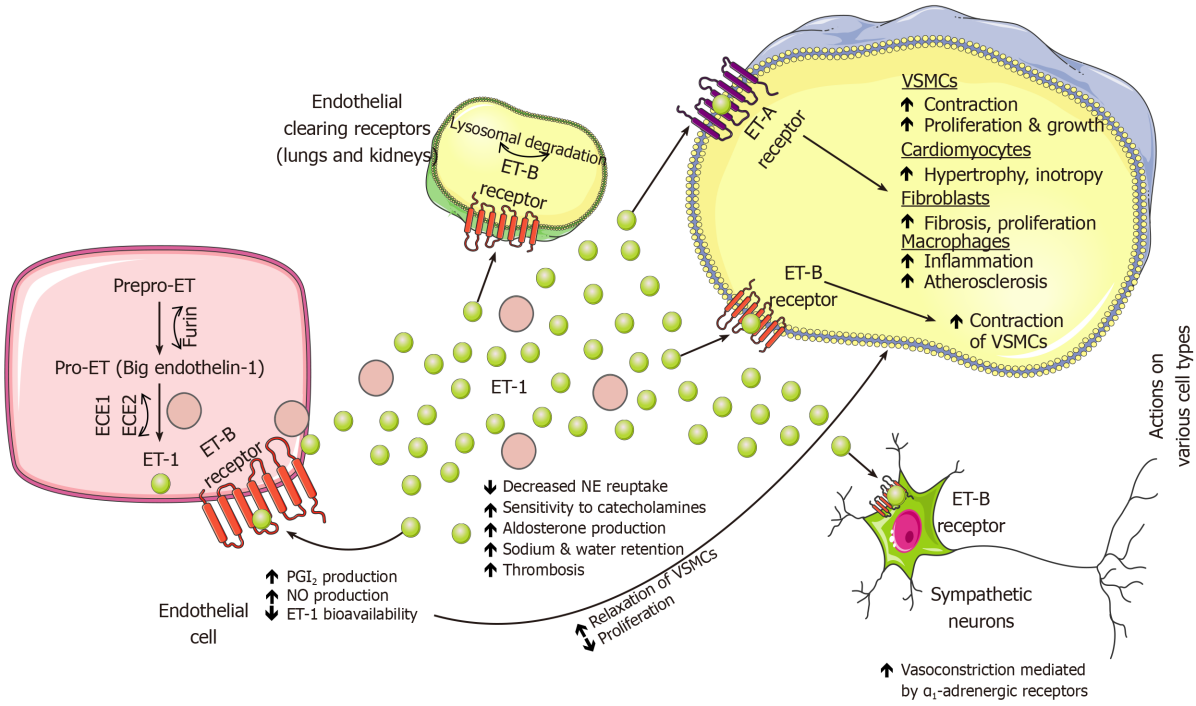

Figure 4 Physiological and pathophysiological implications of endothelin-1 in circulation and on various cell types and adrenergic neurons. ECE: Endothelin converting ezyme; ET-1: Endothelin-1; NE: Norepinephrine; NO: Nitric oxide; PGI_2: Prostacyclin (prostaglandin I2); VSMCs: Vascular smooth muscle cells.

physiological effect is the autocrine action on the chromaffin cells in the adrenal medulla and adrenergic neurons by which it modulates spillover of catecholamines (primarily NE) into peripheral circulation while concomitantly exhibiting paracrine and endocrine effects since it can be readily measured in the venous and arterial blood[290]. Furthermore, CST is potent regulator of arterial blood pressure since it exerts direct vasodilatative effect in humans *in vivo*, activates histamine release from mast cells and stimulates production of NO within endothelial cells[291-293]. In the chromaffin cell, ACh is a physiological agonist that, upon activation of ionotropic nAChR receptor, permits Na^+ entry into the cell which further depolarizes cellular membrane and enables activation of voltage-gated Ca^{2+} channels and subsequent Ca^{2+} entry that mobilizes chromaffin granules and initiates exocytosis of several neurohormones, neuropeptides, and catecholamines[281,294]. Once secreted outside of the cell through the process of exocytosis, extracellular post-translational proteolytic processing of the ChgA molecule will release several bioactive peptides and CST that will ultimately bind nAChR receptors of chromaffin cells in autocrine fashion thus antagonizing ACh actions in the periphery as depicted in Figure 5[295].

In the perspective of previously discussed catecholamine storage vesicle neurotransmitters, Mahapatra and colleagues demonstrated that CST inhibited nicotinically triggered exocytotic release of several co-transmitters from chromaffin granules such as NPY, adenosine triphosphate, chromogranins and catecholamines thereby demonstrating that CST is a potent regulator of neuropeptide transmission in the sympathochromaffin system[296]. However, in the CNS, CST exhibits both sympathoexcitatory and procholinergic effects depending on the region of medulla where its expressed[297,298]. Of established cardiovascular effects, CST suppresses beta-adrenergic activation and acts in a negative inotropic and chronotropic fashion, stimulates angiogenesis and proliferation of vascular smooth muscle cells, decreases thrombogenicity of endothelial cells, suppresses atherosclerosis and inflammation while also exerts cardioprotective effects by abrogating cardiomyocyte ischemia-reperfusion injury[299-306]. A very recent study by Alam *et al*[307] showed that CST has a direct and independent inhibiting effect on hypertrophy elicited by NE in the cultured H9c2 cardiac myoblasts and that is involved in the regulation of a large number of fetal genes that are upregulated during the process of myocardial hypertrophy[307]. Furthermore, the same study showed that CST effectively blunted stimulative effects of NE and other mitogenic signals on β_1 and β_2 adrenergic receptors thus providing novel evidence that CST has a direct modulatory effect on adrenergic transmission at

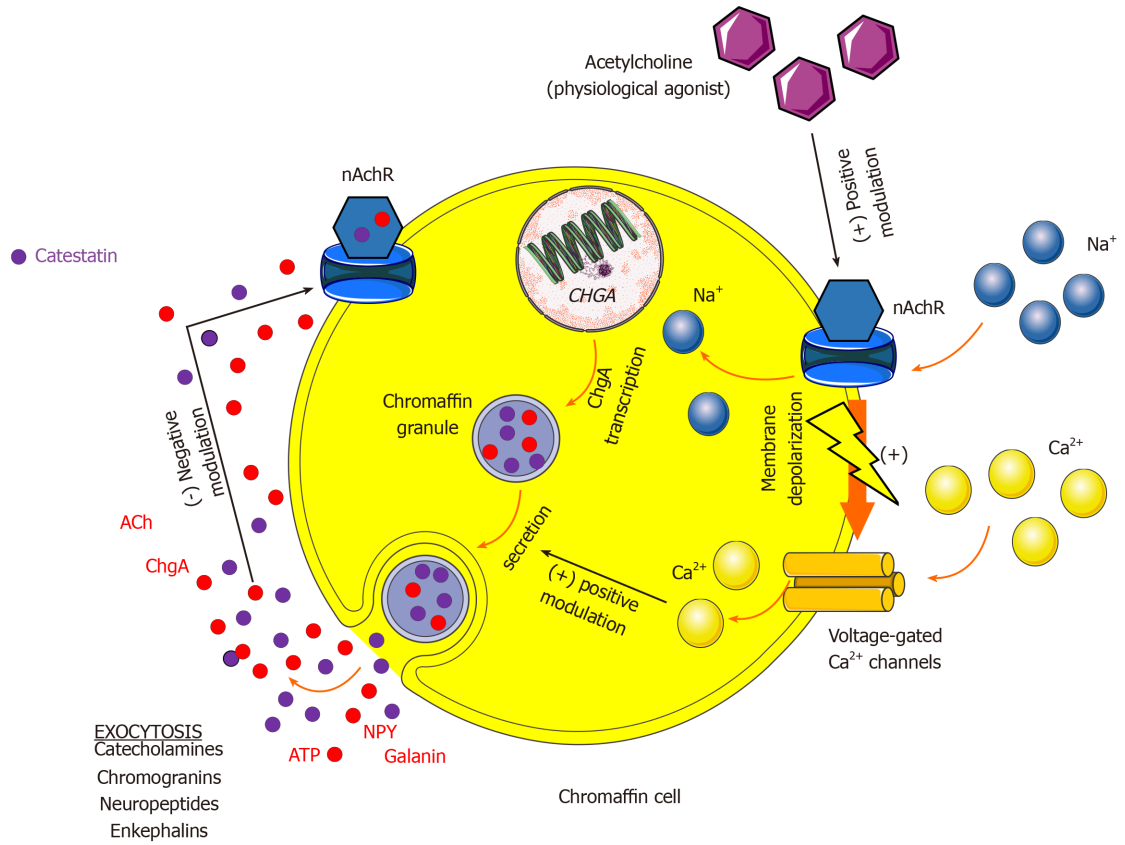

Figure 5 Mechanism of catestatin autocrine modulation of chromaffin cell in the adrenal medulla during sympathetic stimulation. ACh: Acetylcholine; ATP: Adenosine triphosphate; Ca^{2+}: Calcium; ChgA: Chromogranin A; nAchR: Neuronal type of nicotinic cholinergic receptors; Na^{+}: Sodium; NPY: Neuropeptide Y.

the level of adrenergic receptors. Similarly, in the model of rat heart, CST activated β_2 and β_3 adrenergic receptors thus upregulating the activity of eNOS and consequently increasing cyclic GMP and phosphodiesterase type 2 (PDE2) levels[308].

In line with its sympatholytic effects, chronic administration of exogenous CST improved autonomic function, shortened QT interval, and action potential duration and reduced the incidence of experimentally-induced ventricular arrhythmias in a rat model of myocardial infarction[309]. Similarly, in the rat model of hyperadrenergic hypertension, rats with ablated *ChgA* gene showed significantly longer QT interval, R-amplitude, and QRS time-voltage and this was accompanied by increased resting heart rate and QT variability thus demonstrating that arrhythmogenic ventricular assault develops in the status of low circulating CST levels[310]. These preclinical observations were clinically validated as elevated CST levels were an independent predictor of complicated malignant arrhythmias among AMI patients[311]. This observation might seem counterintuitive at first, however, it could be explained in the sense that CST levels reflect a compensatory response for the increased SNS activity and excess catecholamine discharge and are attempting to restore autonomic balance. Therefore, circulating CST levels likely "mirror" biological catecholamine turnover and degree of sympathetic activity as CST co-localizes and is co-released with catecholamines and other neuropeptides. Finally, cardioprotective effects of CST are likely pathophysiologically overpowered by sympathetic discharge in conditions in which cardiovascular homeostasis is disrupted such as AMI or decompensated HF, despite the relatively high circulating CST levels.

There are only a few available studies that examined the role of CST in HF and investigated its prognostic role in this syndrome.

In the first study by Zhu *et al*[312] CST levels gradually decreased from stage A to C of HF while there was a significant difference between stage A and B in terms of CST concentrations with a cut-off value of 19.73 ng/mL providing 90% sensitivity and 50.9% specificity for the detection of B stage of HF[312]. This finding is of clinical relevance since stage B presumes structural cardiac disorder but without symptoms, while stage A assumes patients at high risk for developing HF but without functional or structural heart disorder. Therefore, this study showed that there is a utility for

decreased CST levels implying structural heart disease among asymptomatic patients. In the study by Liu *et al*[313] performed in the similar setting, CST was found higher in patients with HF compared to control subjects and it positively correlated with functional syndrome burden as assessed by the NYHA class[313]. Furthermore, etiology of HF (ischemic or not) and NYHA class predicted plasma CST levels while BNP provided better area under the curve value than CST in terms of detecting moderate to severe HF diagnosis (area under the curve values of 0.831 and 0.626, respectively). Adding CST to BNP did not improve diagnostic accuracy.

Recently, Borovac *et al*[314] showed in CATSTAT-HF study, that patients with acutely decompensated HF had higher serum CST levels if they belonged to a higher NYHA functional class while circulating CST levels were significantly higher among patients with ischemic *vs* non-ischemic etiology of disease thus likely reflecting an augmented SNS neurohumoral activation in patients with ischemic etiology of the disease (in the study defined as those with the history of myocardial infarction)[314]. The same study revealed that CST levels did not differ in respect to LVEF phenotypes while CST levels independently correlated with NYHA class, waist-to-hip ratio, HbA1c, LDL cholesterol, non-HDL cholesterol, high sensitivity cardiac troponin I and the heart rate at admission and rest. Finally, higher CST levels were strongly associated with favorable echocardiographic profile as they positively correlated with smaller LV volumes and dimensions, as well as with decreased left ventricular mass and smaller dimensions of the left atrium and this finding clinically validates the concept that CST locally has cardioprotective effects, attenuates adverse ventricular remodeling and acts in antihypertrophic fashion, as these biological effects were postulated in a few earlier preclinical studies[307,315].

CONCLUSION

Finally, the prognostic value of CST as a biomarker was demonstrated among chronic HF patients. In the multivariate Cox regression analysis, plasma CST was an independent risk factor for all-cause death (HR: 1.84, 95%CI: 1.02-3.32, P = 0.042) and cardiac death (HR: 2.41, 95%CI: 1.26-4.62, P = 0.008), respectively, during the median follow-up of 52.5 mo[316]. If patients had both high CST and BNP levels during hospitalization the risk of all-cause death increased 3-fold while the risk of cardiac death increased 4-fold.

Based on these findings it is plausible that CST could be a reliable indirect marker of SNS activity and it is likely that high CST levels reflect advanced disease burden and high sympathoexcitatory profile of an individual HF patient. Furthermore, CST provides complementary prognostic information to natriuretic peptides in terms of mortality in HF and could aid in the risk stratification of chronic HF patients. However, since the latter finding is based on only one clinical study further large-scale studies are required to validate these findings and clarify the role of circulating CST levels in predicting HF prognosis. Finally, patients with elevated CST levels might be suitable candidates for the introduction or up-titration of sympatholytic agents such as beta-adrenergic blockers, however, the effects of neurohumoral antagonists on circulating CST levels are yet to be determined.

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MINIREVIEWS

Forensic interrogation of diabetic endothelitis in cardiovascular diseases and clinical translation in heart failure

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Abstract

Diabetic heart disease (DHD) can be classified as a primary consequence from several pathophysiological manifestation of diabetes mellitus (DM) on cardiac tissues or secondarily in extracardiac tissues and is encountered as either primary or secondary complications of DM. Endothelitis is inflammation of the vascular endothelium and is likely to be seen in the majority of patients who start to manifest an end organ complication of DM in this case DHD. Diabetes is a leading cause for many cardiovascular syndromes and diseases including congestive heart failure (CHF) however much remains unknown about the transition from diagnosed DM to clinical state and the contribution of the various mechanical and counterregulatory systems in the manifested complaint. Diastolic heart failure or heart failure with preserved ejection fraction (DHF/HFpEF), accounts for half of all CHF presentations, has DM as a major contributor, however, there remain large gaps in clinical and pathophysiological understanding. This review aims to explore the microscopic aspects in diabetic endothelitis and provide a clinical link to with context to HFpEF.

Key words: Cardiovascular disease; Diabetic heart disease; Diabetes mellitus; Diastolic heart failure; Endothelitis; Heart failure with preserved ejection fraction; Inflammation

Core tip: We discuss the concept of diabetes mellitus and inflammation in the endothelium of blood vessels or "diabetic endothelitis". The vascular endothelium permeates every organ in the body. Macro and microvascular inflammation in coronary and related arterial beds contributes to diabetic heart diseases such as congestive heart failure. Heart failure

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with preserved ejection fraction is an important and poorly understood condition. In this review we provide a basic science perspective and a clinical link to this problem.

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INTRODUCTION

Basic sciences are the doorway for forensic analysis of clinical syndromes. Since the early community heart studies such as Framingham Heart Studies two clear delineations of congestive heart failure were observed, systolic heart failure where all cases had diastolic impairment and isolated heart failure where systolic function appeared preserved from all available diagnostic tools hence diastolic heart failure or Heart Failure with preserved Ejection Fraction (DHF/ HFpEF)[1]. The former is well studied however the latter still struggles from a fixed definition, aetiology, pathophysiology and diagnostic taxonomy and a single proven prognostic therapy[2]. The bench will play a critical role in understanding this syndrome. Diabetes Mellitus (DM) and its common end organ complication of *"endothelitis"* are seen in all diabetic heart disease (DHD) and is a valid area to focus to identify a bedside link for HFpEF.

The vascular endothelium, that forms the lining of all blood and lymphatic vessels, is uniquely vulnerable to the effects of chronic or intermittent hyperglycaemia[3]. Being largely dependent on glycolytic metabolism for generating adenosine triphosphate rather than oxidative phosphorylation, the uptake of glucose into endothelial cells is not downregulated as ambient glucose levels rise. This means that increasing glycolytic flux increasingly generates toxic intermediates including reactive dicarbonyls and reactive oxygen species (ROS). This glucotoxicity, along with the additional impacts of lipotoxicity, endoplasmic reticulum (ER) stress, inflammasome activation, oxidative and shear stress in diabetes induce pathophysiological changes in the vascular endothelium that are best characterised as "*endothel-it is*". These changes include increased adhesion and extravasation of leucocytes, production of chemokines/cytokines, exudation of plasma, altered vasomotor tone and haemostasis, endothelial senescence and apoptosis, endothelial to mesenchymal transition (EndoMT) and neo-angiogenesis that contribute not only to accelerated atherosclerosis but also the development of progression of heart failure in diabetes[3-6] (Figure 1). Moreover, although originally considered a consequence of hypertrophy and overload, HFpEF is increasingly viewed as a "microvascular" disorder driven by endothelitis[7]. In this paper, we explore the key inflammatory changes in the vascular endothelium and their potential role in DHD. We also provide a short hypothetical perspective of a contextual bedside translational strategy to advance a clinical focus for diabetic endothelitis ("diabetic endothelitis" is a term the authors use to describe endothelial injury associated with the chronic inflammatory milieu of diabetes. The exacts proponents of the injury and its manifestations are the subject of this paper and ongoing works. Conventional vascular inflammation often associated with connective tissue diseases are well described. The endothelium itself is a component of the vasculature, is less describe in that sense. When endothelial function is altered the term "endothelial dysfunction" is used.).

CELLULAR BASIS OF DIABETIC INFLAMMATION IN THE ENDOTHELIUM AND DYSFUNCTION

Endothelitis, leukocyte recruitment and infiltration

Activation of the vascular endothelium plays a key initiating role in the leucocyte adhesion and the subsequent development of the nascent atherosclerotic plaque. Intrinsically, monolayer of endothelial cells forms a critical interface between circulating immune cells and the tissues of the body. The luminal expression of chemokines, like macrophage chemoattractant protein (MCP-1), attracts leucocytes,

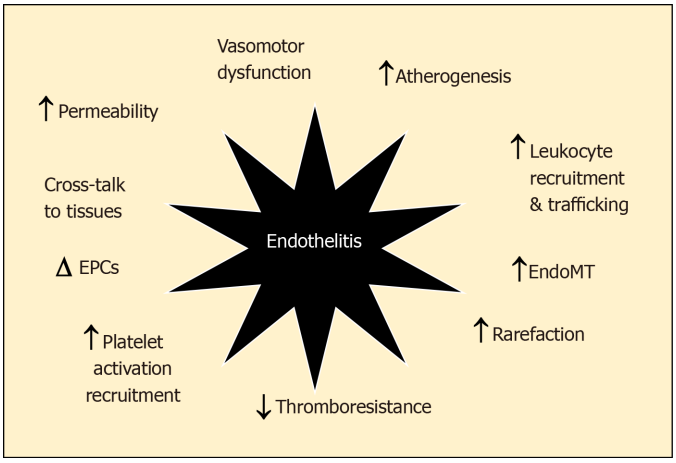

Figure 1 Endothelitis is associated with a range of dysfunctional changes that contribute the development and progression of cardiovascular disease. EndoMT: Endothelial-mesenchymal transition; EPC: Endothelial progenitor cell.

which then roll, arrest and bind to an activated endothelium expressing adhesion molecules, including selectins, intercellular adhesion molecule (ICAM)-1, and vascular cell adhesion molecule (VCAM)-1, Junctional Adhesion Molecules (JAMs), P-selectin and E-selectin, and subsequently migrate into the sub-epithelium and beyond. Subepithelial macrophages become engorged with cholesterol creating lipid-laden macrophages known as foam cells, creating fatty streaks and eventually the necrotic core of atheromatous plaques after their cellular death. In diabetes, the rate at which circulating monocytes enter the atherosclerotic lesion is increased, while plaque regression is reduced[8]. The increased transit of activated inflammatory cells into the vessel wall in diabetes, not only leads to more atherosclerosis, but as a result, atherosclerosis is more complex, multivessel with more unstable plaque in diabetes. Diabetic heart failure in diabetes is also characterised by a leukocytic infiltration, including activated monocytes, T-cells and dendritic cells[9,10]. As with atherosclerosis, these cells largely originate in the bloodstream and therefore must bind to and cross an activated endothelium expressing adhesion molecules to reach the heart in a targeted way.

Endothelitis and endothelial dysfunction

Endothelial dysfunction is one end-result of the phenotypic changes associated with endothelitis. Although there are very many dysfunctional microvascular changes associated with endothelitis, the best characterised is an impairment of endothelium - dependent nitric oxide (NO)- mediated vasodilatation. In healthy vessels, increased shear stress triggers flow-mediated vasodilatation due to increased synthesis and release of NO, the principal regulator of vascular tone, that acts on underlying smooth muscle to relax blood vessels. In patients with diabetes, vaso-relaxation is significantly impaired or even paradoxically reversed. This is partly due to impaired formation of NO due to uncoupling of endothelial nitric oxide synthase and a decrease in tetrahydrobiopterin and L-arginine, the co-factor and the substrate, respectively, for NO synthesis. At the same time, the bioavailability of NO is reduced due to quenching by ROS and reactive dicarbonyls. In addition, elevated levels of asymmetric dimethylarginine, function as an endogenous competitive inhibitor of NO activity.

Endothelial dysfunction can be measured using invasive tests by selective infusion of acetylcholine into the epicardial coronary arteries. However, it is more commonly estimated using non-invasive testing, including flow-mediated dilation (FMD), low-flow-mediated constriction of the brachial artery measured by ultrasound, and peripheral arterial tonometry (EndoPAT) using the finger pulse wave amplitude in response to reactive hyperaemia. More recently, changes in endothelial-mediated blood flow can be identified using monitored by positron emission tomography. Each of these non-invasive are correlated with the results of invasive testing as well as future cardiac outcomes. However, each phenomenon is only partly NO determined. Moreover, none have a clear place in guiding treatment or prognosis in the clinic.

Compromised endothelium - dependent vasodilatation is thought to be an important contributor to increased myocardial ischemia in diabetes. In particular, myocardial ischemia in individuals with relatively normal epicardial coronary arteries (also known as microvascular angina) appears to be partly driven by impaired

endothelium-mediated vasomotion in the cardiac microvasculature. For example, 10-year data from the Women's Ischemia Syndrome Evaluation (WISE) study, found that half of individuals undergoing angiography for investigation of chest pain, but found not to have obstructive coronary disease, had endothelial dysfunction[11], and those that did had worse clinical outcomes. This is not simply an epiphenomenon, as abnormal vasomotion may also be observed before the development of atherosclerotic lesions or overt cardiovascular disease. For example, in individuals at moderate CV risk but without macrovascular disease, endothelial dysfunction is associated with an increased incidence of CV events[12].

The role of microvascular ischemia/hypoxia in the pathogenesis of diabetic heart failure remains controversial. While there is a clear association between endothelial dysfunction and diastolic dysfunction in heart disease, this may simply be because of common risk factors (*e.g.*, diabetes) rather than a linked pathogenesis. Certainly, reduced oxygen delivery impairs myocyte relaxation, especially in the setting of increased oxygen demands associated with increased intramyocardial tension. Chronic ischemia may lead to subclinical micro-scars replacing small foci of dead cardiomyocytes, that cumulatively result in myocardial stiffening. Endothelium-mediated control of the venous vascular tone is also important for the regulation of cardiac filling pressures.

CONSEQUENCES OF ENDOTHELITIS

Endothelitis and increased vascular permeability

The best-known pathophysiological change associated with microvascular dysfunction in diabetes is the accompanying increase in vascular permeability. The healthy endothelium provides an effective semipermeable barrier that prevents the exudation of plasma contents despite high intravascular pressures. In hyperglycaemia, increased permeability and loss of endothelial barrier function, results in extravasation of circulating elements (*e.g.*, large molecular weight proteins, lipoprotein particles, clotting factors) into the interstitial space and tissue oedema. This is simply observed as hard exudates in the retina or with increased albuminuria, both of which are strongly associated with and increased risk of cardiovascular events and heart failure. This is because impaired barrier functions in one site predict endothelial dysfunction at other sites (the so called “Steno hypothesis”).

Increased vascular permeability in diabetes is likely the functional end result of multiple pathophysiological changes in the endothelium including thinning and changes in the composition of the surface glycocalyx, rearrangements of cell-to-cell junctions (paracellular trafficking) and altered vesicular trafficking (transcytosis) associated with endothelitis. One of the most important regulators of barrier function is considered to be Vascular Endothelial Growth Factor (VEGF). The induction of VEGF in diabetes directly increases vascular permeability[13]. Outside of diabetes (*e.g.*, in sepsis), loss of barrier function may also be partly VEGF-dependent.

Endothelitis, thrombo-resistance and fibrinolysis

A healthy endothelium creating an anticoagulant surface for blood flow that inhibits the formation of intraluminal clots (known as thromboresistance). By contrast, an inflamed endothelium is thrombogenic in a number of different ways. For example, endothelial dysfunction is associated with a reduction in expression of the membrane bound anticoagulant glycoprotein, thrombomodulin. At the same time, the release of soluble thrombomodulin is increased, leading to increased circulating levels in patients with diabetes, especially those with vascular complications. Indeed, soluble thrombomodulin levels in diabetes closely correlate with other markers of endothelitis, including circulating cytokines, oxidative stress markers, vascular permeability (*e.g.*, albuminuria, retinal exudates) and impaired FMD. At the same time, an inflamed endothelium also liberates thrombogenic molecules including plasma factor VIII, von Willebrand factor, fibronectin, inhibitor of plasminogen activator type 1, and thrombospondin. Platelet aggregation is also enhanced in diabetes. Although a healthy endothelium produces anti-aggregants, including such as NO and PGI2, to attenuate this process as a negative feedback mechanism. However, both are reduced in the setting of endothelitis.

Endothelitis and vascular rarefaction

Compounding the obvious tissue hypoxia associated with diabetes, there is often a reduction in microvascular density (known as rarefaction or capillary dropout) that is

triggered by endothelitis[14]. This phenomenon has been best described in the diabetic retina, where rarefaction and pericyte dropout is thought to be a key driver of tissue hypoxia and subsequent problematic neo-angiogenesis. However, in the diabetic heart as well, endothelitis also leads to capillary rarefaction, associated with decreased contractility and increased left ventricular wall stiffness[8]. Autopsy studies in individuals specifically with heart failure also demonstrate reduced microvascular density in the heart alongside more severe fibrosis when compared to healthy controls[15,16]. Equally in hypertension, the density of microvascular networks in the heart is reduced[17]. In each case rarefaction is thought to be partly driven by inflammatory changes that lead to an imbalance between inadequate angiogenesis/regeneration and augmented vascular destruction/regression due to apoptosis and/or senescence.

Endothelial senescence is an irreversible phenotypic transition that leads to cell-cycle arrest. It can be initiated by inflammation (*e.g.*, activation of NF-κB) as well as result in a pro-inflammatory pro-atherosclerotic, and prothrombotic state characterised by increased cytokine production and expression of adhesion molecules (*i.e.*, endothelitis) which in turn leads to even more senescence[18]. Exposure to high glucose levels also triggers endothelial senescence[13]. This is probably mediated on a number of levels including the induction of cellular/DNA damage by reactive intermediates (*e.g.*, ROS, dicarbonyls), telomere shortening, mitochondrial damage, replicative cell turnover, and epigenetic reprogramming including the persistent activation of activation of NF-κB[19].

Endothelial senescence is thought to contribute to age-related vascular dysfunction and myocardial hypoxia partly by impairing the repair capacity of the endothelial lining, leading ultimately to microvascular rarefaction. Inflammatory changes associated with senescence also potentially contribute to atherosclerosis, thrombophilia and plaque instability. In addition, recent studies also suggest endothelial senescence may be involved in heart failure. For example, in an accelerated model of ageing triggered by augmented endothelial senescence, both diastolic and endothelial dysfunction are also increased[20]. Moreover, knocking-out endothelial *p53* prevents endothelial senescence as well as myocardial fibrosis induced following pressure overload in mice[21].

Senescence ultimately ends with programmed cell death (apoptosis) of endothelial cells. Whether this is directly triggered by senescence or indirectly due to exposure to inflammation and oxidative damage in senescent cells is unclear. Of course, apoptosis can also be triggered independent of senescence, particularly associated with endothelitis triggered by glucotoxicity, shear stress, angiotensin II, cholesterol and oxidative stress. Increased endothelial cell apoptosis is observed in the endothelium covering unstable atherosclerotic plaques and in atherosclerosis-prone regions of the vasculature[16]. Apoptosis not only leads to disruption of the endothelial barrier with leak of plasma proteins and exposure of a thrombogenic subendothelial matrix. Apoptotic endothelial cells are themselves pro-coagulant and pro-adhesive for leukocytes and platelets. Moreover, products released from apoptotic cells including DNA, ATP, microRNA both free and within micro-vesicles derived from cell fragmentation communicate endothelial dysfunction to other cells[22]. Quantitative measurement of these microparticles may be a useful biomarker for endothelial damage, as well as their response to treatment.

ENDOTHELIUM, MYOCARDIUM AND VASCULATURE

Endothelitis and EndoMT

The central players in cardiac fibrogenesis are myofibroblasts (Figure 2). These can be derived from the activation of local fibroblasts and the recruitment of circulating precursors. In addition, inflamed endothelial cells are able to transform to become myofibroblasts *via* a process known as endothelial-mesenchymal transition (EndoMT)[23]. Indeed, to 27%-35 % of the total pool of cardiac fibroblasts during cardiac fibrosis may be derived from endothelial cells[24]. This TGF-β1-dependent transformation and their subsequent detachment and migration into the cardiac interstitium leads to the generation of increased amounts of extracellular matrix proteins, contributing to diastolic stiffening. In addition, products released from active myofibroblasts have paracrine actions on cardiomyocyte to induce apoptosis[25]. Augmented EndoMT has been documented in the diabetic kidney and retina, where they contribute to increased tissue fibrosis[26,27]. Little is known about the role of endoMT in the diabetic heart although the same factors that promote endoMT at other

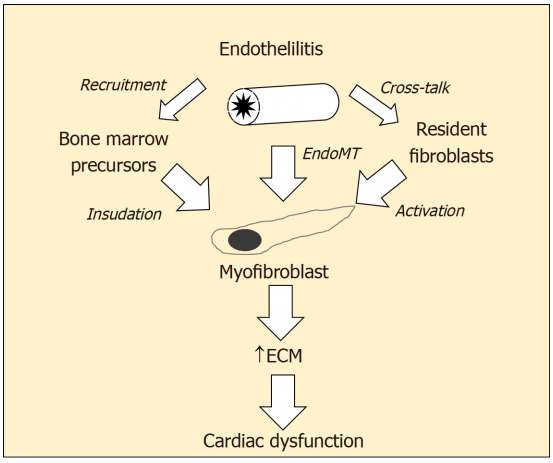

Figure 2 Myofibroblasts the central players in cardiac fibrogenesis. ECM: Extracellular matrix; EndoMT: Endothelial-mesenchymal transition; EPC: Endothelial progenitor cell.

sites (*e.g.*, TGF β-Smad3) are also increased in the diabetic myocardium as the content of cardiac myofibroblasts is also increased.

Endothelitis and endothelial progenitor cells

Circulating endothelial progenitor cells (EPCs) normally participate in the process of new blood vessel formation and vascular repair. In the setting of cardiac ischaemia, the mobilisation of EPCs is thought to contribute to optimal remodelling. Elevated number and the function of EPCs is generally correlated with better endothelial function. Indeed, EPCs have been suggested as useful additional marker of endothelial function. In diabetes, functionality of EPCs is weakened, including impaired migration, mobilization, adhesion and homing, reduced ability to proliferate, differentiate and survive, leading to reduced reparative capacity. In addition, EPCs numbers are paradoxically normal or sometime s reduced. In particular, EPC numbers also fall in patients with diabetic kidney disease, especially with increasing albuminuria or reducing renal function.

Cross talk between endothelium and cardiomyocytes

The endothelium not only regulates and redistributes regional blood flow. Endothelial cells also have significant paracrine effects to regulate regional function. For example, it is well established that vascular smooth muscle function is modified through the altered production of vasoactive substances by endothelial cells (*e.g.*, NO). In addition, in the heart, diffusible factors released from the coronary and endocardial endothelium also modulate underlying cardiomyocyte contractility and remodelling. These factors include NO, prostaglandins, endothelins, interleukins, and other "*angiocrine*" small-molecules[28]. For example, NO released from the endothelium stimulates an earlier onset of relaxation favouring diastolic filling[29]. In co-culture experiments, endothelial cells modulate the contraction and relaxation of cardiomyocytes, and this is substantially altered by the induction of endothelial inflammation (*e.g.*, pre-exposure to TNF-α or interleukin-1β). Consequently, the impact of impaired NO generation associated with endothelial dysfunction extends beyond simply changes in local blood flow to real-time cardiac dynamics.

The paracrine signals emerging from endothelitis also alters the perivascular environment to activate pathways that ultimately converge on converge on myocardial fibrosis. A quiescent endothelium releases factors that suppress fibrogenesis including bone morphogenetic protein 9 (BMP9), while an activated endothelium releases pro-fibrotic cytokines including TGF-β and IL-33, MCP-1, endothelin-1 (ET-1). In addition, paracrine and autocrine signalling of the renin-angiotensin-aldosterone system (RAAS), and its primary mediator Ang II, are known to have a direct influence on the progression of the atherosclerotic process[30], reactive ventricular hypertrophy, and myocardial fibrosis. Diabetes is classically associated with activation of the cardiac RAAS, at least partly through the development of endothelitis. Moreover, blockade of the RAAS in the setting of diabetes, using ACE inhibitors angiotensin receptor blockers or mineralocorticoid receptor blockade, has clear effects on vascular remodelling and myocardial fibrosis, over and above their effects on blood pressure.

The beating heart has only a limited capacity for energy substrate synthesis or storage, relying instead on blood flow and transit across the endothelium for its energy needs. Changes in metabolic status (*e.g.*, feeding or fasting state) and therefore optimal substrate utilisation are communicated both to and from the vascular endothelium. Beyond regulating blood flow and the trans-endothelial supply of metabolic substrates, paracrine signals from the endothelium also have a major impact on cardiac metabolism, including NO, insulinotropic factors, growth factors and enzymes[4]. In addition, endothelial lipase (EL) activity appears to play an important role in liberating free fatty acids from high density lipoproteins to be used in cardiac metabolism, as genetic depletion of endothelial lipase results in heart failure due to reduced fatty acid uptake in the heart[31]. In cardiac hypertrophy, EL activity is increased, possibly to facilitate increased fat supply to the myocardium. Serum EL concentrations in human plasma are associated with circulating inflammatory markers, while plasma levels are increased in diabetes, heart failure and atherosclerosis. This increase in circulating EL may partly reflect the loss from endothelial sites associated with endothelitis that modulates cardiac metabolism and contributes in the long term to dysfunction.

CONNECTING THE DOTS FROM THE LAB TO THE CLINIC PATIENT A FOCUS ON HFPEF

HFpEF is an evolving syndrome where observations have been predominately clinical and some pathophysiological connect. In the transition from Diabetes to HFpEF, knowing the canvas is large and will advance, we highlight 5 areas relevant to our regional context that the authors feel are worth exploring. Creating a clinical link for DM, DHD and finally HFpEF will be difficult, highlights the complexity of the syndrome, but is critical as more elements of the basic science map are revealed. In a recent review we discussed the importance of the primary step of confirming the diagnosis CHF, with solid proof, *i.e.* elevation of left ventricular end diastolic pressure (LVEDP). A rise in LVEDP in the absence of systolic impairment, is then the result of a complex interplay of diastolic phase that is unable to contribute (is impaired/ or failing) to overall cardiac output directly or through adequate counterregulatory compensation or the lack of it (dysfunction). A direction to approach this interplay has been published and we refer readers to Figure 1 in reference 2. From this complex canvas we extract several clinical observations and areas we feel relevant for further study (Figure 3). We break this down in an inflammatory context:

Inflammation: Being selective and finding where to focus in this vast area is important. For example, chronic sterile inflammation has key pathways triggered by dying cells such as IL-1α[32], suggests opportunities exist for identifying other key pathways.

Clinical scenario: (1) Acute Decompensated Heart Failure (ADHF) – is the drawing board to for clinical studies. Often patients present with CHF and are discharged after a short stay. Trials have predominately focused on relieving congestion. The future landscape should be greater forensic interrogation of molecular and clinical observations; (2) Risk factors – pregnancy and future risk of HFpEF, hypertension and metabolic syndromes suggest a haemodynamic connect. Aging is associated diastolic impairment on echocardiography although the clinical manifestation varies suggesting a more complex interaction[33,34]; (3) Obesity (flux) – is difficult to determine if it's a risk factor, association, driver of cardiac decompensation (ADHF) or a combination. However rapid changes in weight in either direction can provide exponential clinical changes in symptoms; and (4) Secondary endorgan – Chronic renal impairment (CRI) and retention of protein bound uremic toxins (PBUT), which have robust pro-inflammatory properties but have been relatively immune to treatments[35].

Tissue/systems: Four arms the mechanical, cytoskeletal (structure), conduction and heart, the interconnectivity (hemodynamical and molecular) shape the clinical picture of HFpEF.

Pathophysiological changes: When tissue and systems are exposed to disease or altered state a series of changes occur that is initially transient then becomes permanent. This remodelling leads to heart failure but also feedback loops. In time an equilibrium sets in that changes baseline levels *e.g.*, biomarkers like natriuretic peptides.

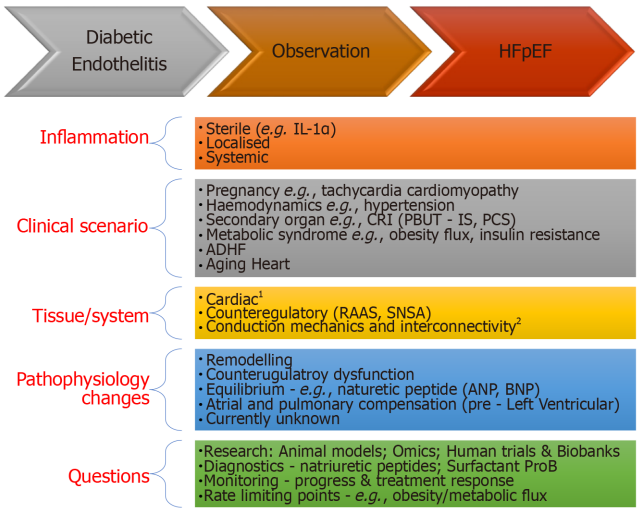

Figure 3 Theoretical bench to bedside flow chart for some commonly encountered clinical scenarios. [1]Cytoskeleton (*e.g.*, Rho kinase activity), contractile apparatus (*e.g.*, Titin phosphorylation), mitochondrial energetics, AGE. [2]Ventricular atrial coupling; Microvasculature; Pericardial restraint; Chronotropic reserve. ADHF: Acute decompensated heart failure; AGE: Advanced glycation end products; ANP: Atrial natriuretic peptide; BNP: Brain natriuretic peptide; IL-1α: Interleukin 1 alpha; CRI: Chronic renal impairment/insufficiency; IS: Indoxyl sulphate; PBUT: Protein bound uremic toxins; PCS: P-cresyl sulphate; RAAS: Renin angiotensin aldosterone syndrome; SNSA: Sympathetic nervous system activity.

Questions: Based on points 1-4 here we need to devise how we examine these areas. Four pressing areas are needed: Firstly – a basic science program including animal models, omics and biobanks to that have close relationship with clinician and scientists; Secondly are diagnostic biomarkers, by understanding disease baseline and changes when under stress to help with bedside stress testing diagnosis and monitoring treatment response[30]; Finally as there are so more causative confounders and counterregulatory pathways identifying key pathways will help focus clinical questions.

In summary while the above list is superficial however the idea of creating flow loops is vital as HFpEF remains a syndrome at its infancy. Much is not known and observations and idea are still welcome additions to this science[36-38].

CONCLUSION

There is an undeniable link between systemic inflammation and primary metabolic comorbidities including diabetes, obesity, obstructive sleep apnoea and secondary associations including aging, hypertension, lifestyle. These are all associated with endothelitis and the resulting vascular dysfunction is a "common soil" for atherogenesis and cardiac failure[39,40]. The cellular mechanism for inflammation in the subendothelial space that leads to atherogenesis and the inflammatory changes in the failing heart begins with (1) Dysfunctional endothelial changes that facilitate the recruitment of inflammatory cells; (2) Furthermore, increased barrier permeability, reduced thromboresistance, and altered paracrine signalling associated with endothelitis contributes to and compounds adverse vascular and cardiac remodelling in diabetes; and (3) In so far as, endothelitis is a driving force of progressive cardiac dysfunction in diabetes, then targeting microvascular dysfunction should provide benefits for patients with diabetes. The bench to bedside link can be seen when optimal glucose and blood pressure control have microvascular as well as macrovascular benefits, albeit largely when instituted early in the course of disease and continued for long periods of time (> 10 years). In addition, blockade of the RAAS in patients with a high cardiovascular risk or CHF is unequivocally associated with improved clinical outcomes, independent of blood pressure reduction, and over short trial intervals. The MICRO-HOPE study, treatment with ramipril was associated with a reduction in CHF hospitalisation and major acute coronary events. With increasing understanding of "diabetic endothelitis" and its contribution to HFpEF specifically, in the future a more direct targeting of endothelial dysfunction with therapies including anti-inflammatory therapies, oral nitrite/nitrate, guanylyl cyclase activators and phosphodiesterase inhibitors may prove useful. An improved understanding of these

sciences will also allow opportunities to advance clinical and pathophysiological understanding; and an improved ability to clinically correlate haemodynamic observations to monitor progress perhaps with novel diagnostic biomarkers.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Impact of cardiologist intervention on guideline-directed use of statin therapy

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Abstract

BACKGROUND

Statins have an important and well-established role in the prevention of atherosclerotic cardiovascular disease (ASCVD). However, several studies have reported widespread underuse of statins in various practice settings and populations. Review of relevant literature reveals opportunities for improvement in the implementation of guideline-directed statin therapy (GDST).

AIM

To examine the impact of cardiologist intervention on the use of GDST in the ambulatory setting.

METHODS

Patients with at least one encounter at the adult Internal Medicine Clinic (IMC) and/or Cardiology Clinic (CC), who had an available serum cholesterol test performed, were evaluated. The 2 comparison groups were defined as: (1) Patients only seen by IMC; and (2) Patients seen by both IMC and CC. Patients were excluded if variables needed for calculation of ASCVD risk scores were lacking, and if demographic information lacked guideline-directed treatment recommendations. Data were analyzed using student *t*-tests or χ^2, as appropriate.

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Analysis of Variance was used to compare rates of adherence to GDST.

RESULTS

A total of 268 patients met the inclusion criteria for this study; 211 in the IMC group and 57 in the IMC-CC group. Overall, 56% of patients were female, mean age 56 years (± 10.65, SD), 22% Black or African American, 56% Hispanic/Latino, 14% had clinical ASCVD, 13% current smokers, 66% diabetic and 63% hypertensive. Statin use was observed in 55% (n = 147/268) of the entire patient cohort. In the IMC-CC group, 73.6% (n = 42/57) of patients were prescribed statin therapy compared to 50.7% (n = 107/211) of patients in the IMC group (P = 0.002). In terms of appropriate statin use based on guidelines, there was no statistical difference between groups [IMC-CC group 61.4% (n = 35/57) *vs* IMC group, 55.5% (n = 117/211), P = 0.421]. Patients in the IMC-CC group were older, had more cardiac risk factors and had higher proportions of non-white patients compared to the IMC group (P < 0.02, all).

CONCLUSION

Although overall use of GDST was suboptimal, there was no statistical difference in appropriate statin use based on guidelines between groups managed by general internists alone or co-managed with a cardiologist. These findings highlight the need to design and implement strategies to improve adherence rates to GDST across all specialties.

Key words: Statin use; Guideline directed statin therapy; Cardiologist; Ambulatory care; Adherence

Core tip: Statins have an important and well-established role in the prevention of atherosclerotic cardiovascular disease. However, several studies have reported widespread underuse of statins in various practice settings and populations. Review of relevant literature reveals opportunities for improvement in the implementation of guideline-directed statin therapy (GDST). We aimed to examine the impact of cardiologist intervention on adherence to GDST in the ambulatory setting. Our evaluation shows that although overall adherence to GDST was suboptimal, there was no statistical difference in appropriate statin use based on guidelines between groups managed by general internists alone or co-managed with a cardiologist. These findings highlight the need to design and implement strategies to improve adherence rates to GDST across all specialties.

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INTRODUCTION

Statins have an important and well-established role in the prevention of atherosclerotic cardiovascular disease (ASCVD). Large-scale clinical trials have shown that statins substantially reduce cardiovascular morbidity and mortality in both primary and secondary prevention. The American College of Cardiology/American Heart Association (ACC/AHA) Guideline on the Treatment of Blood Cholesterol to Reduce ASCVD Risk in Adults emphasizes identifying and treating individuals at the highest risk for developing ASCVD with statins[1,2]. However, despite mounting evidence supporting its use, several studies have reported widespread underuse of statins in the ambulatory setting[3], in secondary prevention[4], and more frequently among women[5], older adults, Blacks, Hispanics/Latinos, and those who are under/un-insured[6]. Early skepticism of the feasibility of implementing the ACC/AHA guidelines in a patient population has been documented. One report found 56% predicted prescriber adherence to those guidelines in a retrospective simulated

analysis of a large academic medical practice[6]. In a more recent study, one-third of patients with ASCVD and almost one-half of patients without ASCVD were not receiving guideline recommended moderate- to high-intensity statin therapy in cardiology practices after the publication of the 2013 ACC/AHA guideline[1]. The most recent cholesterol 2018 guidelines from ACC/AHA continue to emphasize the use of statins as a primary treatment modality for eligible patients to achieve appropriate low-density lipoprotein cholesterol (LDL-C) reduction[2].

Limited information is available regarding the appropriate implementation of the cholesterol guidelines as they pertain to evidence-based statin use. The objective of our study was to examine physician adherence to GDST in the ambulatory setting across multiple subgroups of patients and determine the impact of cardiologist intervention on GDST.

MATERIALS AND METHODS

Design and sample

A retrospective chart review was conducted of patients who had at least one encounter at the adult Internal Medicine Clinic (IMC) and/or Cardiology Clinic (CC) at our community tertiary care teaching hospital from May 2016 to April 2017 and who had an available serum cholesterol test performed. Patients were excluded if the following biometric variables needed for calculation of ASCVD risk score were unavailable: age, sex, race, systolic blood pressure (SBP), total cholesterol (TC), LDL-C, high density lipoprotein cholesterol (HDL-C), history of diabetes mellitus (DM), smoking status, and hypertension treatment status. In addition, patients whose demographics lacked guideline directed treatment recommendations were excluded (*e.g.*, age < 40 or > 79 years). The 2 comparison groups were defined as: (1) Patients only seen by IMC; and (2) Patients seen by both IMC and CC.

Definitions

The presence of clinical ASCVD included history of acute coronary syndrome, history of myocardial infarction, stable or unstable angina, coronary or other arterial revascularization, stroke, transient ischemic attack, or peripheral artery disease. Moderate- and high-intensity statin was defined by statin dose that would lower LDL-C on average by 30%-49% and ≥ 50% with daily dosing, respectively. Statin therapy utilized was categorized as appropriate or not appropriate GDST. As such, the presence of clinical ASCVD or LDL-C greater than 190 mg/dL, would indicate use of high-intensity satin (atorvastatin 40–80 mg or equivalent)[1]. For primary prevention, the presence of a diagnosis of DM and/or ASCVD risk score > 7.5% would indicate the use of at least a moderate-intensity statin (atorvastatin 10-20 mg or equivalent) depending on patient tolerance[1].

Data collection and assessment

Data collected included date of visit, date of lipid panel referenced, gender, age, body mass index, race/ethnicity, TC, HDL-C, SBP, hypertension and its treatment status, presence of clinically diagnosed DM, smoking status, history of ASCVD, clinically diagnosed hyperlipidemia and current statin use including type and dose/intensity. Data were entered into the ASCVD risk calculator, with 10-Year and lifetime ASCVD risk recorded. Treatment assigned by the physician at the last clinic visit was then compared to guideline recommended treatment, and labeled as appropriate or inappropriate. If a patient's LDL-C was greater than 190 mg/dL or if they had clinical ASCVD, then they would be eligible for high-intensity statin and use of the ASCVD risk calculator was not indicated and therefore not calculated, as per guideline recommendations.

Statistical analysis

Descriptive statistics are presented as mean ± SD or number and percent. Baseline characteristics of subgroups were compared using student *t*-tests or χ^2 tests, as appropriate. χ^2 test analysis was used to analyze statistical significance between groups. Analysis of variance was used to test the statistical difference between means of continuous variables. Statistical significance was defined as $P < 0.05$. All statistical analyses were performed using SPSS version 26.0 (SPSS™ Inc., Chicago, IL, United States).

RESULTS

A total of 268 patients met the inclusion criteria for this study. Table 1 describes the demographic characteristics of the study population, 56% were female, mean age was 56 years (± 10.65, SD), 22% identified as Black or African American and 56% identified as Hispanic/Latino. Approximately 14% of the cohort had clinical ASCVD as previously defined, 13% were current smokers, 66% were diabetic, and 63% were hypertensive. Statin use was observed in 55% of the entire cohort, with moderate-intensity statins being the most commonly prescribed.

Of the total 268 patients, 211 and 57 patients were in the IMC only and IMC-CC group, respectively (Table 2). Overall, in the IMC-CC group, 73.6% (n = 42/57) of patients were prescribed statin therapy compared to 50.7% (n = 107/211) of patients in the IMC group (P = 0.002). In terms of appropriate statin use based on guidelines, there was no statistical difference between groups [IMC-CC group 61.4% (n = 35/57) *vs* IMC group, 55.5% (n = 117/211), P = 0.421]. Patients in the IMC-CC group had significantly higher cardiac risk as compared to the IMC group: Clinical ASCVD history (35.1% *vs* 18%, P < 0.001), diabetes (47.3% *vs* 30.3%, P = 0.016), hypertension (80.7% *vs* 59.2%, P = 0.003) and smoking history (47.4% *vs* 26.1%, P = 0.002). Patients in the IMC-CC group were significantly older (mean age 62.1 years *vs* 55.5 years, P < 0.001) and had a higher proportion of non-white patients (49.6% *vs* 29.4%, P = 0.021) compared to the IMC group. Mean LDL-C and TC levels were lower in the IMC-CC group *vs* the IMC group (mean LDL-C, 110.36 mg/dL *vs* 123.98 mg/dL, P = 0.013) and (TC, 187.67 mg/dL *vs* 204.13, P = 0.018).

DISCUSSION

In our evaluation of real-world cholesterol management, we found that adherence to GDST by physicians occurred in about half the patients eligible for statin therapy. The 2013 ACC/AHA Guideline on the Treatment of Blood Cholesterol to Reduce ASCVD Risk in Adults (which were the latest available guidelines during our study period) recommends statins as first-line lipid lowering therapy for both primary and secondary prevention[7]. Furthermore, data from the National Health and Nutrition Examination Surveys estimated an increase in statin-eligible patients by 2.4 million, 2.2 million, and 8.2 million in patients with ASCVD, diabetes, and in primary prevention, respectively[8]. Given recent shifts in the cholesterol treatment paradigm from the older ATP III guidelines[9], Schoen *et al*[6] predicted 56% adherence with the 2013 blood cholesterol guidelines in a retrospective analysis. In another study, Pokharel *et al*[7] evaluated trends in the use of statin therapy and non-statin therapy in cardiology practices, before and after the publication of the 2013 ACC/AHA guidelines. They found modest, yet significant increases in the use of moderate- to high-intensity statins in ASCVD patients and no change in the other statin benefit groups and nearly half of statin eligible patients did not receive a statin[7]. By comparison, our real-world study demonstrated similar trends in that nearly half of statin eligible patients did not receive a statin. Furthermore, adherence to GDST by general internists tended to be lower (55.5%) when compared to patients who were also being managed by a cardiologist (61.4%), although this difference was not statistically significant. Hence, being evaluated by a cardiology specialist did not appear to have any additional impact on the use of GDST at our institution.

The literature also notes that different factors and patient characteristics affect implementation and use of GDST. Schoen *et al*[10] found that women and patients with diabetes were less likely to be treated optimally; and this in turn, could impact cardiovascular outcomes. In our study, there was a higher proportion of patients with clinical ASCVD and diabetes that were seen by the cardiologist. These patients also had lipid profiles that necessitated therapy. This may suggest that at our institution, higher risk patients needing further LDL-C reduction are appropriately being managed by cardiology specialists who are trained to address these complex situations. Furthermore, patients who were managed by the cardiologist achieved significantly lower LDL-C levels, which may translate to greater reduction in future coronary heart disease events[11]. Further long-term studies of these patients may shed light on such outcomes.

Several other studies have reported similar trends in 2013 ACC/AHA guideline implementation, however, very little is known about the barriers to adherence. Clough *et al*[12] found that although community-based physicians often accurately estimated risk, beliefs and approach to statin discussion varied and these variables had minimal

Table 1 Patient Clinic demographics, *n* = 268, *n* (%)

Patient characteristics	Data
Age (yr), mean	56 (± 10.65, SD)
Sex	
Male	118 (44)
Female	150 (56)
Race	
White	180 (67.2)
Black	60 (22.2)
Other	28 (10.4)
Ethnicity	
Hispanic/Latin-o,-a	151 (56.3)
Smoker	
Never	184 (68.7)
Former	48 (17.9)
Current	34 (12.7)
Unknown	2 (0.7)
ASCVD	38 (14.2)
mean ASCVD score	12.1%
Diabetes	177 (66)
Hypertension	171 (63.8)
Statin prescribed	149 (55)
Low	11 (4.1)
Moderate	82 (30.6)
High	56 (20.9)
Clinical laboratory profile	
TC (mg/dL), mean	200.63 (46.7, SD)
LDL-C (mg/dL), mean	121.08 (36.9, SD)
HDL-C (mg/dL), mean	51.17 (15.2, SD)
SBP (mmHg), mean	132 (19.2, SD)

TC: Total cholesterol; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol; SBP: Systolic blood pressure.

impact on low rates of statin prescribing. An inter-professional approach using the patient-centered medical home model made no difference in guideline implementation within a primary care practice[12]. It has been well established that it may take up to 17 years for evidence to be fully implemented into practice, which may explain the low statin use in the overall study cohort[13]. More studies will need to be conducted to fully understand these barriers. However, a recent study has shown that since the publication of the 2013 ACC/AHA guidelines (and subsequent 2018 guidelines), cholesterol levels and statin use have improved in the US[14]. Future studies should be conducted to evaluate the long-term impact of the latest cholesterol guidelines on adherence to GDST[15].

Our study has several limitations including the short period of analysis, its retrospective design, and small sample size. As noted, studies have demonstrated that it may take up to 17 years for evidence to be fully implemented into practice[13], and therefore the period of analysis for our study [which occurred within 3 years (2016 to 2017) following guideline publication], may simply reflect the lag time between guideline publication and implementation. Furthermore, our study relied on the

Table 2 Adherence to guideline-directed statin therapy and patient characteristics by group, *n* (%)

All patients (*n* = 268)	IMC only (*n* = 211)	IMC/CC (*n* = 57)	*P* value
Statin prescribed	107 (50.7)	42 (73)	0.002
Appropriate intensity statin prescribed	117 (55.5)	35 (61.4)	0.421
Distribution of population			
Age(years), mean	55.47 (± 10.11, SD)	62.14 (± 11.08, SD)	< 0.001
ASCVD	18(8.5)	20 (35.1)	< 0.001
DM	64 (30.3)	27 (47.3)	0.016
Hypertension	125(59.2)	46 (80.70)	0.003
Smoking history (current and former)	55 (26.1)	27 (47.4)	0.002
Non-white	62 (29.4)	26 (45.6)	0.021
Hispanic, Latino	127 (60.2)	24 (42.1)	0.015
LDL-C (mg/dL), mean	123.98 (± 34.77, SD)	110.36 (± 42.47, SD)	0.013
TC (mg/dL), mean	204.13 (± 44.96, SD)	187.67 (± 50.77, SD)	0.018
HDL-C (mg/dL), mean	50.4 (± 14.1, SD)	53.9 (± 18.6, SD)	0.126

IMC: Internal Medicine Clinic; CC: Cardiology Clinic; TC: Total cholesterol; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol; SBP: Systolic blood pressure.

accuracy and completeness of physician documentation which may have impacted the determination of physician adherence to guideline recommendations. Our analysis does not account for other reasons why GDST was not implemented (*e.g.*, patient factors, physician attitudes towards prescribing, adverse reactions, and cost of therapy). Additionally, our study did not account for patient adherence to prescriber recommendations. Regardless of statin treatment, we obtained information from the lipid panel which may not have impacted high-risk cohorts but underestimated statin eligibility for other cohorts (*e.g.*, patient previously prescribed statin, but at the time of assessment patient may not have appeared to be eligible for statin therapy based on cholesterol results).

In conclusion, our study compared the use of GDST in patients managed by general internists and those co-managed by a cardiologist. As expected, patients with higher cardiac risk factors and co-morbidities were more likely to be co-managed by a cardiologist and placed on statin therapy. In terms of appropriate statin use based on guidelines, there was no statistical difference in proportion of patients receiving GDST between groups managed by general internists alone or co-managed with a cardiologist; however, there was a significantly greater use of statins in patients co-managed by a cardiologist. Overall, statin use in this population is comparable to what other studies have shown and highlights the need to design and implement strategies to improve prescriber adherence to GDST.

ARTICLE HIGHLIGHTS

Research background

Statins have an important and well-established role in the prevention of atherosclerotic cardiovascular disease (ASCVD). However, several studies have reported widespread underuse of statins in various practice settings and populations.

Research motivation

Review of relevant literature reveals opportunities for improvement in the implementation of guideline-directed statin therapy (GDST).

Research objectives

In this study, we aimed to examine the impact of cardiologist intervention on the use of statin therapy in the ambulatory setting.

Research methods

We conducted a retrospective chart review of patients who had at least one encounter at the adult Internal Medicine Clinic (IMC) and/or Cardiology Clinic (CC) and who had an available serum cholesterol test performed. The 2 comparison groups were defined as: (1) Patients only seen by IMC; and (2) Patients seen by both IMC and CC. Baseline characteristics of subgroups were compared.

Research result

A total of 268 patients met the inclusion criteria for this study. Approximately, 14% had clinical ASCVD, 13% were current smokers, 66% were diabetic, and 63% were hypertensive. Statin use was observed in 55% of the entire cohort, with moderate-intensity statins being the most commonly prescribed. Overall, in the IMC-CC group, 73.6% of patients were prescribed statin therapy compared to 50.7% of patients in the IMC group. There was no statistical difference in the use of GDST between groups.

Research conclusions

Our study compared the use of GDST in patients managed by general internists and those co-managed by a cardiologist. Overall, statin use in this population is comparable to what other studies have shown and highlights the need to design and implement strategies to improve prescriber adherence to GDST.

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META-ANALYSIS

Systematic review and meta-analysis of outcomes of anatomic repair in congenitally corrected transposition of great arteries

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Abstract

BACKGROUND

Treatment of congenitally corrected transposition of great arteries (cc-TGA) with anatomic repair strategy has been considered superior due to restoration of the morphologic left ventricle in the systemic circulation. However, data on long term outcomes are limited to single center reports and include small sample sizes.

AIM

To perform a systematic review and meta-analysis for observational studies reporting outcomes on anatomic repair for cc-TGA.

METHODS

MEDLINE and Scopus databases were queried using predefined criteria for reports published till December 31, 2017. Studies reporting anatomic repair of minimum 5 cc-TGA patients with at least a 2 year follow up were included. Meta-analysis was performed using Comprehensive meta-analysis v3.0 software.

RESULTS

Eight hundred and ninety-five patients underwent anatomic repair with a pooled follow-up of 5457.2 patient-years (PY). Pooled estimate for operative mortality was 8.3% [95% confidence interval (CI): 6.0%-11.4%]. 0.2% (CI: 0.1%-0.4%) patients required mechanical circulatory support postoperatively and 1.7% (CI: 1.1%-2.4%) developed post-operative atrioventricular block requiring a pacemaker. Patients surviving initial surgery had a transplant free survival of 92.5% (CI: 89.5%-95.4%) per 100 PY and a low rate of need for pacemaker (0.3/100 PY; CI: 0.1-0.4). 84.7% patients (CI: 79.6%-89.9%) were found to be in New York Heart Association

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(NYHA) functional class I or II after 100 PY follow up. Total re-intervention rate was 5.3 per 100 PY (CI: 3.8-6.8).

CONCLUSION

Operative mortality with anatomic repair strategy for cc-TGA is high. Despite that, transplant free survival after anatomic repair for cc-TGA patients is highly favorable. Majority of patients maintain NYHA I/II functional class. However, monitoring for burden of re-interventions specific for operation type is very essential.

Key words: Congenitally corrected transposition of great arteries; Anatomic repair; Double switch operation; Atrial switch Rastelli; Hemi-Mustard Rastelli; Atrio-ventricular block

Core tip: This is a systematic review and meta-analysis looking at short- and long-term outcomes with the anatomic repair strategy (double switch or atrial switch Rastelli operation) for patients with congenitally corrected transposition of great arteries. Updated outcomes of operative mortality, long term survival free of transplantation and re-operation/re-intervention rates are provided. We find favorable long-term survival after anatomic repair despite the initial high operative mortality.

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INTRODUCTION

Congenitally corrected transposition of the great arteries (cc-TGA) is an uncommon cardiac defect accounting for less than 0.5% of congenital heart disease[1,2]. Associated anatomic cardiac abnormalities include ventricular septal defects and pulmonary and subpulmonary obstruction, coarctation and Ebstein's anomaly of the tricuspid valve (TV)[1]. Surgical anatomic correction, depending on underlying associated cardiac anomalies, can be obtained with either a double switch operation (Senning/Mustard atrial switch and arterial switch) or an atrial switch (Senning/Mustard) with a Rastelli operation (ventricular septal defect closure with a baffle to the aorta and a right ventricle to pulmonary artery conduit). An additional variation includes a hemi-Mustard with a bidirectional Glenn shunt. The anatomic repairs allow for the left ventricle to become the systemic ventricle. This reduces the long-term deleterious risk of systemic right ventricular (RV) failure, and the propensity for progressive systemic TV insufficiency[3,4].

There are multiple long-term complications that can occur after anatomic repair for cc-TGA. Patients with cc-TGA are prone to developing atrioventricular block (AVB) requiring pacemaker implantation regardless of operative intervention[5,6]. Furthermore, the baffles for systemic and pulmonary venous return have been shown to place patients at long-term risk for the development of atrial arrhythmia, though this risk might be mitigated by having a systemic left ventricle[6,7]. Postoperative baffle leaks and stenoses are an additional complication that can lead to a variety of symptomology including cyanosis, paradoxical embolism, or venous congestion. Finally, the long-term transplant free survival remains unknown without comparison to survival following a physiologic repair strategy[8].

Anatomic repair for cc-TGA is the preferred treatment strategy for many institutions; however, the short and long-term outcomes are mostly limited to single center studies with a limited number of patients. We therefore sought to better delineate the short-term surgical outcomes and long-term risks including need for pacing, baffle complications requiring reintervention, symptoms, and transplant free survival by pooling the data from multiple observational studies.

MATERIALS AND METHODS

MEDLINE and Scopus databases were queried for manuscripts published till December 31, 2017 with the search items "transposition great arteries"; "TGA"; "double switch" and "anatomical repair". All manuscripts reporting outcomes of anatomic repair in cc-TGA patients were considered in the initial review. Final inclusion criteria were a minimum sample size of 5 patients with at least 2 years of follow up. Many centers have published multiple reports of their experience at varying follow up durations. Thus, the most up to date manuscript from each group was selected. Two authors (Chatterjee A and Law MA) independently reviewed all studies considered to ensure no overlap amongst included studies. Figure 1 shows the relevant details of the study selection process.

Full texts for all potentially relevant studies were extracted and examined for alignment with inclusion criteria and verification of outcomes reported. All initially considered studies were discussed formally amongst experienced cardiologists (Chatterjee A, Cribbs MG, Law MA) and a final list was drawn up. Relevant data was then extracted from these manuscripts and reviewed for accuracy by two authors (Law MA, Chatterjee A) independently. Any disagreement was discussed in the group for resolution.

Primary outcome studied was operative mortality of the anatomic repair strategy. We also evaluated immediate operative complications including the need for extra corporeal membrane oxygenation (ECMO) or left ventricular assist devices (LVAD) and AVB requiring a pacemaker. Long term outcomes evaluated were transplant free survival, New York Heart Association (NYHA) class of patients, need for pacemaker, and rate of re-interventions. We also pooled data for development of left ventricular (LV) systolic dysfunction: LV dysfunction was defined as LV ejection fraction < 40% or when reported as moderate or severe.

Comprehensive meta-analysis (version 3; Biostat, Englewood, NJ) software was used to perform the meta-analysis. Short-term outcomes are reported as events (%) and long-term outcomes or reinterventions are reported as events per 100 patient years (PY). Heterogeneity in the data was assessed with the I^2 test (I^2 > 50 and Cochran's Q statistic P value < 0.05 implying significant heterogeneity)[9]. Random effects modelling was used in keeping with the observational nature of the reports included and also heterogeneity. Publication bias was assessed using the standard funnel plot method using standard errors and any corrections assessed using the Duvall and Tweedie *trim and fill* method. Two-tailed P values were used with P < 0.05 implying statistical significance and confidence intervals (CI) were reported at the 95% level. PRISMA guidelines were followed in reporting the meta-analysis results[10].

RESULTS

Twenty-one reports of anatomic repair were included in the final analysis[4,11-30]. Table 1 lists the studies included and the type of anatomic repair used. A total of 895 patients with cc-TGA underwent anatomic repair: The pooled analysis yielded a total follow up of 5457.2 PY. The median/mean age at operation varied from 0.75–11.1 years. Four hundred and thirteen patients underwent the double switch operation (DS) while 482 patients underwent either an atrial switch-Rastelli operation or a hemi-Mustard Glenn-Rastelli operation (ASR). Sixteen studies reported patients with both types of operations; 4 studies reported experience with ASR operations only and 1 study reported only DS outcomes. Fifteen studies reported prevalence of moderate or more tricuspid regurgitation (TR): 22.7% (150/677) of the pooled sample had significant TR. Fifteen studies reported data on pre-existing AVB: This reveals that 15.6% (104/667) patients have a need for pacing even before any surgical repair.

Short term outcomes

A total of 64 patients did not survive to discharge after initial operation giving a pooled estimate for operative mortality of 8.3% (95%CI: 6.0%-11.4%). Figure 2 shows the funnel plot for operative mortality for included studies which would suggest over-reporting of small studies with low operative mortality. Correcting for publication bias would raise the estimate of operative mortality to 10.9% (CI: 7.6%-15.5%). Need for mechanical circulatory support with ECMO / LVAD post operatively was 0.2% (CI: 0.1%-0.4%). Only 1.7% (CI: 1.1%-2.4%) patients developed AVB needing implantation of a permanent pacemaker.

Table 1 Baseline characteristics of included studies

Ref.	Country	Total patients	Double (arterial/atrial) switch	Atrial switch-Rastelli or hemi-Mustard Glenn-Rastelli	Age at operation (yr)	Mean/median follow up (yr)
Ilbawi *et al*[11], 2002	United States	12	2	10	0.75	7.6
Duncan *et al*[12], 2003	United States	46	26	20	2.3	2
Hörer *et al*[13], 2008	Germany	6	0	6	3.5	7
Gaies *et al*[14], 2009	United States	65	35	30	2.2	4.6
Ly *et al*[15], 2009	France	20	20	0	2.2	5
Sharma *et al*[16], 2009	India	68	31	37	5.2	4.9
Lim *et al*[17], 2010	South Korea	44	10	34	-	5.4
Malhotra *et al*[4], 2011	United States	48	23	25	3	4.9
Murtuza *et al*[18], 2011	United Kingdom	113	68	45	3.2	6.9
Hiramatsu *et al*[19], 2012	Japan	90	18	72	6.8	12.5
Sojak *et al*[20], 2012	Netherlands	8	2	6	2.9	4.5
Hoashi *et al*[21], 2013	Japan	47	0	47	5.5	11.6
Bautista-Hernandez *et al*[22], 2014	United States/Spain	106	64	42	1.2	5.2
Hsu *et al*[23], 2015	Taiwan	18	13	5	8.4	5.0
Tocharoenchok *et al*[24], 2016	Thailand	22	0	22	10.9	5.3
Brizard *et al*[25], 2017	Australia	32	27	5	1.9	5.4
De León *et al*[26], 2017	United States	26	16	10	3	10
Hraska *et al*[27], 2017	United States/Germany	63	38	25	1.6	5
Ibrahimiye *et al*[28], 2017	United States	18	14	4	3	5
Marathe *et al*[29], 2017	Australia	12	6	6	2.3	7.2
Zhang *et al*[30], 2017	China	31	0	31	5.4	3.3
		895	413	482	Median age – 3 yr	Median f/u 5.2 yr

Follow up outcomes

Long term outcomes are tabulated in Table 2. In patients surviving the initial operation, mortality (Figure 3) was estimated to be 0.6 per 100 PY (CI: 0.4-0.8). Fourty-eight patients died over the cumulative follow up of 5457.2 PY. In 20 studies that reported need for transplantation, 10 patients underwent heart transplantation giving an estimated overall survival free of death or needing transplant of 92.5% (CI: 89.5%-95.4%) per 100 PY. Thirteen studies reported data on functional class of surviving patients: 84.7% (CI: 79.6%-89.9%) patients were estimated to be in NYHA functional class I or II. Eighteen studies reported data on LV systolic function in long term follow up and LV dysfunction was estimated to happen in 1.7 patients/100 PY (CI: 1.0%-2.4%).

In long term follow up, further need for pacemaker was noted to be 0.3%/100 PY; (CI: 0.1%-0.4%).

Re-interventions

Twenty studies reported data on total re-interventions and the pooled estimate was 5.3 per 100 PY (CI: 3.8-6.8) with incidence of baffle stenosis being estimated to be 1.1 (CI: 0.8%-1.5%)/100 PY. The most frequently needed reoperations/reinterventions were for conduit replacements or rehabilitation (1.5/100 PY; CI: 0.9-2.1).

Table 2 Outcomes of anatomic repair in long term follow up

Variable	No. of studies	Pooled estimate	95%CI
Mortality after initial operation survival (*n*/100 PY)	21	0.6	0.4-0.8
Transplant free survival (%/100 PY)	20	92.5	89.5-95.4
NYHA I/II symptoms (%/100 PY)	13	84.7	79.6-89.9
Left ventricular systolic dysfunction (*n*/100 PY)	19	1.7	1.0-2.4
Left ventricular outflow tract obstruction (*n*/100 PY)	16	0.2	0.1-0.3
Permanent pacemaker	19	0.3	0.1-0.4
Total Reinterventions (*n*/100 PY)	20	5.3	3.8-6.8
Conduit interventions/operations	20	1.5	0.9-2.1
Baffle stenosis/leak	19	1.1	0.8-1.5
Tricuspid valve operations	21	0.2	0.1-0.3

CI: Confidence intervals; PY: Patient years; NYHA: New York Heart Association.

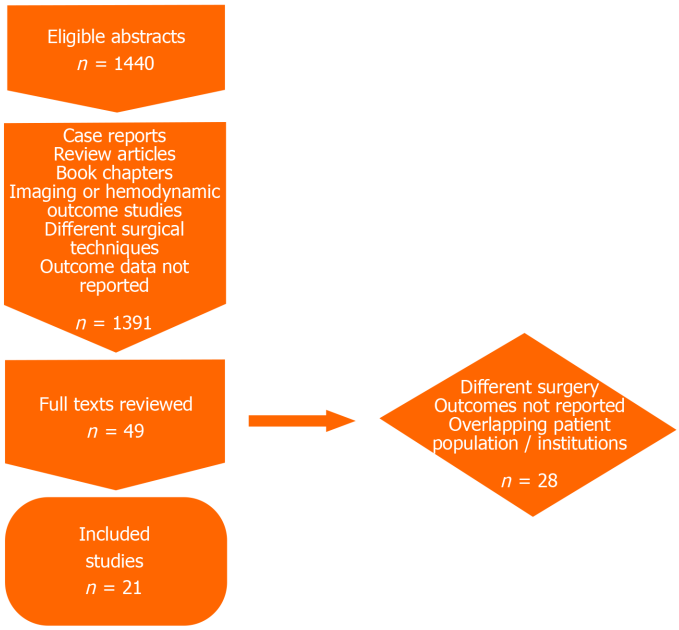

Figure 1 Flowchart depicting study selection process.

DISCUSSION

This is a thorough attempt to use a meta-analysis study design in order to estimate the long-term outcomes with an anatomic repair strategy in this rare congenital heart disease subset. We report pooled outcomes from 895 patients with a sizeable follow up of > 5000 PY. Management patterns for patients with cc-TGA are dictated by variations in anatomic substrate and often by the experience of individual centers[3]. The long-term outcomes with a physiologic repair strategy keeping the right ventricle as the systemic ventricle, although considered safe unmistakably leads to progressive congestive heart failure. Graham *et al*[31] report that 67% patients with ccTGA and associated abnormalities develop congestive heart failure by age 45 and Hraska *et al*[32] estimate the 10-year survival after physiologic repair to be 68% only. With a low follow up mortality of 0.6/100 PY in a very large pooled sample, our results would certainly argue for anatomic repair being a default strategy for patients unless the substrate is not suitable. Most reports of anatomic repair would identify this unsuitable group as older patients whose morphological LV may not be able to sustain systemic pressures or not respond to training with a pulmonary artery band[33]. Other

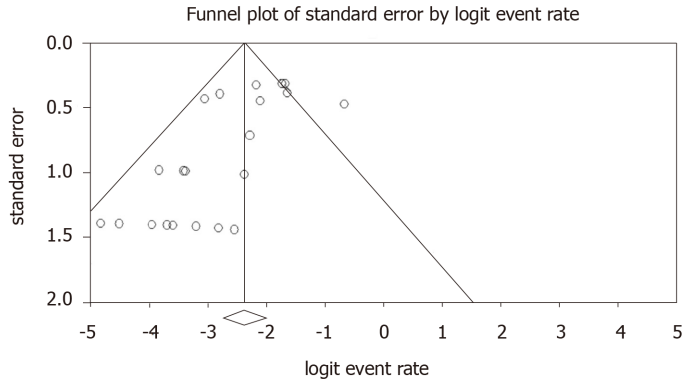

Figure 2 Funnel plot for operative mortality after anatomic repair for congenitally corrected transposition of great arteries showing publication bias to the left of mean.

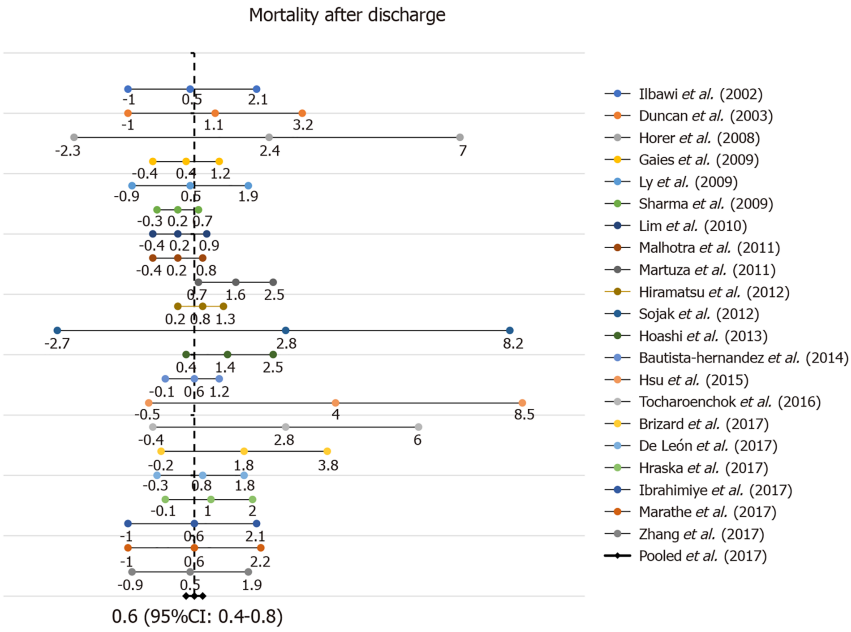

Figure 3 Forest plot for long term mortality after anatomic repair for congenitally corrected transposition of great arteries. CI: Confidence interval.

predictors of poor outcomes after anatomic repair are significant pre-existing TR, RV dysfunction and need for pacing[17,22].

This improved longer term survival outcome over physiological repair needs to be weighed over the apparent short-term safety of only correcting associated abnormalities in a physiologic repair strategy[34]. However, the surgical mortality in physiologic repair population is also not negligible, being reported at 3% in operations done after 1986 in a Mayo Clinic series (16% overall) and 6.7% in the Dutch series by Bogers *et al*[35,36]. The operative mortality in our pooled sample is slightly higher than previously reported estimates[37]. However, this is also a sample with a greater proportion of DS operations versus ASR operations. This is notable since multiple reports have considered the latter to have lower risk of operative mortality[38].

Staged single ventricle palliation with Fontan completion has been advocated as an alternative strategy in the management of cc-TGA[29,39]. The short-term results are comparable in regard to survival and symptomatology, but one must consider that long-term complications are not trivial with many patients experiencing morbid complications. In a study by Dennis *et al*[40], survival of patients was only 80% at the age of 40 years with many patients experiencing arrhythmia, embolic complications, and cardiac failure only after 16 years of age.

AVB in this population is consistently associated with poor outcomes[6]. However,

our study results suggest that operative strategy may not make a huge incremental difference in the development of AVB. The post-operative need for pacing was only 1.7% with the long-term additional need for pacing in another 0.3% patients only.

Re-interventions after anatomic correction for cc-TGA are not rare as corroborated by our analysis. Especially in the ASR cohort, conduit replacements are very common and likely most patients will need at least one exchange to a larger conduit suitable for transcatheter replacement. However, after the first reoperation, multiple transcatheter replacements or rehabilitation procedures can be done avoiding the need for reoperation[41,42].

Baffle complications of residual leaks or stenosis are well described after the Mustard/Senning operations and thus account for the other common reoperations after anatomic repair for cc-TGA[43]. However, in the current era, most of the baffle related complications can be adequately managed with transcatheter techniques minimizing the need for re-operations[44,45]. Furthermore, residual LVOT obstruction is known after both types of anatomic repair[46]. Our analysis shows the incidence of this is low but not completely negligible.

However, the need for re-interventions should also be noted in the context of similar or higher need for reoperations and reinterventions in the physiologic repair strategy. In a sample of 111 children who underwent physiologic repair only, 41% patients required reoperations for conduit exchanges or TV repair/replacement at a mean follow up duration of 11.4 years[36]. Similarly, Bogers *et al*[35] have also reported a re-intervention rate of 32% at 20 years with a physiologic repair.

Limitations

The meta-analysis study design lends itself to certain characteristic limitations. Systematic pooling of observational studies with differences in baseline patient characteristics, varied anatomic substrate, different sample sizes, surgical technique and different follow-up durations make the pooled sample more heterogenous. This is also not a patient level meta-analysis which can overcome some of these limitations. In congenital heart disease literature, reports often consist of small sample sizes and limited follow up, hence pre-disposing to lower than actual pooled estimates when included in a meta-analysis. Since all the studies included are observational, there is also the consideration of under-reporting of outcomes or exclusion of patients with poor outcomes. There is also no way to adequately account for refinement in surgical technique as experience with anatomic repair grew.

Conclusion

This large pooled analysis supports the overall favorable outcomes after anatomic repair for cc-TGA, especially beyond the early initial operative period. Majority of patients have good quality of life, falling in NYHA class I or II. Despite increase in the complexity of repair, there does not seem to be a large increase in the prevalence of heart block. Re-interventions are required but can be accomplished in many situations with transcatheter techniques in the modern era.

ARTICLE HIGHLIGHTS

Research background

Anatomic repair for congenitally corrected transposition of great arteries (cc-TGA) is accomplished by either a double switch operation or one of many modifications of an atrial switch operation combined with a Rastelli operation. However, these operations are complex and a simpler physiologic repair strategy of correcting only associated defects like tricuspid valve regurgitation or ventricular septal defects is also adopted in many patients. Anatomic repair strategy has the benefit of restoring the left ventricle to the systemic circulation, thus decreasing the chances of development of congestive heart failure from a systemic right ventricle.

Research motivation

There are variations in practice regarding anatomic versus physiologic repair for cc-TGA. Long term data from a large set of patients regarding safety and outcomes of anatomic repair are lacking.

Research objectives

The objective of this study was to pool high quality observational studies reporting

outcomes after anatomic repair in cc-TGA patients and perform a systematic review and meta-analysis to provide more comprehensive outcomes.

Research methods

A search of MEDLINE and Scopus was conducted using pre-defined search criteria to identify manuscripts reporting outcomes after anatomic repair. Studies meeting inclusion criteria were reviewed and information regarding variables of interest were extracted. Meta-analysis was performed according to standard methods using Comprehensive meta-analysis software (version 3).

Research results

Eight hundred and ninety-five patients who were treated with an anatomic repair strategy were pooled from 21 studies with a total follow-up of 5457.2 patient-years (PY). Estimated operative mortality was 8.3%. Survivors had a transplant free survival of 92.5% (CI: 89.5%-95.4%) per 100 PY. 84.7% patients experienced a New York Heart Association functional class I or II after 100 PY follow up. There were 5.3 re-operations/re-interventions per 100 PY (CI: 3.8-6.8).

Research conclusions

Our study reports a high operative mortality rate for anatomic repair strategy in cc-TGA patients. However, the long-term survival is excellent for survivors.

Research perspectives

Our study suggests that the anatomic repair is worth pursuing in most patients with cc-TGA because of favorable long-term outcomes despite a high operative mortality risk. Re-intervention/reoperation risk remains – however with the advent of transcatheter therapies, most of these issues can be managed without a re-operation in the modern era.

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