Gdański Uniwersytet Medyczny

Rozprawa doktorska

„Niskie stężenie katestatyny jako czynnik ryzyka rozwoju chorób sercowo-naczyniowych – ocena u pacjentów z incydentaloma nadnercza".

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Składam podziękowania wszystkim współautorom i osobom zaangażowanym w uzyskanie materiału do badań będących podstawą publikacji składających się na niniejszą rozprawę doktorską.

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SPIS TREŚCI

I. WYKAZ PRAC WCHODZĄCYCH W SKŁAD ROZPRAWY DOKTORSKIEJ

Praca oryginalna:

Autorzy: Zalewska Ewa, Kmieć Piotr, Sobolewski Jakub, Koprowski Andrzej, Sworczak Krzysztof;

tytuł artykułu: „Low catestatin as a risk factor for cardiovascular disease – assessment in patients with adrenal incidentalomas.";

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IF 5.2| MNiSW 100| Q1

Praca poglądowa:

Autorzy: Zalewska Ewa, Kmieć Piotr, Sworczak Krzysztof;

tytuł artykułu: "Role of Catestatin in the Cardiovascular System and Metabolic Disorders.";

Frontiers in Cardiovascular Medicine, 2022, doi:10.3389/fcvm.2022.909480.

IF 3.6| MNiSW 40| Q2

Łączna wartość wskaźnika oddziaływania (IF): 8.8

Łączna punktacja MNiSW: 140

II. WYKAZ STOSOWANYCH SKRÓTÓW

ABPM – *24-hour ambulatory blood pressure monitoring;* 24 godzinny pomiar ciśnienia tętniczego metodą holter,

AI – *adrenal incidentaloma;* incydentaloma nadnercza,

BMI – *body mass index;* wskaźnik masy ciała,

BP – *blood pressure;* ciśnienie tętnicze,

Cts – *catestatin;* katestatyna,

CgA – *chromogranin A;* chromogranina A,

CVDs – *cardiovascular diseases;* choroby sercowo - naczyniowe,

FRS - *Framingham risk score;* skala Framingham oceniająca 10-letnie ryzyko miażdżycowej choroby sercowo-naczyniowej oszacowane za pomocą kalkulatora udostępnionego online w 2018r. przez American Heart Association i American College of Cardiology na podstawie Framingham Heart Study,

HDL-C – *high density lipoprotein cholesterol*; frakcja lipoprotein o wysokiej gęstości,

hs-CRP – *high sensitive C-reactive protein;* białko C-reaktywne wysokiej czułości,

HT – *hypertension;* nadciśnienie tętnicze,

LDL-C – *low density lipoprotein cholesterol;* frakcja lipoprotein o niskiej gęstości,

MACS – *mild autonomous cortisol secretion;* łagodne autonomiczne wydzielanie kortyzolu,

MetS – *metabolic syndrome*; zespół metaboliczny,

RVLM - *rostral ventrolateral medulla*; dogłowowy brzuszno-boczny obszar rdzenia przedłużonego,

SCORE2/-OP - *Systematic Coronary Risk Estimation 2 /-Older People*; kalkulator ryzyka-naczyniowego według Europejskiego Towarzystwa Kardiologicznego 2021,

TTE – *transthoracic echocardiography;* echokardiografia przezklatkowa.

III. STRESZCZENIE W JĘZYKU POLSKIM

Zwiększona aktywność współczulnego układu nerwowego odgrywa istotną rolę w patofizjologii nadciśnienia tętniczego (HT) i chorób układu krążenia (CVDs), które są główną przyczyną zgonów na świecie. Katestatyna (Cts) ze względu na swoją zdolność do hamowania dalszego uwalniania katecholamin w mechanizmie ujemnego sprzężenia zwrotnego przeciwdziała nadmiernej aktywacji współczulnej. Na podstawie badań eksperymentalnych wykazano na modelach zwierzęcych, że Cts może prowadzić do normalizacji ciśnienia tętniczego, hamowania ekspresji cytokin prozapalnych, poprawy wrażliwości na insulinę oraz zmniejszenia nasilenia miażdżycy, masy lewej komory, a także grubości kompleksu intima-media. Korzystny wpływ Cts na układ sercowo-naczyniowy i homeostazę metaboliczną został szczegółowo omówiony w mojej pracy poglądowej, będącej częścią rozprawy doktorskiej. Badania kliniczne wskazują, że Cts może stać się nowym biomarkerem, służącym do oceny ryzyka rozwoju i monitorowania przebiegu CVDs. W pracy oryginalnej, zawartej w niniejszej rozprawie doktorskiej, badam związek między Cts a modyfikatorami ryzyka sercowo-naczyniowego, w tym: HT, dyslipidemią, hiperurykemią, a także stężeniem wysokoczułego białka-C-reaktywnego w populacji pacjentów z incydentaloma nadnerczy, którzy są uznawani za grupę osób o podwyższonym ryzyku sercowo-naczyniowym. Ponadto badam związek Cts z bezobjawowym uszkodzeniem narządowym wywołanym przez HT przy pomocy echokardiografii i oceny kompleksu intima-media tętnicy szyjnej wspólnej. Jako pierwsza wykazałam poniższe zależności: 1) wśród osób bez jawnej CVDs innej niż pierwotne HT stężenia osoczowe Cts są porównywalne u pacjentów z gruczolakami nadnercza i tymi z prawidłową morfologią nadnerczy; 2) stężenia Cts są niższe u dorosłych pacjentów z zespołem metabolicznym w porównaniu do osób bez niego; 3) zachodzi ujemna korelacja pomiędzy Cts a ryzykiem sercowo-naczyniowym wyliczonym za pomocą skali Framingham. Konieczne są dalsze, długofalowe badania na większej populacji, aby zastosować Cts jako biomarker CVDs w praktyce klinicznej.

IV. STRESZCZENIE W JĘZYKU ANGIELSKIM

Dissertation topic: "Low catestatin as a risk factor for cardiovascular disease – assessment in patients with adrenal incidentalomas"

Dysregulation of the sympathetic nervous system plays an important role in the pathophysiology of hypertension (HT) and cardiovascular diseases (CVDs), which are the leading cause of death globally. Catestatin (Cts) due to its high capacity to inhibit further release of catecholamines in a negative feedback mechanism counteracts the sympathetic outflow. In the field of experimental research, in animal models, it has been demonstrated that Cts can lead to normalization of blood pressure, inhibition of the expression of pro-inflammatory cytokines, improvement of insulin sensitivity, and reduction of: the severity of atherosclerosis, the left ventricular mass, as well as the thickness of the intima-media complex. The beneficial effects of Cts on the cardiovascular system and the metabolic homeostasis have been discussed in detail in my review paper. Clinical studies point to Cts as a novel biomarker for assessing the risk of development as well as monitoring the course of CVDs. In my original paper I investigated the association between Cts and cardiovascular risk modifiers including HT, dyslipidemia, hyperuricemia, as well as high sensitive-C-reactive protein level in the population of adrenal incidentaloma patients, who are considered to be at an increased cardiovascular risk. Moreover, I examined associations between Cts and asymptomatic HT-mediated organ damage based on echocardiography and examination of the intima-media complex of common carotid artery. I was the first to report: 1) comparable plasma Cts concentrations in adrenal adenoma patients and those of matched controls with normal adrenal morphology among persons without overt CVD other than primary HT, 2) lower Cts in adult patients with metabolic syndrome than those without it; 3) a correlation between Cts and Framingham risk score. Still, longitudinal assessment of larger populations is required to apply serum Cts as a biomarker in clinical practice.

V. WPROWADZENIE

W ciągu ostatnich trzech dekad nastąpił wzrost liczby zgonów z powodu chorób sercowo-naczyniowych (CVDs), które odpowiadają obecnie za blisko jedną trzecią wszystkich zgonów na świecie [World Heart Report 2023]. Szacuje się, że na całym świecie około 1,28 miliarda dorosłych w wieku od 30 do 79 lat cierpi na nadciśnienie tętnicze (HT), które leży u podłoża sztywnienia tętnic prowadząc do: zmniejszenia dopływu krwi i tlenu do serca, uszkodzenia nerek oraz pęknięcia lub zablokowania tętnic zaopatrujących mózg [World Heart Report 2021].

W patofizjologii HT i CVDs zwiększona aktywność układu współczulnego odgrywa niezaprzeczalnie istotną rolę [de Lucia et al. 2019; Valensi 2021]. Układ współczulny wraz z układem przywspółczulnym należą do układu autonomicznego, który unerwia narządy wewnętrzne i reguluje procesy niezależne od woli. Harmonijne współdziałanie układu współczulnego, odpowiadającego za mobilizację organizmu, i przywspółczulnego, którego działanie przejawia się w spoczynku, jest niezbędne do prawidłowej pracy układu sercowo-naczyniowego. Regulacja aktywności współczulnej odbywa się głównie w mechanizmie odruchów. Ewolucyjnie większe znaczenie dla przeżycia osobnika miała odpowiedź na spadek ciśnienia tętniczego (BP) w wyniku utraty objętości krwi krążącej lub utrudniony dostęp do wody i soli niż przed nadmiernym wzrostem BP. Stąd wynika asymetria odruchu i skuteczniejsza ochrona przed obniżeniem niż nadmiernym podwyższeniem BP [Traczyk 2015].

Wzrost BP pobudza mechanoreceptory zlokalizowane w zatokach szyjnych i w łuku aorty, tzw. baroreceptory tętnicze, co przekazywane jest za pośrednictwem jądra pasma samotnego do doogonowego obszaru brzuszno-bocznego rdzenia przedłużonego w którym zgrupowane są neurony hamujące dogłowowy brzuszno-boczny obszar rdzenia przedłużonego (RVLM). W RVLM zlokalizowane są neurony przedwspółczulne, podtrzymujące toniczną aktywność współczulną skierowaną głównie do układu krążenia. W konsekwencji hamowania RVLM dochodzi do odruchowego obniżenia BP. Z kolei obniżenie BP powoduje odbarczenie baroreceptorów tętniczych, co skutkuje odhamowaniem tonicznej aktywności współczulnej, prowadząc do zwiększenia całkowitego oporu obwodowego i pojemności minutowej serca a w konsekwencji powrót prawidłowego BP [Traczyk 2015].

Neurony przedwspółczulne RVLM pobudzają współczulne neurony przedzwojowe rdzenia kręgowego do produkcji acetylocholiny, która działa na receptory cholinergiczne typu N zlokalizowane w neuronach zazwojowych i komórkach chromochłonnych rdzenia nadnerczy prowadząc do ich aktywacji i produkcji katecholamin (CAs): noradrenaliny i adrenaliny [Gaede et al. 2010; Callingham 1965]. CAs łączą się z receptorami a1-adrenergicznymi naczyń prowadząc do ich skurczu, co powoduje wzrost BP, oraz ß1-adrenergicznymi serca, skutkiem czego jest: zwiększenie siły skurczu (tzw. dodatni efekt inotropowy), częstości akcji serca (tzw. dodatni efekt chronotropowy), tępa relaksacji (tzw. dodatni efekt luzytropowy) oraz prędkości przewodzenia (tzw. dodatni efekt dromotropowy) [Motiejunaite et al. 2021]. Ponadto pobudzenie receptorów β1-adrenergicznych w nerkach powoduje uwolnienie reniny, która stymuluje produkcję angiotensyny II, ta z kolei uwalnianie aldosteronu przez korę nadnerczy. Stymulacja osi renina-angiotensyna-aldosteron przyczynia się do dalszego wzrostu BP przez zwiększenie całkowitego oporu obwodowego i reabsorpcji wody w nerkach [Traczyk 2015].

CAs są uwalniane przez egzocytozę z pęcherzyków ziarnistych wraz z innymi białkami, w tym chromograniną A (CgA), która do niedawna uznawana była za białko nieaktywne. W 1988 po raz pierwszy wykazano, że CgA podlega proteolitycznej hydrolizie i jest prekursorem dla peptydu mającego zdolność regulacji wydzielania CAs [Simon et al. 1988]. W 1997 Mahata i in. udało się zidentyfikować ten peptyd i nadali mu nazwę "katestatyna" (Cts) ze względu na wysoką zdolności do hamowania dalszego uwalniania CAs w mechanizmie ujemnego sprzężenia zwrotnego [Mahata et al. 1997]. Cts wiąże się z receptorem cholinergicznym typu N, blokując wychwyt jonów sodu, co hamuje depolaryzację błony komórkowej i napływ jonów wapnia przez bramkowane napięciem kanały wapniowe [Taupenot et al. 2000; Herrero et al. 2002]. W konsekwencji dochodzi do zahamowania zarówno dalszego uwalniania CAs przez egzocytozę, jak i transkrypcji genu CgA. Kolejne badania wykazały, iż Cts jest zaangażowana nie tylko w regulację układu sercowo-naczyniowego, ale również immunologicznego, jak i w homeostazę metaboliczną [Bozic et al. 2021; Pasqua et al. 2019; Muntjewerff et al. 2018; Bourebaba et al. 2021]. Dotychczas nie jest znany optymalny zakres stężeń Cts, zapewniający jej plejotropowe działanie biologiczne.

Postulowano, iż Cts może stać się nowym biomarkerem służącym do monitorowania przebiegu i oceny ryzyka rozwoju CVDs [Bozic et al. 2021; O'Connor et al. 2002; 2008; Durakoğlugil et al. 2015; Zhao et al. 2016]. Klasyczne czynniki ryzyka rozwoju CVDs określone zostały na podstawie „Framingham Heart Study" i są nimi: palenie wyrobów tytoniowych, nieaktywny tryb życia, otyłość, wysokie BP, obniżone stężenie frakcji cholesterolu o wysokiej gęstości (HDL-C) i podwyższone stężenie frakcji cholesterolu o niskiej gęstości (LDL-C) [Jahangiry et al. 2017; Mahmood et al. 2014]. Przykładami innych nowych biomarkerów, służących do wyróżnienia osób, które szczególnie skorzystałyby z interwencji medycznych (np. obniżenia LDL-C) są kwas moczowy [Yu et al. 2020] i wysokoczułe białko C-reaktywne (hs-CRP) [Koosha et al. 2020]. Wysokie stężenie kwasu moczowego sprzyja rozwojowi CVDs poprzez regulację sygnałów molekularnych takich jak odpowiedź zapalna, stres oksydacyjny, insulinooporność, dysfunkcja śródbłonka oraz stres retikulum endoplazmatycznego [Yu et al. 2020]. Rola hs-CRP w patogenezie CVD polega głównie na zapoczątkowaniu i rozwoju zmian miażdżycowych, zwiększeniu prawdopodobieństwa pęknięcia blaszek miażdżycowych oraz promowaniu insulinooporności i cukrzycy typu 2 [Koosha et al. 2020].

Obecność incidentaloma nadnercza o radiologicznym charakterze gruczolaka lub przerostu kory nadnerczy (AI), a zwłaszcza łagodne autonomiczne wydzielanie kortyzolu (MACS) w jej przebiegu, wiązane jest z zaburzeniami metabolicznymi i podwyższonym ryzykiem sercowo-naczyniowym [YAĞIZ et al. 2022]. Częstość występowania AI waha się od 1,05% do 8,7% (w zależności od źródła) i rośnie z wiekiem, osiągając szczyt w piątej i szóstej dekadzie życia [Sherlock et al. 2020]. Te dwa fakty – wysoka częstość występowania oraz możliwa podkliniczna aktywność hormonalna – sprawiają, że pacjenci z AI stanowią szczególnie wartą zbadania populację w kontekście ryzyka CVDs. Co istotne, stężenie Cts u pacjentów z AI nie było do tej pory oceniane.

VI. CELE PRACY

Obrałam następujące cele:

1) określenie stężenia Cts u pacjentów z przypadkowo wykrytą zmianą ogniskową nadnercza o radiologicznym charakterze gruczolaka lub rozrostu kory nadnerczy bez jawnej CVD innej niż HT,

2) zbadanie zależności pomiędzy stężeniem Cts a zaburzeniami metabolicznymi, BP oraz wykładnikami podklinicznego uszkodzenia narządowego w przebiegu HT u tych chorych.

VII. OMÓWIENIE PUBLIKACJI WCHODZĄCYCH W SKŁAD ROZPRAWY DOKTORSKIEJ

Na niniejszą rozprawę składa się jedna publikacja oryginalna i jedna poglądowa. W artykule poglądowym przeanalizowana została rola Cts w układzie sercowo-naczyniowym i zaburzeniach metabolicznych na podstawie aktualnego piśmiennictwa [Zalewska et al. 2022]. W ramach pracy oryginalnej zrealizowane zostały zaplanowane cele rozprawy doktorskiej [Zalewska et al. 2023].

A. ARTYKUŁ ORYGINALNY

Artykuł oryginalny „Low catestatin as a risk factor for cardiovascular disease – assessment in patients with adrenal incidentalomas", (tłumaczenie własne: "Niskie stężenie katesatyny jako czynnik ryzyka rozwoju chorób sercowo – naczyniowych – ocena u pacjentów z incydentaloma nadnercza" przedstawia wyniki przekrojowego, obserwacyjnego badania klinicznego, które zostało przeprowadzone za zgodą Niezależnej Komisji Bioetycznej do spraw Badań Naukowych przy Gdańskim Uniwersytecie Medycznym (NKBB/659/2019).

Podstawowym celem badania była ocena związku między stężeniem Cts a czynnikami ryzyka rozwoju CVD jak i podklinicznym uszkodzeniem narządowym w przebiegu HT u pacjentów z AI bez jawnej CVD innej niż HT.

Uczestników badania rekrutowano kolejno spośród pacjentów z AI hospitalizowanych w Klinice Endokrynologii i Chorób Wewnętrznych Uniwersyteckiego Centrum Klinicznego Gdańskiego Uniwersytetu Medycznego

w okresie od listopada 2018 do lutego 2020 roku celem oceny funkcji wydzielniczej nadnerczy. Do badania włączono 64 pacjentów z radiologicznymi cechami gruczolaka/rozrostu nadnerczy ujawnionymi w tomografii komputerowej lub rezonansie magnetycznym, którzy zgodzili się na udział w nim i nie spełniali żadnego z poniższych kryteriów wykluczenia, którymi były m.in.:

- utrwalona i/lub jawna CVD inna niż pierwotna HT w tym: przewlekła choroba wieńcowa, ostry zespół wieńcowy, udar, przemijający atak niedokrwienny, choroba tętnic obwodowych, patologiczne arytmie, istotne wady zastawkowe, tamponada serca, kardiomiopatia, wrodzone wady serca, niewydolność serca, żylna choroba zakrzepowo-zatorowa i zapalenie naczyń;
- wiek powyżej 75. roku życia lub poniżej 40. roku życia;
- choroba nerek z eGFR <60 ml/min/1,73m^2 i/lub białkomoczem >0,25 g/24h);
- otyłość III stopnia (body mass index [BMI] >40 kg/m2);
- czynna choroba nowotworowa i/lub immunologiczna i/lub zakaźna;
- okres przedmenopauzalny;
- terapia antagonistami receptora mineralokortykoidego;
- występowanie nieprawidłowej funkcji wydzielniczej nadnerczy innej niż MACS, zdefiniowanego jako stężenie kortyzolu w teście nocnym („overnight") hamowania z 1 mg deksametazonu między 50 a 138 nmol/l przy niewystępowaniu cech fenotypowych zespołu Cushinga (tzw. MACS); wykluczono zatem osoby z:
    - autonomicznym wydzielaniem kortyzolu charakteryzującym się kortyzolemią ponad 138 nmol/l w teście nocnym z 1 mg deksametazonu oraz z jawnym klinicznie zespołem Cushinga,
    - pierwotnym aldosteronizmem oraz te z dodatnim wskaźnikiem aldosterononowo-reninowym (powyżej 2 ng/dL:mIU/mL) i jednocześnie występującym HT,
    - osoby z hiperandrogenizmem i tych z
    - guzem chromochłonnym czy nieprawidłowym dobowym wydalaniem pochodnych metoksykatecholamin z moczem.

Na podstawie dokumentacji medycznej naszego szpitala, obejmującej badania zlecone w przychodniach specjalistycznych i na oddziale ratunkowym, zidentyfikowano 24 osoby z prawidłową morfologią nadnerczy w badaniu CT/MRI wykonanym w ciągu 5 lat poprzedzających niniejsze badanie, którzy nie spełnili

żadnego z ustalonych kryteriów wykluczenia i zgodzili się wziąć udział w naszym badaniu jako grupa kontrolna.

Zarówno pacjenci z AI, jak i z grupy kontrolnej przeszli następującą ocenę:

- wywiad lekarski i badanie fizykalne;
- ocenę laboratoryjną stężenia surowiczego/osoczowego: Cts, kreatyniny, elektrolitów (sód, potas), aldosteronu, reniny, lipidogramu (cholesterol całkowity, HDL-C, LDL-C, triglicerydów), kwasu moczowego oraz hs-CRP;
- spoczynkową, 12-odporwadzeniową elektrokardiografię;
- echokardiografię przezklatkową (TTE);
- ocenę maksymalnego wymiaru kompleksu intima-media tętnicy szyjnej wspólnej;
- 24-godzinny, ambulatoryjny pomiar ciśnienia tętniczego metodą holter (ABPM).

Ponadto u pacjentów z AI oceniono stężenie porannego kortyzolu w surowicy, siarczanu dehydroepiandrosteronu i 24-godz. wydalanie kortyzolu, białka i albuminy z moczem, a także wykonano nocny test hamowania kortyzolu z 1 mg deksametazonu (test „overnight"). W teście tym o godz. 23:00 pacjent przyjmuje doustnie 1 mg deksametazonu, a kolejnego dnia o godz. 8:00 ocenia się kortyzolemię.

Na podstawie uzyskanych danych wyliczone zostało:

- 10-lenie ryzyko rozwoju CVD przy użyciu kalkulatora z 2018r. udostępnionego online przez American Heart Association and the American College of Cardiology na podstawie Framingham Heart Study (FRS) – https://www.framinghamheartstudy.org/fhs-risk-functions/cardiovascular-disease-10-year-risk/;
- dla osób bez cukrzycy 10-letnie ryzyko rozwoju CVD obliczone za pomocą Systematic Coronary Risk Estimation 2 (SCORE2) dla osób w wieku 40-69 lat i SCORE2-Older People (SCORE2-OP) dla osób w wieku 70-75 lat dla krajów o wysokim ryzyku sercowo-naczyniowym. Byliśmy świadomi, że SCORE2/-OP został opracowany w celu oszacowania ryzyka u osób nieotrzymujących dotychczas leków obniżających BP i poziom lipidów, a znaczna część naszych pacjentów stosowała już przynajmniej jedną z tych terapii. Niemniej jednak doszliśmy do wniosku, że zastosowanie tego najpopularniejszego w Polsce narzędzia do oszacowania ryzyka sercowo-naczyniowego będzie wartościowe.

Grupy pacjentów z AI i kontrolna były porównywalne pod względem wieku, płci, BMI, częstości występowania nikotynizmu i HT, cukrzycy typu 2., blaszek miażdżycowych i dyslipidemii. FRS było również porównywalne między pacjentami z AI a grupą kontrolną.

Rozkład Cts był bimodalny zarówno u pacjentów z AI jak i w grupie kontrolnej. W obu grupach u ok. 75% chorych wykazano Cts poniżej 45.2 ng/ml, nie obserwowano pacjentów z Cts w zakresie 45.2-99, a u ok. 25% badanych Cts była w zakresie 100-133 ng/ml. Przed uwzględnieniem potencjalnych czynników zakłócających (płeć, wiek, BMI) stężenie Cts było nieco wyższe u pacjentów z AI: 6,45 (zakres międzykwartylowy: 4,9-37) vs. 4,5 (3,5-28) ng/ml, p=0,047. Jednak po przeprowadzeniu analizy regresji wielorakiej obecność AI nie była istotnie związana ze stężeniem Cts (ß=-8,1, p=0,44). Ponieważ CgA nie ulega ekspresji w tkance gruczołowej kory nadnerczy, uzyskany wynik porównywalnych stężeń Cts między pacjentami z AI i grupą kontrolną po uwzględnieniu potencjalnych czynników zakłócających był przeze mnie oczekiwany.

Wśród pacjentów z AI, stężenie Cts było wyższe:

- u kobiet niż u mężczyzn: 7,3 (5,5-103) vs. 6 (4,26-7,6) ng/ml, p=0,03,
- u osób z HT w porównaniu do osób z prawidłowym BP: 5,6 (4,36-6,82) vs. 21,7 (6,85-107), p < 0,001),
- oraz u osób z zespołem metabolicznym (MetS) w porównaniu do osób bez MetS: 26 (6,4-116) vs. 5,6 (4,4-7,5) ng/ml, p < 0,01,

niezależnie od potencjalnych czynników zakłócających.

Ponadto u pacjentów z AI stwierdzono ujemne, słabe korelacje między Cts a: BMI (r=-0,31) i FRS (r=-0,42) oraz dodatnie z HDL-C (r=0,32) niezależnie od leczenia statynami. Co ciekawe, wśród uczestników bez terapii obniżającej stężenie cholesterolu wystąpiły również dodatnie korelacje między Cts a: cholesterolem całkowitym i LDL-C (r=0,36 dla obu zależności). Ponadto, analizując uczestników badania z grupy badanej i kontrolnej jako całość, zaobserwowałam ujemną korelację między Cts a kwasem moczowym (r=-0,27, p=0,01), podczas gdy dla każdej grupy analizowanej osobno nie osiągnięto istotności statystycznej dla tej zależności prawdopodobnie ze względu na wielkość próby.

W dalszej analizie wykazałem, że wśród pacjentów z AI ze stężeniem Cts poniżej mediany wynoszącej 6,5 ng/ml (4,9-37 ng/ml) w porównaniu z pozostałymi częściej występowało HT (iloraz szans (OR) 0,17, przedział ufności (CI) 0,05-5,37, p=0,003)

i MetS (OR 0,21, CI 0,06-7,51, p=0,018). Co więcej BMI, 24-godzinne średnie skurczowe BP i FRS były również wyższe u tych ze stężeniami Cts poniżej mediany (odpowiednio 30,1 ± 4 vs. 27,2 ± 3,6 kg/m2, p=0,004; 123 ± 7,4 vs. 117 ± 9,5 mmHg, p=0,022; 13,2% (8,9-19,2) vs. 6,3% (4,2-10,8), p=0,002).

Ponadto podzieliłam uczestników AI na cztery podgrupy w oparciu o rozkład Cts, tj. na tych z: „bardzo niskim" (<4,9 ng/ml, n=17), „niskim" (≥4,9 i <6,5 ng/ml, n=15), „pośrednim" (≥6,5 i ≤45,2 ng/ml, n=17) i „wysokim" (≥100 ng/ml, n=15) stężeniem Cts. Analiza wykazała, że płeć męska występowała częściej w podgrupie z „bardzo niskim" w porównaniu do tej z „wysokim" stężeniem Cts (53% vs. 6,7%). Odsetek osób z HT oraz tych z MetS był taki sam w czterech podgrupach stężeń Cts, a istotność statystyczna dla różnic w częstości występowania obu schorzeń uzyskana została dla porównań między osobami z „bardzo niskim" a: „pośrednim" i „wysokim" stężeniem Cts (odpowiednio 82,4% wobec 35,3% i 33,3%, p=0,04 w obu przypadkach). Co więcej, HDL-C było niższe w podgrupie z „bardzo niskim" w porównaniu do trzech pozostałych podgrup Cts (42,9 ± 42,9 wobec 61,6 ± 17,8, 56,7 ± 13,7 i 55,8 ± 9,5 mg/dl, odpowiednio p=0,001, 0,01 i 0,03); a 24-godzinne średnie skurczowe BP było wyższe w podgrupie „niskiego" Cts niż „pośredniego" (124,7 ± 6,7 vs. 114,5 ± 8,5 mmHg, p=0,008). FRS w podgrupie „bardzo niskiego" Cts było wyższe niż w podgrupie „pośredniego" i „wysokiego" Cts: 14,3% (19,2-24,5) vs 7,1% (4,7-14,2) i 5,6% (3,8-9.8), odpowiednio p=0,014 i 0,005, zgodnie z różnicami w proporcjach płci, częstości zaburzeń metabolicznych i HT między podgrupami. Nie odnotowano istotnych statystycznie zależności między Cts a parametrami z TTE oraz ultrasonografii tętnicy szyjnej wspólnej.

Najistotniejszym ograniczeniem danych jest niska liczebność badanej populacji w kontekście istotnej heterogenności uczestników (np. w zakresie rozkładu wieku badanych osób czy BMI) i szczególnie niska liczba mężczyzn. Inne wady to brak oznaczania CgA (iloraz Cts do CgA mógłby dostarczyć dodatkowych danych) i badań hormonalnych w grupie kontrolnej. Ponadto bardziej czułe metody oceny śródbłonka i serca mogłyby wykazać zależność ze stężeniem Cts, których nie udało się zaobserwować przy zastosowaniu klasycznych parametrów TTE i ultrasonografii tętnicy szyjnej wspólnej.

Należy jednak podkreślić mocne strony pracy. Co najważniejsze, przeprowadziłam dogłębną analizę związku między Cts a czynnikami ryzyka sercowo-naczyniowego i po raz pierwszy wykazałam, że:

- wśród osób bez jawnej CVD innej niż pierwotne HT stężenia Cts w osoczu pacjentów z gruczolakami nadnercza są porównywalne z osobami o prawidłowej morfologii nadnerczy;

- u dorosłych pacjentów z MetS Cts jest niższa niż u osób bez niego;

- zachodzi ujemna korelacja między Cts a FRS.

Konieczne są dalsze, długofalowe badania na większej populacji w celu potwierdzenia, czy niskie stężenie Cts jest czynnikiem ryzyka rozwoju CVD.

B. PRACA POGLĄDOWA

Niniejsza rozprawa doktorska zawiera artykuł przeglądowy „Role of Catestatin in the Cardiovascular System and Metabolic Disorders" (tłumaczenie własne: „Rola katestatyny w układzie sercowo-naczyniowym i zaburzeniach metabolicznych") [Zalewska et al. 2022]. Artykuł został sporządzony na podstawie przeglądu piśmiennictwa przeprowadzonego w kwietniu 2022r. za pomocą bazy Pubmed z użyciem kluczowego terminu „catestatin". Na podstawie tytułów i streszczeń wybrałam artykuły kwalifikujące się do omówienia w kolejnych częściach pracy przeglądowej, dotyczących roli Cts w regulacji BP, funkcji i chorób serca oraz zaburzeń metabolicznych, jak i miażdżycy. W każdej z części omówiono badania eksperymentalne *in vitro* i *in vivo* na modelach zwierzęcych, a także badania kliniczne.

W kontekście regulacji BP badania eksperymentalne wskazują, iż Cts oprócz hamowania uwalniania CAs z komórek chromochłonnych i neuronów noradrenergicznych, o czym wspomniałam wcześniej, może wykazywać silne działanie rozszerzające naczynia krwionośne, w którym pośredniczy – przynajmniej częściowo – uwalnianie histaminy [Kennedy et al. 1998; Krüger et al. 2003]. Ujemne efekty inotropowe i luzytropowe Cts mogą również prowadzić do obniżenia BP [Tommaso Angelone et al. 2008]. Ponadto Cts odgrywa rolę w centralnej kontroli krążeniowo-oddechowej [Gaede et al. 2009; 2010]. Na podstawie badań klinicznych wykazano niskie stężenie Cts w stanie przednadciśnieniowym [O'Connor et al. 2002], jednakże wciąż istnieją kontrowersje dotyczące wysokości stężenia Cts u osób z pierwotnym HT. Niektóre badania wykazały porównywalne stężenie Cts u chorych z pierwotnym HT i osób z prawidłowym BP [Durakoğlugil et al. 2015;

O'Connor et al. 2002], inne – niższe w HT [O'Connor et al. 2008; Zalewska et al. 2023], kolejne – wyższe [Meng et al. 2011; Kumric et al. 2022]. Wysunięto hipotezę, że niskie stężenie Cts może predysponować do rozwoju HT, jednakże wyższa aktywność współczulnego układu nerwowego, która wzrasta wraz z postępem HT, przyczynia się do kompensacyjnego wzrostu stężenia Cts [Meng et al. 2011]. Rozwój HT może być również związany ze zmniejszoną konwersją CgA do Cts [Fung et al. 2011; O'Connor et al. 2008; Biswas et al. 2008] i zależeć od wariantów proteolitycznych Cts, uwarunkowanych genetycznie i wywierających różny efekt na BP [Rao et al. 2007].

W kontekście wpływu Cts na funkcję serca *in vitro* i *in vivo* z udziałem modeli zwierzęcych wykazano, że Cts ma potencjalne działanie kardioprotekcyjne, działając jako peptyd kardiodepresyjny [Mazza et al. 2012; Tommaso Angelone et al. 2008; 2011; Imbrogno et al. 2010; T. Angelone et al. 2015], a także zmniejszając apoptozę kardiomiocytów indukowaną stresem oksydacyjnym [D. Wang et al. 2016; Penna et al. 2010; Perrelli et al. 2013; Liao et al. 2015; Chu et al. 2020; Tamm et al. 1994; Brar et al. 2010]. Badania kliniczne sugerują patogenną rolę niskiego stężenia Cts w niedokrwieniu mięśnia sercowego [Xu et al. 2017] oraz na jej udział w przebiegu choroby wieńcowej i niewydolności serca [H. Chen et al. 2019; Zhu et al. 2011; O'Connor et al. 2002]. Podwyższone wartości Cts były obserwowane w momencie dekompensacji niewydolności serca [Liu et al. 2013] i w 3. dobie od wystąpienia ostrego zespołu wieńcowego [X. Wang et al. 2011; Meng et al. 2013; Zhu et al. 2015], co wydaje się potwierdzać kompensacyjny wzrost stężenia Cts do wyższej aktywności współczulnego układu nerwowego. Na podstawie wyników powyższych badań postulowano, iż Cts może znaleźć zastosowanie w monitorowaniu przebiegu CAD i HF [H. Chen et al. 2019; Zhu et al. 2011].

Jeśli chodzi o udział Cts w rozwoju miażdżycy i zaburzeń metabolicznych, badania eksperymentalne wskazują, że Cts może hamować odpowiedź zapalną i interakcje leukocytów z komórkami śródbłonka [Y. Chen et al. 2019; Ying et al. 2021; Kojima et al. 2018; Muntjewerff et al. 2022; Egger et al. 2008], zapobiegać miażdżycy wywołanej przez makrofagi [Y. Chen et al. 2019; Kojima et al. 2018], regulować migrację monocytów [Egger et al. 2008] oraz wytwarzanie i uwalnianie cytokin [Muntjewerff et al. 2022; Egger et al. 2008; Aung et al. 2011]. Wykazano, że Cts jako nowy regulator metabolizmu u gryzoni pomaga w osiągnięciu wrażliwości na insulinę, przezwyciężeniu stresu retikulum endoplazmatycznego [Dasgupta et al.

2020], redukcji tkanki tłuszczowej poprzez zwiększenie lipolizy, nasilenie utleniania kwasów tłuszczowych i ich asymilacji do lipidów [Bandyopadhyay et al. 2012]. Na podstawie badań klinicznych wysunięto podejrzenie, iż niskie stężenie Cts związane jest z niekorzystnym profilem metabolicznym, w tym z otyłością, nieprawidłowym stężeniem lipidów i insulinoopornością [Durakoğlugil et al. 2015; O'Connor et al. 2002; Sahu et al. 2012; Simunovic et al. 2019; Kim et al. 2010; Borovac et al. 2019]. Powyższe obserwacje, wraz z wykazanym obniżonym stężeniem Cts w surowicy osób z dodatnim wywiadem rodzinnym w kierunku HT [O'Connor et al. 2002], skłoniły do wysunięcia hipotezy, iż Cts może znaleźć zastosowanie jako nowy czynnik ryzyka rozwoju CVDs [O'Connor et al. 2002; Durakoğlugil et al. 2015].

VIII. PODSUMOWANIE

Aktualne dane wskazują, że endogenny, bioaktywny peptyd Cts jest czynnikiem regulującym homeostazę sercowo-naczyniową i immunometaboliczną, który być może w przyszłości znajdzie zastosowanie jako nowy biomarker CVDs i zaburzeń metabolicznych. Wyniki przeprowadzonej przeze mnie pracy oryginalnej wskazują na związek niskiego stężenia Cts z ryzykiem sercowo-naczyniowym w populacji osób z przypadkowo wykrytymi gruczolakami nadnercza bez jawnej CVDs innej niż HT. Niewystępowanie istotnych różnic w rozkładzie i stężeniu Cts w grupie pacjentów z AI i kontrolnej sugerują, iż uzyskane wyniki badań mogą być odniesione do populacji z prawidłową morfologią nadnerczy. Należy podkreślić, że dotychczas badania nad Cts prowadzono głównie na modelach zwierzęcych i potrzebne są kolejne prace, aby zastosować oznaczenie Cts w praktyce klinicznej czy – w dalszej perspektywie – wykorzystać korzystne działanie Cts w terapii.

IX. PIŚMIENNICTWO

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Low catestatin as a risk factor for cardiovascular disease – assessment in patients with adrenal incidentalomas

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Background: Catestatin (Cts) is a peptide derived from proteolytic cleavage of chromogranin A, which exhibits cardioprotective and anti-inflammatory properties. Cts has been proposed as a potential biomarker for cardiovascular (CV) disease.

Objectives: examining Cts in patients with incidentally discovered adrenocortical adenomas (AI), and its associations with CV risk factors and blood pressure (BP).

Materials and methods: In this cross-sectional study, 64 AI patients without overt CV disease other than primary hypertension were recruited along with 24 age-, sex-, and body-mass-index (BMI)-matched controls with normal adrenal morphology. Laboratory, 24-h ambulatory BP monitoring, echocardiography, and common carotid artery sonography examinations were performed.

Results: Unadjusted Cts was higher in AI patients (median 6.5, interquartile range: 4.9-37 ng/ml) versus controls (4.5 (3.5 – 28)), p=0.048, however, the difference was insignificant after adjusting for confounding variables. Cts was lower in subjects with metabolic syndrome than in those without it (5.2 (3.9- 6.9) vs. 25.7 (5.8-115) ng/ml, p<0.01), and in men compared to women (4.9 (4-7.4) ng/ml vs. 7 (4.8-100), p=0.015). AI patients in the lower half of Cts levels compared to those in the upper had a higher prevalence of hypertension (OR 0.15, 95% CI: 0.041-0.5, p<0.001) and metabolic syndrome (OR 0.15, 95% CI 0.041-0.5, p<0.001). In AI patients Cts correlated positively with high-density lipoprotein cholesterol (Spearman's r=0.31), negatively with BMI (r=-0.31), and 10-year atherosclerotic CV disease risk (r=-0.42).

Conclusions: Our data indicate associations between CV risk factors and Cts. More clinical research is needed to apply serum Cts as a biomarker.

KEYWORDS

catestatin, adrenal incidentaloma (AI), cardiovascular disease(s), risk predictor, metabolic syndrome

1 Introduction

Risk factors for atherosclerotic cardiovascular disease (ASCVD) can be divided into nonmodifiable (e.g. age or sex) and modifiable (smoking, elevated blood pressure (BP), dyslipidemia, diabetes (DM), and obesity). Apart from established risk factors, new are sought (e.g. uric acid (UA) (1) and high-sensitivity C-reactive protein [hs-CRP) (2)]) to help distinguish persons at higher risk of developing cardiovascular disease (CVD), who would benefit more from medical interventions such as low-density lipoprotein cholesterol (LDL-C) reduction (3).

The sympathetic nervous system plays a pivotal role in CVD development. Chromogranin A (CgA) is co-stored and co-released with catecholaminec from sympathetic neuronal vesicles and the adrenall medulla. One of CgA's proteolytic cleavage products is catestatin (Cts), a cardioprotective, anti-hypertensive, and anti-inflammatory peptide (4, 5). *In vitro*, Cts was shown to bind to nicotinic acetylcholine receptors, which inhibits membrane depolarization and blocks calcium influx, and, consequently, suppresses catecholamine release and activation of the sympathetic nervous system (6). Studies with animal models demonstrated Cts exerts anti-inflammatory effects, cardioprotection, and reduces obesity and insulin resistance (7–9). Clinical studies indicate Cts is involved in the course of hypertension (HT), coronary artery diseases (CAD), and heart failure (HF) (10–12). Adolescents with metabolic syndrome (MetS) had decreased Cts, which was postulated as a novel CVD risk factor (12–14).

In the current study we aimed at 1) determining Cts levels in patients with an incidentally-discovered adrenocortical adenoma/hyperplasia (AI) and without overt CVD other than HT, as well as 2) investigating associations between Cts and ASCVD risk modifiers, and asymptomatic HT-mediated organ damage (15). The presence of an AI *per se*, and particularly mild autonomous cortisol secretion (MACS) in its course, have been associated with metabolic disorders, elevated CV risk and mortality (16). So far, Cts has not been investigated in this patient population.

2 Subjects and methods

2.1 Study population

Study participants with an AI were recruited among 376 consecutive adult patients hospitalized in the Department of Endocrinology and Internal Medicine of the University Clinical Center of the Medical University of Gdańsk between November 2018 through February 2020 due to an adrenal lesion. We included 64 patients with radiological features of an adrenal adenoma/hyperplasia revealed by computed tomography (CT) or magnetic resonance (MR), who agreed to participate in the study, and met none of the following exclusion criteria: 1) age over 75 or under 40; 2) obesity grade III (BMI >40 kg/m^2); 3) premenopausal period; 4) adrenal hormone excess other than MACS, i.e. cortisolemia between 50 and 138 nmol/l in the overnight 1-mg dexamethasone suppression test (DST) and no phenotypic features of Cushing's syndrome (17); 5) kidney disease with eGFR<60 ml/min/1.73m^2 and/or proteinuria >0.25 g/24h); 6) treatment with a mineralocorticoid receptor antagonist; 7) established and/or overt CVD other than primary HT, including: a) ASCVD (CAD, stroke, transient ischemic attack, peripheral artery disease), b) significant cardiac disease (e.g. pathological arrhythmia, severe valvular heart disease, cardiac tamponade, cardiomyopathy, congenital heart disease, HF), c) vascular diseases (among others venous thromboembolism and vasculitis); 8) active malignancy; 9) decompensated autoimmune disease or immune disease associated with CV and/or renal complications; 10) infectious diseases; 11) current or past addiction to alcohol and/or illicit drugs. Study participants were recruited based on anamnesis, physical examination, additional examinations available for review prior to enrollment and performed in the course of the study. Initially, 73 patients were included, however, three withdrew their consent to participate due to the COVID-19 pandemic, in four patients transthoracic echocardiography (TTE) revealed cardiac post-ischemic lesions, and two were diagnosed with primary aldosteronism.

Based on medical records of our hospital, which included examinations ordered in outpatient clinics and the emergency department, we identified 153 persons with normal adrenal morphology in a CT/MRI scan performed within five years preceding this study. There were 129 who met at least one of the above-listed exclusion criteria, declined participation or were unreachable, therefore, 24 subjects without an AI were enrolled as controls.

The research complied with the Declaration of Helsinki and was approved by the Independent Bioethics Committee for Research of our University. Informed consent for inclusion in the study was obtained in writing from each participant.

2.2 Study design

Both AI patients and controls underwent the following evaluation: 1) medical interview; 2) physical examination; 3) antecubital venous blood sampling for laboratory analyses; 4) resting 12-lead electrocardiography (ECG); 5) TTE; 6) common carotid artery (CCA) ultrasonography (USG) including CIMT determination, 7) 24-hour ambulatory blood pressure monitoring (ABPM).

Body-mass-index (BMI) was calculated by dividing body weight (W) in kg by the square of height (H) in meters. 2009 International Diabetes Federation criteria were used to diagnose MetS (18). Subjects with HT received hypotensive medications at the time of enrollment or were diagnosed by ABPM based on mean systolic and diastolic BP (SBP and DBP, respectively) of at least 135/85 mmHg for daytime, 120/75 mmHg for nighttime, and/or 130/80 mmHg for the 24-h period (15). Atherogenic dyslipidemia was defined as triglycerides (TGL) ≥150 mg/dL and serum high-density lipoprotein cholesterol (HDL-C) <40 mg/dL for men and <45 mg/dL for women.

In all study participants, 10-year ACSVD risk was estimated using the 2018 calculator provided online by the American Heart Association and the American College of Cardiology based on Framingham Heart Study (FHS-ASCVD Risk) (19, 20). The calculator estimates 10-year risk of developing ASCVD including coronary death, myocardial infarction, coronary insufficiency, angina, ischemic stroke,

hemorrhagic stroke, transient ischemic attack, peripheral artery disease, and HF for individuals aged 30 to 74 and without CVD at baseline based on the following predictors: age, type 2 DM (DMt2), smoking, treated and untreated SBP, total cholesterol (TC), HDL-C, and LDL-C.

For nondiabetic subjects, 10-year CVD risk was also calculated using Systematic Coronary Risk Estimation 2 (SCORE2) for subjects aged 40-69 and SCORE2-Older People (SCORE2-OP) for those aged 70-75 for high CV risk countries, which include Poland (21). Predictors used in SCORE2/-OP are: age, sex, smoking, SBP, and non-HDL-C. We are aware SCORE2/-OP was developed to estimate risk in treatment-naïve persons, and that a significant portion of our subjects received lipid- and BP-lowering therapy. Nevertheless, we concluded applying this estimation tool along with FHS-ASCVD Risk calculation is of value.

2.3 Laboratory examinations

Blood was drawn between 8 and 10 a.m. after a fasting period of at least 8 hours from an antecubital vein, and used for regular examinations in the laboratory of our hospital apart from samples preserved for the determination of plasma Cts in all subjects, serum aldosterone and plasma direct renin concentration (DRC) in controls. These were centrifuged at 2,000 rpms for 20 minutes at 4 degrees C, aliquoted and stored at -80 degrees C until analysis.

Samples were analyzed in Central Clinical Laboratory in Gdańsk using standard laboratory methods (with a Siemens IMMULITE 1000 Immunoassay System for most biochemical tests, and an Abbott Architect analyzer, which applies the spectrophotometric method). Serum Cts was determined by an enzyme-linked immunosorbent assay (ELISA) by using a commercially-available diagnostic kit (SunRedBio, catalogue no: 201-12-8276; sensitivity: 0.268 ng/mL; assay range: 0.3-90 ng/mL). Cts concentrations above 90 ng/ml (n=15) were extrapolated based on ELISA standard curve.

Serum Cts, creatinine, sodium, potassium, aldosterone, renin, lipid profile (TC, HDL-C, LDL-C, and TGL), UA, hs-CRP, 24-h urinary protein and albumin excretion were determined both in AI patients and controls. Morning serum cortisol, dehydroepiandrosterone sulphate (DHEA-S), overnight 1 mg-DST cortisol and 24-h urinary cortisol were determined in AI patients. In most (n=50) AI patients, 24-h urinary meta- and normetanephrine excretion was determined, in others it had been performed prior to hospitalization. Screening for primary hyperaldosteronism based on aldosterone-to-renin ratio (ADRR) was performed without modifying antihypertensive medications in both AI patients and controls; there were no study participants with both HT and an ADRR above 2 ng/dL:μIU/mL.

2.4 Ambulatory blood pressure monitoring

24h ABPM was conducted using a Spacelabs Ontrak 90227 monitor on the non-dominant arm. During the day BP was recorded every 20 minutes, while during nighttime rest every 30 minutes. ABPM was repeated or not considered in the analysis if more than 30% of measurements were invalid. Normal results were adopted according to the European Society of Cardiology/European Society of Hypertension 2018 guideline: <130/80 mmHg for the 24-h period, <135/85 mmHg for daytime, and <120/70 mmHg for nighttime (22). Patients were classified as 'non-dippers' if their mean diurnal SBP and DBP were not at least 10% higher than nocturnal (22).

2.5 Transthoracic echocardiography

All measurements were performed in accordance with the recommendations endorsed by the American Society of Echocardiography and the European Association of Cardiovascular Imaging (23). Three on-site cardiology consultants with an expertise in ultrasonography performed TTE using the GE Vivid E9/E95 ultrasound system.

Measurements included left-ventricular (LV) internal dimension in diastole (LVIDd) and systole (LVIDs), LV ejection fraction (LVEF) according to modified Simpson's rule (24), posterior LV wall thickness (LVPWd), and interventricular septal thickness (IVSd). LV mass (LVM) was calculated with the cube formula: LVM (g)=0.8×1.04 ×[(LVEDd+IVSd+LVPWd)^3–LVEDd^3]+0.6. LVM was indexed to body surface area (BSA) calculated using the DuBois formula (BSA=0.007184×H$^{0.725}$×W$^{0.425}$): LVM index (LVMI)=LVM/BSA. Relative wall thickness (RWT) was calculated with the formula: RWT=(2×LVPWd)/LVEDd. Left ventricular hypertrophy (LVH) was defined as LVMI >95 g/m^2 for females and >115 g/m^2 for males. RWT was used to further classify LVH as either concentric (RWT >0.42) or eccentric (RWT ≤ 0.42).

Disk summation technique from apical four and two-chamber views was used to determine left atrial volume (LAV), which was indexed to BSA: LAV index (LAVI) (ml/m^2) = LAV/BSA (15). Apical four-chamber view was used to record peak blood flow velocity from LV relaxation in early diastole (E) and peak velocity flow in late diastole (A). Since LVEF was normal in all study participants, four criteria were applied to assess diastolic function: (1) LAVI ≥34 ml/m^2, (2) tricuspid regurgitation velocity (TR) ≥2.8 m/s, (3) ratio of E to average early mitral annular velocity (e') ≥14, (4) septal e'<7 cm/s or lateral e'<10 cm/s. Indeterminate diastolic function was stated if two criteria were met, and dysfunction if three or four (15).

2.6 Common carotid artery USG

Maximum carotid intima-media thickness (CIMT) measurements were recorded using echo-tracking technology on the distal wall of the right carotid artery, 1 to 3 cm below the carotid artery bifurcation. The presence of atherosclerotic plaques (ASP) defined as a CIMT ≥1.5 mm, or by a focal increase in thickness of 0.5 mm or 50% of the surrounding CIMT value was also recorded.

2.7 Statistical analysis

Data were analyzed using R-studio. Discrete variables were presented as number (n) or n (percentage). Continuous

quantitative data with a normal distribution were presented as mean ± standard deviation (SD), and in the case of a non-normal distribution as median (interquartile range, IQR). We used the Shapiro-Wilk test to determine if a data set was well-modeled by a normal distribution. To compare differences between two independent groups Welch's t-test was used when variables were normally distributed or the Mann-Whitney U test in the case of non-normal distribution. One-way ANOVA and Tukey's honestly significant difference (HSD) tests were used to compare three or more independent groups. Simple (bivariate) correlations were computed with the non-parametric Spearman rank-order method (correlation coefficient r is given). Associations between dichotomous categorical variables were examined with Fisher's exact test, and Benjamini-Hochberg Method was applied to correct for multiple testing.

Multiple regression models were applied to adjust for differences in Cts concentrations depending on potential confounding variables including gender, age, BMI, smoking status, comorbidities (HT, DMt2, MetS), and medications (ACEI/ARB, CCB, BB, diuretics, statins, and PPIs). An exhaustive search method was used to select factors that had the strongest relationship with Cts, i.e.: 1) gender, presence of 2) MetS, 3) HT, therapy with 4) statins, and 5) PPIs. The final multivariate model had a R-squared of 0.1831. Out of five variables included in the model only the presence of MetS (ß=-30, p=0.005) was significantly and negatively associated with Cts. For dichotomous dependent variables (Cts halves, HT, DMt2, and MetS) binary logistic regression was used to adjust for gender, age, and BMI. Significance was set at 0.05.

3 Results

3.1 Comparison of examined parameters between AI patients and controls

To assess whether the presence of an AI affected Cts levels, verification of matching between AI patients and controls was undertaken. These groups did not differ in regard to age, sex, BMI, smoking status, and comorbidities (incidence of HT, DM t.2, ASP, and dyslipidemia), see Table 1. Concerning subjects with HT, the number of patients on mono-, dual- and triple-drug therapy (including betablockers) was also comparable (Supplementary Table 1).

Among AI patients, there were 14 with MACS and 31 classified as NFAI; analyses for AI patients and these two subgroups were performed separately.

Ten-year FHS-ASCVD Risk was comparable between patients with an AI/NFAI/MACS and controls, while in nondiabetic subjects, SCORE2/-OP was significantly higher in patients with MACS than in controls and patients with NFAI: 14% (11-18) vs. 8% (4.5-14), p = 0.021, and 8% (6-12), p = 0.005, respectively (Table 1). Still, this CVD risk index was comparable between controls and all AI patients (p=0.31), which illustrates effective matching between these groups.

Cts distribution was bimodal both in AI patients and controls. Unadjusted Cts was slightly higher in AI patients: 6.45 (4.9-37) vs. 4.5 (3.5-28) ng/ml, p=0.047 (Figure 1). However, after adjusting for potential confounding variables (gender, age, and BMI), solely BMI and male gender were significantly (negatively) associated with Cts (ß=-28.3, p=0.01 and ß=-2.3, p=0.04, respectively) but not the presence of an AI (ß=-8.1, p=0.44).

Lipid profile, hs-CRP, as well as UA were comparable between controls and AI patients, be it with a NFAI or MACS. Proteinuria and albuminuria were normal in all study participants (respectively below 150 and 30 mg/24h). ABPM parameters (SBP, DBP, and pulse rate, PR) were comparable between AI patients and controls (Table 1 and Supplementary Table 1).

Concerning TTE, there were significant differences in IVSd, LVPWd, and LVMI between AI patients and controls (respectively 11 (10-12) vs. 10 (9-10) mm, p=0.003; 10 (9-11) vs. 9 (8-9.5) mm, p=0.007; 86.4 ± 119.2 vs. 84.7 ± 18.5 g/m^2, p=0.001). Moreover, LVH was more prevalent in MACS patients than controls (42.9% vs. 4.4%, p=0.007) and NFAI patients (42.9% vs. 14%, p=0.028).

Maximum CIMT was higher in patients with an AI, be it with a NFAI or MACS, than in controls: 1 (0.9-1.1) vs. 0.8 (0.8-0.9) mm, p<0.01. However, there were no differences in maximum CIMT between patients with a NFAI and MACS (Table 1). A trend toward a higher prevalence of an ASP in AI, NFAI, and MACS patients (29.7%, 32%, 21.43%, respectively) than controls (9.5%) could be observed (p=0.12) (Table 1).

3.2 Catestatin in clinically-specified patient groups

Upon comparing Cts levels between controls with normal adrenal morphology and AI patients, peptide's levels were tested in different patient groups. Cts was higher in women than in men: 7 (4.8-100) vs. 4.9 (4-7.4) ng/ml, p=0.015, and the difference between sexes was significant in both AI patients (7.3 (5.5-103) vs. 6 (4.26-7.6) ng/ml, p=0.03) and controls (5.1 (3.8 - 62.6) vs. 2.8 (1.7 - 3.5) ng/ml, p = 0.043), see Figure 2.

Further, in AI patients and controls analyzed together Cts was lower in hyper- versus normotensive subjects: 5.6 (4-7.1) vs. 15.8 (5.2-103) ng/ml, p=0.003, which was also found for AI patients alone: 5.6 (4.36-6.82) vs. 21.7 (6.85-107), p < 0.001) (Figure 3). Cts was also significantly lower in subjects with MetsS than in those without it: 25.7 (5.8-115) vs. 5.2 (3.9- 6.9) ng/ml, p<0.01 (Figure 4), regardless of potential confounders (gender, age, BMI, presence of an AI and/or HT, statin and PPI use). We confirmed these differences (normo- versus hypertensive subjects as well as those without and with MetS) in women but not men (probably due to their low number). Cts in hypertensive AI females was lower than in normotensive ones: 5.6 (4.7 – 11.6) vs. 45.2 (8.2 – 118) ng/ml, p <0.01, and also lower in those with MetS than without it, both among AI patients: 5.6 (4.8-6.9) vs. 34.3 (7.7 - 121) ng/ml, p= <0.01 and controls: 3.8 (3.7-5.1) vs. 61.2 (8.4-112) ng/ml, p = 0.025 (Supplementary Figures 1, 2).

There were no differences in Cts between obese and non-obese subjects, smokers and non-smokers, or, among subjects with HT, 'dippers' and 'non-dippers'.

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TABLE 1 Clinical, laboratory, ABPM, echocardiographic, and CCA sonography parameters in AI patients and controls.

	Controls	AI patients			p	adjusted p		
		all	NFAI	MACS	Cont. vs. AI	Cont.vs. NFAI	Cont. vs. MACS	NFAI vs. MACS
n	24	64	50	14	–	–	–	–
F:M ratio	19:5	45:19	35:15	10:4	0.592	1	1	1
Age [years]	62.2 ± 7.4	60.9 ± 8.8	60.1 ± 8.8	63.7 ± 9.3	0.487	0.575	0.854	0.334
BMI [kg/m^2]	27.9 ± 4.6	28.6 ± 4.1	28.8 ± 4.2	28.3 ± 3.9	0.48	0.685	0.968	0.911
Obesity [n (%)]	8 (33.33%)	24 (37.5%)	20 (40%)	4 (28.6%)	0.807	0.928	1	0.928
HT [n (%)]	10 (41.7%)	36 (56.3%)	27 (54%)	9 (64.3%)	0.327	0.555	0.555	0.555
DMt2 [n (%)]	1 (4.17%)	12 (18.8%)	11 (22%)	1 (7.1%)	0.103	0.266	1	0.411
MetS [n (%)	11 (45.8%)	36 (56.3%)	27 (54%)	9 (64.3%)	0.527	0.621	0.621	0.621
Smokers [n (%)]	24 (33.3%)	28 (43.8%)	20 (40%)	8 (57.1%)	0.521	0.619	0.543	0.543
SCORE2/-OP [%] *	8 (4.5-14)	9 (7-13)	8 (6-12)	14 (11-18)	0.31	0.978	**0.021**	**0.005**
FHS-ASCVD score [%]	5.9 (2.8-12)	10.1 (4.8-16.4)	9.3 (4.6-16.4)	12.5 (8.4-15.6)	0.085	0.338	0.253	0.813
Catestatin [ng/ml]	4.5 (3.5-28)	6.5 (4.9-37)	7.2 (5-101)	6.1 (5-7.8)	**0.048**	0.71	0.7	0.274
HDL-C [mg/dl]	58.7 ± 12.1	54.0 ± 14.7	52.7 ± 14	58.7 ± 17	0.13	0.196	1	0.33
LDL-C [mg/dl]	120 ± 36.6	129 ± 48.2	130 ± 49.1	129 ± 46.8	0.32	0.663	0.828	0.998
TC [mg/dl]	202 ± 42.4	211 ± 53.1	210 ± 53.4	213 ± 53.8	0.45	0.813	0.803	0.978
TGL [<150 mg/dl]	106 (96.8-126)	130 (87-162)	130 (88.5-160)	129 (86.2-162)	0.25	0.414	0.965	0.727
UA [2.5-7 mg/dl]	5.3 (4.8-5.8)	5.1 (4.2-6.1)	5.1 (4.4 -6.1)	4.8 (4.2-5.8)	0.97	0.87	0.983	0.817
hs-CRP [<5 mg/l]	1.4 (1.1-2.5)	1.2 (0.7 - 1.7)	1.2 (0.7-1.6)	1.3 (0.6-2)	0.14	0.811	0.443	0.155
DST cortisol [<50 nmol/L]	–	26.9 ± 34	11.3 ± 17	79.1 ± 22.3	–	–	–	**<0.001**
24h SBP [mmHg]	118 ± 8.4	121 ± 9.7	120 ± 9.3	118 ± 7.7	0.5	0.62	0.982	0.612
24h DBP [mmHg]	70.6 ± 5.9	71.4 ± 7.8	71.7 ± 8.1	70.3 ± 6.5	0.61	0.818	0. 994	0.82
Non-dipper status [n (%)]†	6 (40%)	9 (28.1%)	9 (37.5%)	0	0.442	0.61	0.447	0.46
IVSd [mm]	10 (9-10)	11 (10-12)	11 (10-12)	12 (10.2-12)	**0.003**	**0.045**	**0.006**	0.295
LVIDd [mm]	46.1 ± 4.1	44.8 ± 4.5	45.2 ± 4.7	43.5 ± 3.7	0.193	0.651	0.183	0.427
LVIDs [mm]	29.9 ± 3.3	27.3 ± 3.1	27.3 ± 3.2	27.2 ± 3	**0.002**	**0.005**	**0.036**	0.991
LVPWd [mm]	9 (8-9.5)	10 (9-11)	10 (9-11)	11 (10-11.8)	**0.007**	0.08	**0.009**	0.268
LVM [g]	149 ± 27.2	165 ± 41.8	164 ± 42	169 ± 42.6	**0.047**	0.307	0.289	0.887
LVMI [g/m^2]	86.4 ± 19.2	84.7 ± 18.5	92.7 ± 21	77.9 ± 8.7	**0.006**	**0.037**	**0.023**	0.21
LVH [n (%)] #	1 (4.4%)	13 (20.3%)	7 (14%)	6 (42.9%)	0.101	0.421	**0.007**	**0.028**
LAVI [ml/m^2]	24.6 (15.1-31.9)	24.9 (15.9-31.6)	23.8 (13.1-31.8)	25.5 (20.5-30)	0.29	0.639	0.498	0.864
CIMT max [mm]	0.8 (0.7 -0.8)	1 (0.9 - 1.1)	1 (0.9 - 1.1)	0.9 (0.9 - 1)	< **0.01**	**<0.01**	**0.007**	0.996
ASP [n (%)]	2 (9.5%)	19 (29.7%)	16 (32%)	3 (21.4%)	0.117	0.215	0.526	0.526

Data are presented as number, n, (percentage, %), mean ± standard deviation or median (interquartile range) depending on distribution; p values were adjusted for multiple comparisons with Benjamini-Hochberg adjustment (for qualitative variables) and TukeyHSD test (for quantitative variables); bold font denotes significant (<0.05) p values; *only nondiabetic patients were included in SCORE2/-OP risk estimation (n= 23, 52, 39, and 13, respectively for Cont., AI, NFAI and MACS patients); #LVH was defined as values of LVMI exceeding 95 or 115 g/m2 in females and males respectively; † dipper status was considered only in patients with HT. ASP, atherosclerotic plaques; BMI, body mass index; CIMT, carotid intima media thickness; con., controls; DBP, diastolic blood pressure; DMt2, diabetes mellitus t2; DST, dexamethasone suppression test; FHS-ASCVD Risk – 10-year atherosclerotic cardiovascular disease risk calculated based on the Framingham Heart Study; HDL-C, high density lipoprotein cholesterol; HT, hypertension; hs-CRP, high sensitivity C-reactive protein; IVSd, interventricular septal end diastole; LAVI, left atrial volume index; LDL-C, low density lipoprotein cholesterol; LVH, left ventricular hypertrophy; LVIDd, left ventricular internal diameter end-diastole; LVIDs, left ventricular internal diameter end systole; LVM, left ventricular mass; LVMI, LVM index; LVPWd, left ventricular posterior wall end diastole; MetS, metabolic syndrome; SBP, systolic blood pressure; SCORE2/-OP, Systematic Coronary Risk Estimation 2/-Older People; TC, total cholesterol; TGL, triglycerides, UA, uric acid.

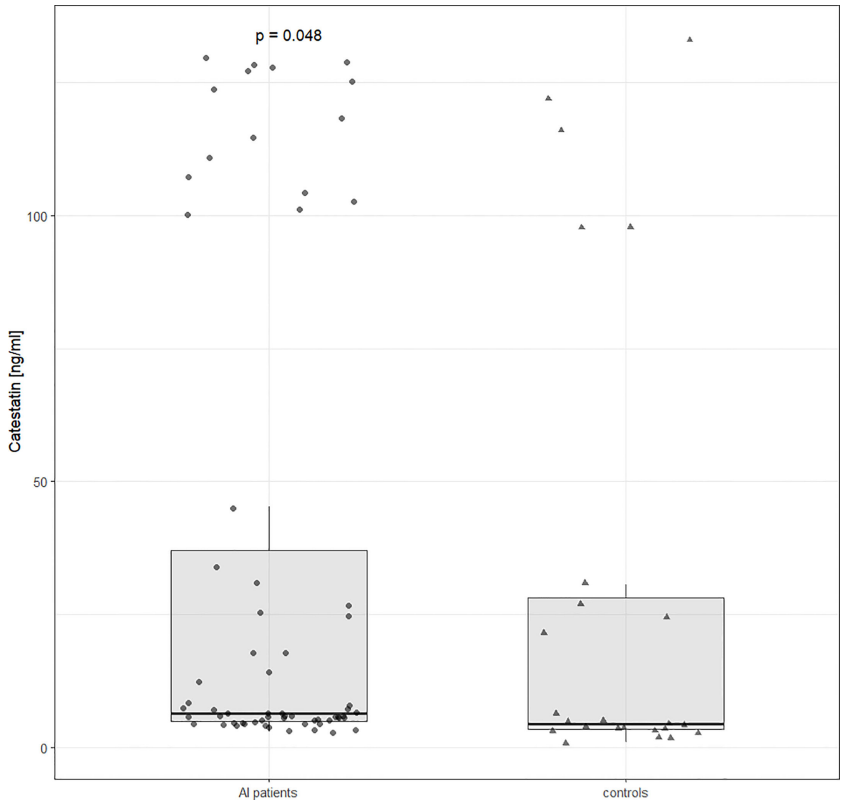

FIGURE 1
Catestatin distribution in controls and AI patients. Boxplot and data distribution with dots (AI patients) and triangles (controls) indicating individual datapoints. Unadjusted p value was determined using the Mann Whitney U test. AI, adrenal incidentaloma.

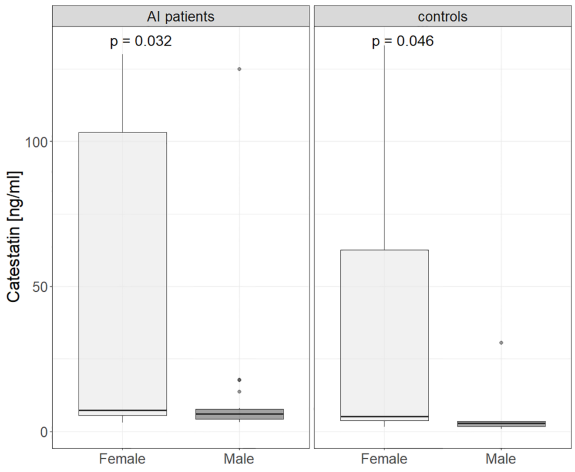

FIGURE 2
Catestatin in male and female controls and AI patients. Boxplot chart. P-value was determined using the Mann-Whitney U test. AI, adrenal incidentaloma.

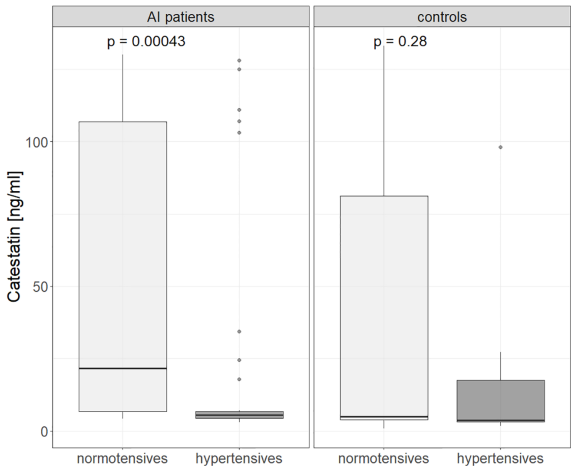

FIGURE 3
Catestatin in normotensive and hypertensive in controls and AI patients. Boxplot chart. P-values were determined using the Mann-Whitney U test. AI, adrenal incidentaloma.

3.3 Correlations between catestatin and laboratory, TTE, and CCA USG parameters

To further investigate associations between Cts and CVD risk, correlations were tested between peptide's levels and other parameters. In AI patients, weak correlations were found between Cts and: BMI (r=-0.31) (Figure 5A), FHS-ASCVD Risk (r=-0.42) (Figure 5B), and HDL-C (r=0.32) regardless of statin therapy (Figure 5C). Interestingly, among participants without it, there were also positive correlations between Cts and: TC and LDL-C

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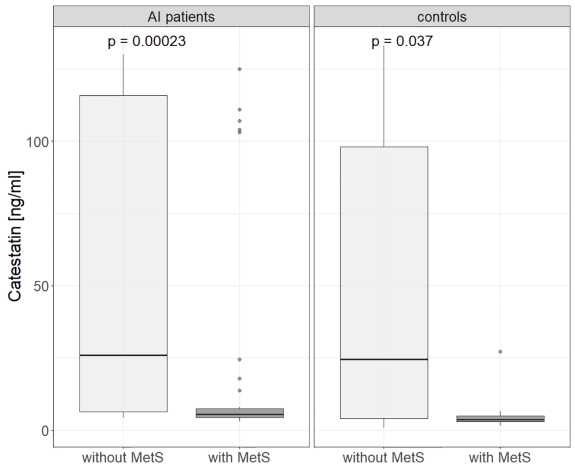

FIGURE 4
Catestatin in subjects without and with metabolic syndrome in controls and AI patients. AI, adrenal incidentaloma; MetS, metabolic syndrome. P-values determined using the Mann-Whitney U test.

(r=0.36 for both) (Table 2 and Supplementary Figures 3, 4). In AI patients and controls analyzed as a whole a negative correlation between Cts and UA was also observed (r=-0.27, p=0.01), while for each group analyzed separately, significance has not been reached probably due to sample size.

Analyses in subjects of each sex revealed Cts correlated with HDL (r=0.31, p=0.014), BMI (r=-0.29, p=0.019), and UA (r=-0.27, p=0.031) in women, but not in men (respectively r=0.13, p=0.53; r=-0.06, p=0.78; r=-0.12, p=0.56). A negative correlation between Cts and FHS-ASCVD Risk was also recorded in women with an AI (r=-0.3, p=0.049) but not in female controls (r =0.025, p=0.92), nor men with an AI (n=19, r=- 0.37, p=0.12). Correlations for male controls were not tested due to a low number of these subjects (n=5).

Concerning hormonal tests, only a weak correlation between Cts and DRC was observed, but not with aldosterone, nor ADRR (Supplementary Table 2). Cts did not correlate with ABPM, CCA USG, nor TTE parameters (LVPWd, IVSd, LVMI, LAVI) (Table 2 and Supplementary Table 2).

3.4 Clinical, laboratory, and TTE parameters according to catestatin categories

Further analyses were performed among AI patients based on Cts categories. First, adjustment for gender, age and BMI in binary logistic regression analysis revealed AI patients in the lower half of Cts concentrations (median 6.5, IQR 4.9-37 ng/ml) compared to those in the upper had a higher prevalence of HT (OR 0.17, CI 0.05-5.37, p=0.003), and MetS (OR 0.21, CI 0.06-7.51, p=0.018). Moreover, BMI, 24-SBP, and FHS-ASCVD Risk were also higher in the former (respectively 30.1 ± 4 vs. 27.2 ± 3.6 kg/m^2, p=0.004; 123 ± 7.4 vs. 117 ± 9.5 mmHg, p=0.022; 13.2%(8.9-19.2) vs. 6.3% (4.2-10.8), p=0.002), as summarized in Table 3.

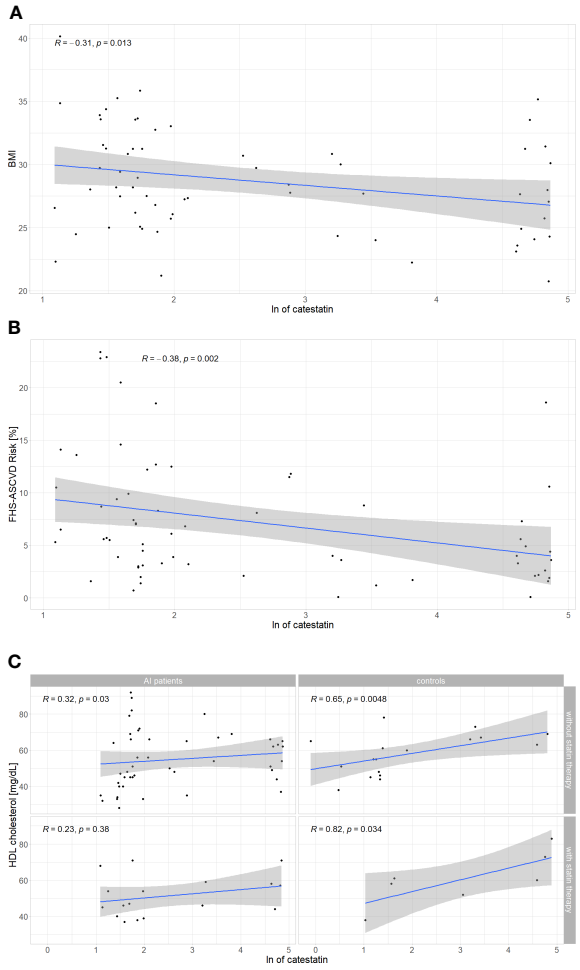

FIGURE 5
Correlations between catestatin and: **(A)** BMI in AI patients; **(B)** FHS-ASCVD Risk in AI patients; **(C)** HDL-C in AI patients and controls. Correlations were computed by the Spearman rank-order method. For HDL-C, correlations were tested separately in subjects with and without statin therapy. AI, adrenal incidentaloma, BMI, body mass index, FHS-ASCVD Risk - 10-year atherosclerotic cardiovascular disease risk calculated based on the Framingham Heart Study; HDL-C, high density lipoprotein cholesterol, ln, natural logarithm.

Second, based on Cts distribution, we divided AI participants into four subgroups, i.e. with: 'very low' (Cts <4.9 ng/ml, n=17), 'low' (≥4.9 and <6.5 ng/ml, n=15), 'intermediate' (≥6.5 and ≤45.2 ng/ml, n=17), and 'high' (≥100 ng/ml, n=15) Cts levels (Table 3). The first two comprised subjects from two lower quarters, while the 'high Cts' subgroup corresponded to almost all patients in the fourth quarter (15 instead of 16 patients were included since there were none in the 45.2 - 100 ng/ml range, see Figure 1).

These four Cts subgroups differed significantly in male-to-female ratio, prevalence of HT and MetS, mean/median BMI, HDL-C, 24h SBP, and FHS-ASCVD Risk (Table 3). *Post hoc* analysis revealed male gender was more prevalent in the 'very low' versus 'high' Cts subgroup (53% vs. 6.7%), while HT and MetS in the 'very low' versus 'intermediate' (82.4% vs. 35.3%, p=0.04 for both) and 'high' Cts subgroups (82.4% vs. 33.3%, p=0.04 for both). HDL-C was lower in the 'very low' than in the three remaining Cts subgroups (42.9 ± 42.9 vs. 61.6 ± 17.8, 56.7 ±

TABLE 2 Correlations between catestatin and examined parameters.

Correlation between Cts and	Control group (n = 24)		AI patients (n = 64)		Both groups (n = 88)	
	r	p	r	p	r	p
Age	0.246	0.247	-0.142	0.262	-0.016	0.7
BMI	-0.293	0.164	**-0.308**	**0.013**	**-0.27**	**0.009**
HDL-C	**0.704**	**< 0.001**	**0.306**	**0.014**	**0.344**	**0.001**
HDL-C *	**0.649**	**0.005**	**0.317**	**0.03**	**0.317**	**0.011**
LDL-C	0.102	0.634	0.15	0.236	0.153	0.15
LDL-C *	**0.573**	**0.016**	**0.361**	**0.003**	**0.361**	**0.003**
TC	0.215	0.313	0.118	0.353	0.16	0.137
TC *	**0.568**	**0.017**	**0.295**	**0.044**	**0.364**	**0.003**
TGL	-0.216	0.145	-0.19	0.131	-0.143	0.183
TGL *	-0.085	0.746	0.215	0.313	-0.142	0.263
UA $	-0.37	0.07	-0.209	0.1	**-0.27**	**0.01**
hs-CRP	0.191	0.407	0.005	0.97	-0.045	0.68
LVMI	0.149	0.488	-0.009	0.942	0.05	0.645
LAVI	0.13	0.545	-0.202	0.109	-0.121	0.263
Maximum CIMT	-0.09	0.697	-0.136	0.286	-0.03	0.774
SCORE2/-OP #	0.127	0.563	- 0.064	0.652	0.05	0.651
FHS-ASCVD Risk [%]	-0.237	0.265	**-0.42**	**< 0.001**	**-0.24**	**0.022**

Correlations were computed by the Spearman rank-order method; bold font denotes statistically significant correlations; *denotes scorrelations in participants without statin therapy: n=17 and 47, respectively for controls and AI patients; $ patients with and without medications that could lower uric acid (allopurinol, n = 1) were analyzed separately and the results were the same; # denotes correlations in nondiabetics only: n= 23 and 52, respectively for controls and AI patients; BMI, body mass index; CIMT, carotid intima media thickness; FHS-CVD, 10-year atherosclerotic cardiovascular disease risk calculated based on the Framingham Heart Study; HDL-C, high density lipoprotein cholesterol; hs-CRP, high sensitivity C-reactive protein; LAVI, left atrial volume index; LDL-C, low density lipoprotein cholesterol; LVMI, left ventricular mass index; r, correlation coefficient; SCORE2/-OP, Systematic Coronary Risk Estimation 2/-Older People; TC, total cholesterol; TGL, triglycerides; UA, uric acid.

13.7, and 55.8 ± 9.5 mg/dL, adjusted p=0.001, 0.01, and 0.03, respectively). What is more, 24h SBP in the 'low' Cts subgroup was higher than in the 'intermediate' (124.7 ± 6.7 vs. 114.5 ± 8.5 mmHg, adj. p=0.008).

Ten-year FHS-ASCVD Risk in the 'very low' Cts subgroup was higher than in the 'intermediate' and 'high': 14.3% (19.2-24.5) vs. 7.1% (4.7-14.2) and 5.6% (3.8-9.8), respective adjusted p=0.014 and 0.005, in line with differences in gender proportions, prevalence of metabolic disorders and HT between the subgroups.

Fisher's exact test revealed differences in LVH prevalence between four Cts subgroups, yet, without significance in pairwise comparisons with correction for multiple testing (Holm–Bonferroni method). No other significant differences were recorded between Cts halves and subgroups in TTE and CCA USG parameters (Table 3). Finally, clinical, laboratory and TTE parameters were also analyzed in the same Cts subgroups for females only (Supplementary Table 3). Significant differences were recorded for: HT and MetS prevalence, HDL-C and hs-CRP concentrations, mean 24h DBP, and FHS-ASCVD Risk. Calculations for male AI subjects were not performed due to their low number.

4 Discussion

Clinical research on Cts is scarce, even though it deserves attention due to its protective effects on the CV system demonstrated *in vitro* and *in vivo*. To our knowledge, our study is the first to examine Cts in patients with an AI, and to show lower Cts in adult patients with MetS than those without it, as well as correlations between Cts and FHS ASCVD risk index.

A thorough assessment was undertaken to investigate associations between Cts and CV risk factors. It must be highlighted that Cts changes dynamically in response to sympathetic nervous system activation in a negative feedback mechanism (25). Also, multiple diseases and drugs lead to CgA secretion, which may affect the concentration of its derivatives, including Cts. For these reasons, we excluded patients with established CVD, stage 3-5 chronic kidney disease, cancer, etc., and controlled the use of PPIs (10, 12, 26, 27). Limitations of our study include a small, heterogeneous patient sample, lack of CgA determination (Cts : CgA ratios may have provided further insights) and hormonal work-up in controls.

Since CgA is not expressed in the adrenocortical adenoma tissue, Cts levels are unlikely to differ between subjects with and

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TABLE 3 Clinical, laboratory, ABPM, echocardiographic, and CCA sonography parameters in AI patients according to catestatin category.

Cts half [ng/ml]	Lower (Cts < 6.5)		Upper (Cts ≥6.5)		p subgroups	p halves
Cts subgroup [ng/ml]	Very low (< 5)	Low (5 ≤ Cts < 6.5)	Intermediate (6.5 ≤ Cts ≤ 45.2)	High (Cts ≥ 100)		
n	17	15	17	15	-	-
F:M ratio	8:9	12:3	11:6	14:1 *	**0.027**	0.274
Age [years]	61.9 ± 9.4	61.7 ± 5.9	60.7 ± 11.5	59.2 ± 7.2	0.827	0.412
BMI [kg/m²]	30.4 ± 4.6	29.9 ± 3.4	27.1 ± 3.2	27.4 ± 4.2	**0.034**	**0.004**
Obesity [n (%)]	8 (47.1%)	8 (53.3%)	3 (17.7%)	5 (33.3%)	0.158	0.07
Smokers [n (%)]	7 (41.2%)	9 (40%)	5 (29.4%)	7 (46.7%)	0.378	0.45
PPI therapy [n (%)]	4 (23.5%)	4 (26.7%)	2 (11.8%)	3 (20%)	0.793	0.536
HT [n (%)]	14 (82.4%)	11 (73.3%)	6 (35.3%) *	5 (33.3%) *	**0.005**	< **0.001**
>1 hypotensive drug [n(%)] †	7 (50%)	7 (63.6%)	3 (50%)	1 (20%)	0.495	0.470
DMt2 [n (%)]	6 (35.3%)	4 (26.7%)	1 (5.9%)	1 (6.7%)	0.09	**0.022**
MetS [n (%)]	14 (82.4%)	11 (73.3%)	6 (35.3%) *	5 (33.3%) *	**0.005**	< **0.001**
Statin use [n (%)]	6 (35.3%)	3 (20%)	4 (23.5%)	4 (26.7%)	0.837	1
HDL-C [mg/dL]	42.9 ± 10.8	61.6 ± 17.8 *	56.7 ± 13.7 *	55.8 ± 9.5 *	**0.001**	0.076
LDL-C [mg/dL]	124 ± 49.4	121 ± 41.6	127 ± 36.3	146 ± 63.6	0.491	0.27
TC [mg/dL]	202 ± 52.6	206 ± 51	211 ± 41	225 ± 68.4	0.677	0.31
TGL [mg/dL]	143 (98 - 198)	133 (118 - 141)	121 (87 - 168)	105 (87.5 - 142)	0.159	0.36
Uric acid [mg/dL]	5.8 (4.9 - 6.8)	4.8 (4.1 - 5.8)	5.1 (4.2 - 6.1)	4.9 (4.3 - 5.8)	0.112	0.36
MACS [n (%)]	4 (23.5%)	4 (26.7%)	5 (29.4%)	1 (6.7%)	0.417	0.763
Hs-CRP [mg/L]	1.2 (0.8 - 3.9)	1.5 (1 - 2.1)	0.8 (0.6 - 1.5)	1.2 (0.8 - 2)	0.243	0.16
24h SBP [mmHg]	120.8 ± 7.8	124.7 ± 6.7	114.5 ± 8.5 #	119.2 ± 10.5	**0.01**	**0.009**
24h DBP [mmHg]	71.1 ± 9.6	74.9 ± 5.9	68.2 ± 6.1	72 ± 7.9	0.126	0.13
24h PR [bpm]	71.2 ± 9.8	72.9 ± 7.5	71.2 ± 6.5	72 ± 8.3	0.929	0.84
Non-dipper status [n (%)] †	4 (28.6%)	2 (18.2%)	1 (16.7%)	2 (40%)	0.723	0.685
LVMI [g/m^2]	86.1 ± 18.1	84.2 ± 17	86.6 ± 19	88.9 (± 24.0)	0.931	0.61
LVH [n (%)]	1 (5.9%)	3 (20%)	2 (11.76%)	7 (46.7%)	**0.036**	0.213
LAVI [ml/m^2]	26.6 ± 9.6	24 ± 8.6	24 ± 9.44	20.3 ± 7.1	0.258	0.2
Maximum CIMT [mm]	1 (0.9 - 1.2)	1 (0.9 - 1.1)	1 (0.9 - 1.1)	0.9 (0.9 -1.1)	0.685	0.393
ASP [n(%)]	7 (41.2%)	4 (26.7%)	4 (23.5%)	4 (26.7%)	0.709	0.585
SCORE2/-OP [%] ‡	8 (7 - 12)	12 (8 - 15.5)	7 (5 - 14.5)	8.5 (8 - 11.5)	0.572	0.29
FHS-ASCVD Risk [%]	14.3 (9.2 - 24.5)	11.2 (7.8 - 16.2)	7.1 (4.7 - 14.2)*	5.6 (3.8 - 10)*	**0.003**	**0.002**

AI patients were categorized based on catestatin concentrations into those in the lower and upper half, and further into four subgroups (two lowest were identical with first and second quartiles). Data are presented as number (percentage), mean ± standard deviation or median(interquartile range) depending on distribution; p-values were calculated with one-way ANOVA and Tukey's HSD tests (quantitative variables) or Fisher's exact test (categorical variables) with Benjamini-Hochberg correction for multiple testing. Bold font denotes significant (<0.05) p values; * denotes datapoints significantly different versus those for the 'very low' Cts subgroup in post hoc test; # denotes datapoints significantly different versus those for the 'low Cts' subgroup in post hoc test; † dipper status was considered only in patients with HT. ‡ Only nondiabetic patients were included in SCORE2/–OP risk estimation (n=11, 11, 16, and 14, for consecutive subgroups). ABPM, ambulatory blood pressure monitoring; ASP, atherosclerotic plaques; BMI, body mass index; bpm, beats per minute; CCA, common carotid artery; CIMT, carotid intima media thickness; DBP, diastolic blood pressure; DMt2, diabetes mellitus type 2; FHS-ASCVD Risk, 10–year atherosclerotic cardiovascular disease risk calculated based on data from the Framingham Heart Study; HDL-C, high density lipoprotein cholesterol; HT, hypertension; hs–CRP, high sensitivity C–reactive protein; LDL-C, low density lipoprotein cholesterol; LVH, left–ventricular hypertrophy; LVMI, left ventricular mass index; max, maximum; MACS, mild autonomous cortisol secretion; MetS, metabolic syndrome; PPI, proton pump inhibitor; PR, pulse rate; SCORE2/–OP, Systematic Coronary Risk Estimation 2/–Older People; SBP, systolic blood pressure; TC, Total cholesterol; TGL, triglycerides.

without an adrenal adenoma (28, 29), which is what we found here for AI patients compared to age-, sex-, and BMI-matched controls after adjusting for confounding factors. Other researchers showed higher plasma CgA in patients with an adrenal adenoma than in subjects without one, which may underlie a slightly higher unadjusted Cts in our AI patients than controls (24, 25). What should be noticed is a similar distribution of Cts levels in controls and AI patients, including the proportion of subjects with high (>97 ng/ml) Cts: 21% in the former and 23% in the latter. This further suggests that the presence of an AI does not affect Cts. Regarding hormonal activity, we found no differences in Cts between patients with MACS and NFAI, nor correlations between Cts and UFC or 1-mg DST cortisol, possibly due to small sample size.

Despite comparable age, BMI, male-to-female ratio, smoking status and comorbidities, IVSd, LVPWd, LVM, and LVMI were higher in AI patients than controls, which was driven by values recorded in subjects with MACS. Iacobellis et al. reported similar results (higher LVM in AI subjects versus controls, the difference depended on patients with MACS) (30). In our study, SCORE2/-OP was higher in MACS compared to NFAI patients and controls, which indicates increased CV risk associated with subclinical hypercortisolemia. The data support the hypothesis that chronic mild elevation of cortisol levels in AI patients adversely affect the CV system rather than the presence of an adrenal adenoma *per se*. Low Cts was associated here with a higher prevalence of male gender, HT, MetS, as well as BMI, 24h SBP, UA and lower HDL-C. Consequently, an association between Cts and ASCVD risk was recorded (Figure 6). In line with our results, Cts was lower in obese children with MetS than in those without it, and in normal-weight controls (13); O'Connor et al. showed Cts correlated negatively with BMI (12), and Durakoğlugil et al. reported a positive correlation between Cts and HDL-C (14). In the latter study, a negative correlation between plasma Cts and TGL concentration was also observed (14), which was not confirmed here. Surprisingly, among participants without statins, we recorded weak positive correlations between Cts and TC as well as LDL-C. The former may be connected with a positive correlation between Cts and HDL-C, however, the latter is difficult to explain, and requires further clarification.

To date, there are controversies regarding Cts in HT; lower Cts levels have been associated with this disease (12, 14, 31–35) (Figure 6). Here, Cts in AI subjects with HT was lower than in normotensive ones, however, the difference was not significant in the controls and we recorded no correlations between Cts and ABPM results. Our data add important facts to the discussion: low Cts levels are more common in HT, however, some patients do exhibit intermediate and high concentrations of the peptide. This was observed in individuals with effective hypotensive treatment revealed by 24-h monitoring.

Further, no significant associations were recorded here between Cts and TTE as well as CCA USG parameters. Small sample size clearly limits conclusions that can be drawn from these data. More sensitive methods (e.g. global longitudinal strain and microvasculature assessment) may have yielded different results.

Possibly, the most intriguing question is the clinical significance of high versus very low/low Cts in individuals with similar established CV risk factors. For instance, non-smoking females aged ca. 60, with overweight and HT (FHS-ASCVD Risk between 10 and 20%) were recorded both in the first half and highest quarter of Cts levels among AI patients. Longitudinal assessment of much larger populations is required to determine whether Cts provides protection against CVD. If so, determining therapeutic strategies that stimulate Cts would be beneficial.

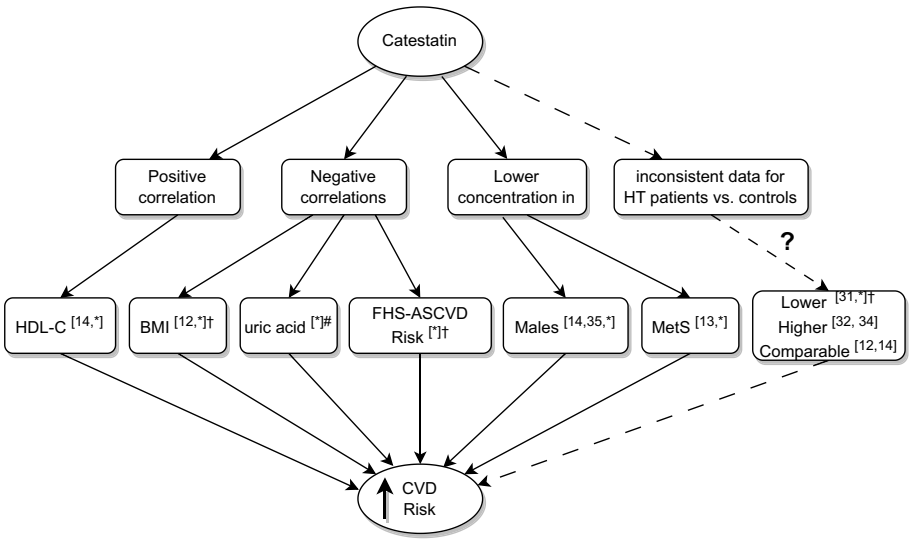

FIGURE 6
Summary of research on associations between low catestatin and cardiovascular risk. Superscript numbers indicate references to previous studies; *indicate results of the current study; #correlation between uric acid and Cts did not reach significance in AI patients analyzed here, it did upon analyzing AI patients and controls together; †significance achieved in AI patients. AI, adrenal incidentaloma, BMI, body mass index; Cts, catestatin; CVD, cardiovascular disease; FHS-ASCVD Risk, 10-year atherosclerotic cardiovascular disease risk calculated based on the Framingham Heart Study; HDL-C, high density lipoprotein cholesterol; HT, hypertension; MetS, Metabolic Syndrome.

5 Conclusions

We are the first to report that among persons without overt CVD other than primary HT, plasma Cts concentrations in patients with an AI are comparable to those of matched controls with normal adrenal morphology. Correlations between Cts and: HDL-C (positive) as well as BMI, UA and FHS-ASCVD Risk (negative) point at cardioprotective effects of the peptide. Data from ABPM, TTE and CCA intima-media assessment did not yield associations between Cts and BP or HT-mediated organ damage. It must be highlighted that many factors influence Cts, and further research is necessary to apply it as a biomarker.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Independent Bioethics Committee for Scientific Research at Medical University of Gdańsk (NKBB/659/2019). The patients/participants provided their written informed consent to participate in this study.

Author contributions

EZ secured ethical approval for the study, analyzed the data, and reviewed the literature. EZ and PK collected the data and wrote the manuscript. JS and AK performed echocardiography and ultrasound examination of the common carotid artery. PK and KS carried out critical interpretations. All authors contributed to the article, approved the submitted version, and are accountable for the content of the work.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1198911/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Catestatin in normotensive and hypertensive AI patients and controls - analyses in subjects of each sex. Boxplot chart. P-values determined using the Mann-Whitney U test. AI – adrenal incidentaloma.

SUPPLEMENTARY FIGURE 2
Catestatin in subjects without and with metabolic syndrome in controls and AI patients - analyses in subjects of each sex. Boxplot chart. P-values determined using the Mann-Whitney U test. AI – adrenal incidentaloma; MetS – metabolic syndrome.

SUPPLEMENTARY FIGURE 3
Correlations between catestatin and LDL-C level in AI patients and controls. Correlations were computed by the Spearman rank-order method and were tested separately in subjects with and without statin therapy. AI – adrenal incidentaloma, LDL-C – low density lipoprotein cholesterol, ln – natural logarithm.

SUPPLEMENTARY FIGURE 4
Correlations between catestatin and total cholesterol level in AI patients and controls. Correlations were computed by the Spearman rank-order method and were tested separately in subjects with and without statin therapy. AI – adrenal incidentaloma, ln – natural logarithm.

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REVIEW
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Role of Catestatin in the Cardiovascular System and Metabolic Disorders

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Catestatin is a multifunctional peptide that is involved in the regulation of the cardiovascular and immune systems as well as metabolic homeostatis. It mitigates detrimental, excessive activity of the sympathetic nervous system by inhibiting catecholamine secretion. Based on *in vitro* and *in vivo* studies, catestatin was shown to reduce adipose tissue, inhibit inflammatory response, prevent macrophage-driven atherosclerosis, and regulate cytokine production and release. Clinical studies indicate that catestatin may influence the processes leading to hypertension, affect the course of coronary artery diseases and heart failure. This review presents up-to-date research on catestatin with a particular emphasis on cardiovascular diseases based on a literature search.

Keywords: catestatin, cardiovascular system, hypertension, heart failure, coronary artery disease, immunometabolism, metabolic disorder

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INTRODUCTION

The sympathetic nervous system is crucial in preserving homeostasis in humans. However, its excessive activity has been recognized to underlie pathologic processes of many cardiovascular diseases (CVDs), which are the leading cause of death globally (1). Among others, upregulated sympathetic nervous system activity has been associated with hypertension (HT), adverse myocardial remodeling, arrhythmias, sudden cardiac death, and overall poor prognosis in patients with heart failure (HF) (2).

Basal and reflex control of the sympathetic activity associated with cardiovascular function occurs in the rostral ventrolateral medulla (3). It sends catecholaminergic projections to the sympathetic preganglionic neurons of the spinal cord. In turn, preganglionic neurons release acetylcholine to activate postganglionic neurons and chromaffin cells of the adrenal medulla, which synthesize and secrete catecholamines (CAs): norepinephrine and epinephrine (stored in sufficient quantities within the cells, which is the reason for a positive chromaffin reaction, and, hence, their name) (3, 4). CAs target α- and β-adrenergic receptors, a family of G protein-coupled receptors. Adrenoceptors are divided into α1, α2, β1, β2, and β3 subtypes. In the smooth muscle cells of blood vessels, there are mainly α1-adrenergic receptors, coupled to stimulatory Gq proteins, which induce constriction by activating phospholipase C. In the heart, the predominant receptor subtype β1 activates the Gs-adenylyl cyclase – adenosine monophosphate – protein kinase A signaling cascade to induce positive inotropic and chronotropic effects (5). α2 receptors are coupled to inhibitory Gi proteins that inactivate adenylyl cyclase and are mainly found in the central nervous system, where their activation lowers arterial blood pressure (BP) (5).

In 1965, Banks and Helle reported that CA secretion from chromaffin granules of the adrenal medulla is associated with the release of soluble proteins (6). In July 1967, Blaschko's group coined the term "chromogranin A" (CgA) for the major component of these proteins (7, 8). In 1988, Simon et al. reported for the first time that proteolytic hydrolysis of CgA, obtained from cultured bovine adrenal medullary chromaffin cells, generated a peptide product that is capable of inhibiting CAs release, a promising and novel mechanism for counteracting the sympathetic outflow (9, 10). However, the exact identity of this functional peptide remained elusive until 1997, when Mahata et al. synthesized 15 peptides spanning ∼80% of the entire CgA molecule and demonstrated that only one, bovine $CgA_{344-364}$ [RSMRLSFRARGYGFRGPGLQL], inhibited CA secretion induced by nicotine (11). They named it "catestatin" (Cts) due to its high capacity to suppress the release of CAs (11).

Cts was initially considered a regulatory peptide that inhibits CA secretion by acting as a noncompetitive mediator of the nicotinic cholinergic stimulation of chromaffin cells and sympathetic neurite outgrowth (11, 12). Further studies showed that Cts inhibits CA release also due to adenylate cyclase-activating polypeptide stimulation and regulates dense core vesicle quanta (13) (**Figure 1**). Moreover, Cts has emerged as a pleiotropic peptide, which – among others – is involved in the regulation of the cardiovascular and immune system, as well as metabolic homeostasis (14, 17, 25–27).

This review presents up-to-date research on the role of Cts in the cardiovascular system and metabolic disorders contributing to CVDs, and Cts as a putative clinical biomarker. It is based on an electronic literature search of PubMed database performed April 20, 2022, using the key term 'catestatin', which yielded 235 results. Papers were included based on screening of titles and abstracts.

CATESTATIN BIOLOGY

Cts (human $CgA_{352-372}$, bovine $CgA_{344-364}$, and rat $CgA_{367-387}$) is a neuroendocrine peptide that is derived from the proteolytic cleavage of its precursor compound CgA. Human chromogranin A (hCgA) consists of nine dibasic sites and is cleaved by prohormone convertases, namely, cathepsin L, plasmin, and kallikrein, which generates: 1) Cts, which is a 21-amino acid, hydrophobic peptide derived from hCgA's C terminal fragment, 2) a dysglycemic peptide pancreastatin (hCgA250–301), 3) a vasodilating, antiadrenergic, and antiangiogenic peptide vasostatin-1 (hCgA1–76), 4) a peptide that acts as an antigen for diabetogenic CD4+ T-cell clones WE14 (hCgA324–337), and, 5) a proadrenergic peptide serpinin (hCgA411–436) (15).

Abbreviations: Akt, Protein kinase B; AMI, acute myocardial infarction; BP, blood pressure; Ca^{2+}, calcium ions; CAD, coronary artery disease; CA, catecholamine; CgA, Chromogranin A; Cts-KO, catestatin knockout; CVD, cardiovascular disease; Chga-KO, Chromogranin knockout; Cts, catestatin; hCgA, human CgA; HF, heart failure; HT, hypertension; I/R injury, ischemia/reperfusion injury; nAChR, nicotinic acetylcholine receptor; NO, nitric oxide; nNOS, neuronal nitric oxide synthase; NYHA, New York Heart Association; OSA, obstructive sleep apnea; PMNs, Polymorphonuclear neutrophils; WT, wild type.

Cts includes a sequence [SSMKLSFRARAYGFRGPGPQL] that is highly conserved across various species and is flanked by proteolytic cleavage sites (11, 28). Five single-nucleotide polymorphisms have been identified in the hCgA gene *(CHGA)* region expressing Cts: Gly364Ser (rs9658667), Pro370Leu (rs965868), Arg374Gln (rs9658669) (29), Tyr363Tyr (rs9658666), and Gly367Val (rs200576557) (15, 30, 31). Intriguingly, a genome-wide association study identified two loci that affect Cts concentrations in regions that include genes encoding kallikrein and Factor XII; these enzymes participating in a proteolytic cascade (Factor XII activates prokallikrein to kallikrein, which activtes FXII) were shown to generate active compounds from chromogranin A and B cleavage *in vivo* and *in vitro* (32). In particular, kallikrein produced a 12-amino-acid CgA-fragment (CgA361-372) with preserved biological activity of 21-amino-acid Cts (32).

Apart from limiting CA secretion, Cts is a potent inhibitor of the release of other chromaffin cell cotransmitters, including neuropeptide Y, adenosine triphosphate, and CgA; it is widely distributed in secretory granules of the chromaffin cells, diffuse neuroendocrine system, neuronal cells, bone-marrow derived cells, the auditory system, and the heart (20, 24, 33, 34) (**Figure 1**). In the latter, processing of CgA can be carried out by extracellular proteases on both cardiomyocyte cell membrane and in the extracellular matrix (33, 35). In a rat model, it was shown that the heart generates intracardiac CgA fragments, including locally derived Cts, in response to hemodynamic and excitatory challenges (36). Moreover, recently, a CgA1-373 fragment that encompasses the Cts domain was found to elicit direct cardiac effects both *in vitro* and *ex vivo* (37).

CATESTATIN AND REGULATION OF BLOOD PRESSURE

In vitro and *in vivo* Animal Studies

In vitro, it was shown Cts binds to α, β, δ, and γ subunits of nicotinic acetylcholine receptors (nAChR) with a high affinity binding site on the β subunit near the membrane surface, which blocks sodium ions' uptake, thus inhibiting membrane depolarization, and blocking the influx of calcium ions (Ca^{2+}) through voltage-gated calcium channels (38, 39). Inhibition of Ca^{2+} influx suppresses both CA release by exocytosis (all-or-none secretion) and *CHGA* transcription through a pathway involving the activation of protein kinase C and mitogen-activated protein kinase (40) (**Figure 1**).

Human Cts polymorphic variants were shown to exhibit varying potency of nAChR inhibition *in vitro* using a rat pheochromocytoma cell line and the human receptor (30, 41, 42). One should bear in mind that lower potency of a Cts variant in this respect does not necessarily translate to higher plasma CAs, since in such a case lower desensitization to CA secretion occurs (i.e., an effect due to repeated exposure of the nAChR to Cts). Further, since Cts blood-lowering effect depends partly on the release of nitric oxide (NO), human Cts Gly364Ser variant (see Clinical Studies) was shown to display lower NO-triggering and

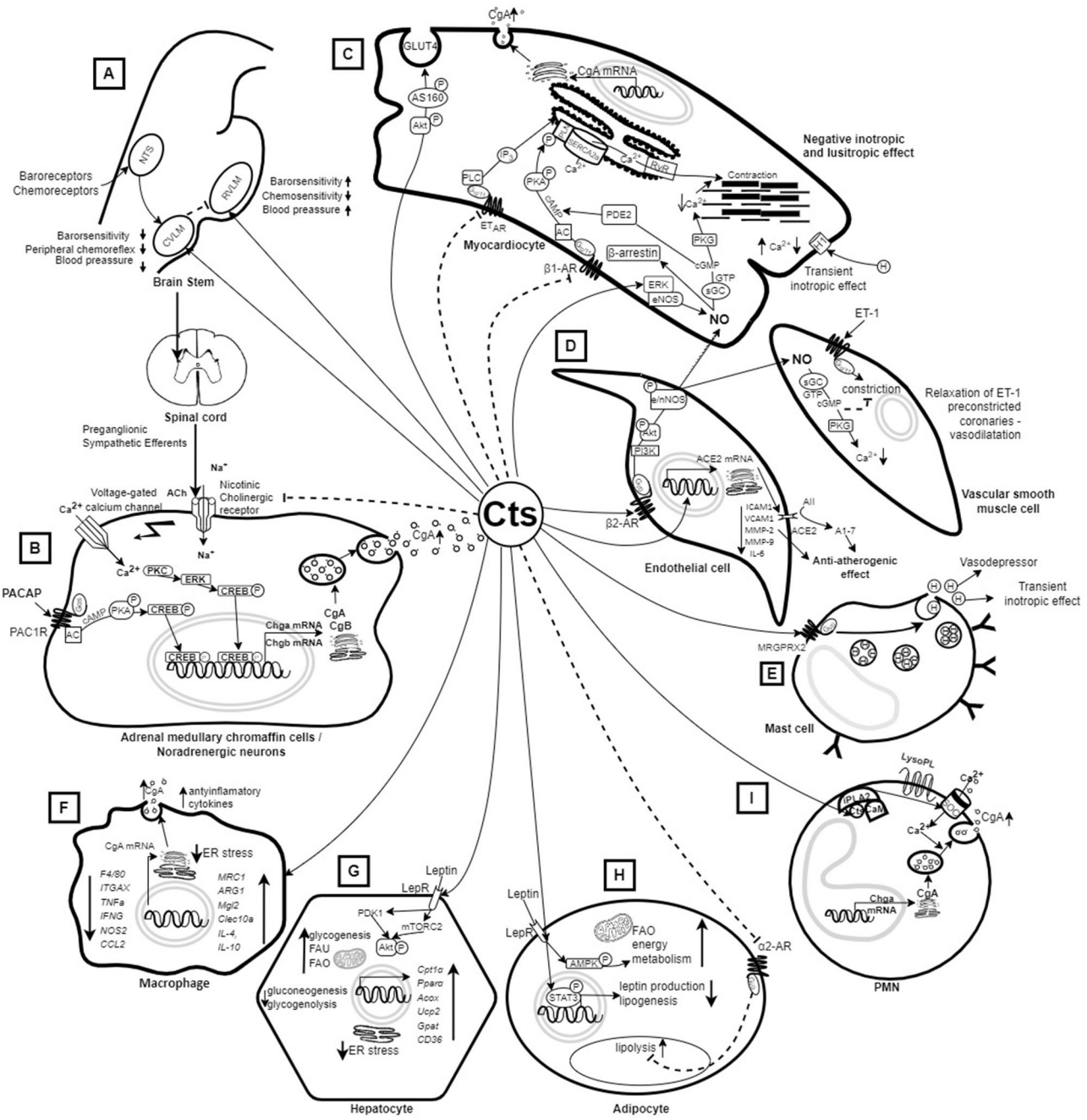

FIGURE 1 | Mechanism of action of catestatin based on *in vitro* and *in vivo* animal studies. **(A)** Injection of Cts into the CVLM or the central amygdala (not shown) of rats decreases sympathetic barosensitivity and attenuates peripheral chemoreflex with consequent hypotension. On the other hand, injection of Cts into the RVLM increases barosensitivity and attenuates chemosensitivity with consequent elevation of blood pressure (14). **(B)** Cts inhibits CA release by binding to nicotinic acetylcholine receptors that block Na^+ uptake (15) as well as due to PACAP stimulation (13). **(C)** Cts inhibits the PKA/PLN signaling pathway and induces NO synthesis in myocardiocytes, and the released NO reduces cellular Ca^{2+}, resulting in decreased cardiac contractility (16) and relaxation of ET-1 preconstricted coronaries (17). Cts also induces glucose uptake and Glut4 translocation (18). **(D)** Cts induces NO synthesis from endothelial cells, and activates ACE2, which has an anti-atherogenic effect (15, 16, 19). **(E)** Cts induces histamine release leading to vasodepression and transient inotropic effect in myocardiocytes (16). **(F)** Treatment with Cts results in polarization of macrophages toward an anti-inflammatory phenotype (20). Macrophages also produce Cts (21). **(G)** Cts up-regulates genes promoting fatty acid oxidation (22) and enhances insulin-induced Akt phosphorylation, which helps in overcoming ER stress and achieving insulin sensitivity (23). **(H)** Cts promotes lipid flux from adipose tissue toward the liver and lowers plasma leptin in Chga-KO mice leading to resensitization of leptin receptors (22). **(I)** PMNs are able to produce and secrete CgA-derived peptides, including Cts, which may penetrate into PMNs and activate the release of innate immune factors (24). A1-7, Angiotensin 1-7; AII, Angiotensin II; AC, Adenylyl cyclase; ACE2, activates angiotensin-converting enzyme-2; Ach, acetylcholine; Acox1, acyl-CoA oxidase 1;

(Continued)

FIGURE 1 | Akt, Protein kinase B; AMPK, AMP-activated protein kinase; ARG1, Arginase 1 gene; β1AR, β1 adrenergic receptors; β2AR, β2 adrenergic receptors; Ca^{2+}, calcium ions; cAMP, adenosine monophosphate; CAs, catecholamines; CCL2, C-C Motif Chemokine Ligand 2; CD36, cluster of differentiation 36; CgA, Chromogranin A; CgB, Chromogranin B; Chga-KO, Chromogranin knockout; cGMP, cyclic guanosine monophosphate; Cpt1α, Carnitine palmitoyltransferase 1α; CREB, cAMP response element-binding protein; Cts, catestatin; CVLM, caudal ventrolateral medulla; DNL, *de novo* lipogenesis; eNOS, endothelial nitric oxide synthase; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinas; ET-1, endothelin 1; ETAR, Endothelin receptor type A; ETBR, Endothelin receptor type B; FAO, Fatty acid oxidation; FAU, Fatty acid uptake; Gpat4, lipogenic gene glycerol-3-phosphate acyltransferase; GTP, guanosine-5' triphosphate; H, histamine; ICAM1, Intercellular Adhesion Molecule 1; IFNG, Interferon Gamma gene; IL-4, interleukin 4; IL-6, interleukin 6; IL-10, interleukin 10; iPLA2, calcium-independent phospholipase A2; ITGAX, Integrin Subunit Alpha X; LepR, Leptin receptor; LysoPL, lysophospholipids; MMP-2 Matrix Metallopeptidase 2; MMP-9, Matrix metallopeptidase 9; MRC1, Mannose Receptor C-Type 1 gene; MRGPRX2, Mas-Related G Protein-Coupled Receptor-X2; Na+, sodium; NO, nitric oxide; NOS2, Nitric Oxide Synthase 2; nNOS, neuronal nitric oxide synthase; NTS, Nucleus tractus solitarius; P, phosphor; PACAP, Pituitary adenylate cyclase-activating polypeptide; PAC1R, Pituitary adenylate cyclase-activating polypeptide receptor; PDE2, Phosphodiesterase 2; Pi3K, Phosphoinositide 3-kinase; PKA, Protein kinase A; PKC, protein kinase C; PKG, protein kinase G; PLN, phospholamban; PMNs, Polymorphonuclear neutrophils; Pparα, Peroxisome proliferator-activated receptor-α; RVLM, rostral ventrolateral medulla; RyR, Ryanodine receptor; sGC, soluble guanylyl cyclase; SERCA, Sarcoplasmic reticulum Ca-ATPase; SOC, Store-Operated Calcium Channels; STAT3, Signal Transducer And Activator Of Transcription 3; TAG, Triacylglycerols; TNFa, TNF alpha gene; Ucp2, uncoupling protein 2; VCAM1, vascular cell adhesion molecule 1. Dashed arrow – inhibition; continuous arrow – stimulation.

absent anti-adrenergic activity compared to wild-type peptide (WT-Cts) *in vitro* (31, 43).

This *in vitro* research concerning CA release suppression by Cts is supported by *in vivo* studies with genetically modified rodents. First, knockout of the CgA gene region encoding Cts (Cts-KO) in mice resulted in HT, left ventricular hypertrophy, and elevated CAs, yet, high BP was abated by intraperitoneal injection of exogenous Cts (21). Moreover, this strain exhibited marked cardiac and adrenal macrophage infiltration as well as increased proinflammatory cytokine levels, which may trigger CA release and escalate HT (21). Chlodronate depletion of macrophages, and bone marrow transfer between Cts-KO and WT mice demonstrated that immunosuppression of macrophages by Cts partly underlies its antihypertensive and anti-inflammatory effects (21).

Second, ablation of the CgA gene in another mouse model (Chga-KO) led to – among others – high baseline BP and elevated plasma CAs as well as exaggerated pressor and depressor responses to phenylephrine and sodium nitroprusside (44–46). Exogenous Cts replacement selectively diminished stress-induced increments in BP and HR (45) and restored the sensitivity of high-pressure baroreceptors, i.e., attenuation of both reflex tachycardia (due to sodium nitroprusside-induced hypotension) and reflex bradycardia (following phenylephrine-induced HT) occured (45). This indicates Cts functions efficiently as an antihypertensive peptide even under stressful conditions.

Third, in a genomically humanized mice strain expressing insufficient hCgA amounts, increased plasma CA levels as well as elevated systolic and diastolic BP were recorded in comparison to WT controls and a strain with sufficient hCgA expression (47).

However, Kennedy et al. showed Cts did not affect plasma norepinephrine levels and actually significantly (11-fold) increased those of epinephrine following electrical stimulation of male Sprague-Dawley rats, although it did reduce BP increases (even with anti-adrenergic pretreatment) (48). The possible explanation of these results, which contrast with findings from *in vitro* and Cst- and CgA-knockout studies, is that epinephrine release was compensatory to effects triggered by Cts in acute injury by electric stimulation, which could not have been observed *in vitro* (due to study limitations) and in KO mice owing to significant genetic alterations. Mitigation of pressor responses by Cts in the study by Kennedy et al. was attributed to histamine release: it increased 21-fold within 2 min of Cts injection, and the BP lowering effect was abolished by hydroxizine pretreatment, which was also the case for epinephrine elevation (48). Krüger et al. confirmed this hypothesis *in vitro*: Cts lead to histamine release from rat mast cells *via* a peptidergic pathway – by activating the G protein, because this effect was suppressed by the pertussis toxin, a Gi/Go inactivator (49) (**Figure 1**).

Furthermore, although plasma Cts concentrations gradually increased with the progression of HT in spontaneously hypertensive rats, exogenous Cts reduced HR (indicating anti-sympathetic activity) (50), as well as ameliorated vascular, renal and cardiac proliferation (51). *In vitro*, Cts was also reported to act as a potent inhibitor of isoproterenol- and endothelin-1-mediated activities in the frog (52) and rat heart (53). Angelone et al. showed that WT-Cts increased heart rate and decreased left ventricular pressure, rate-pressure product, and, both positive and negative left ventricular contractility. The authors suggested that these negative inotropic and lusitropic effects of WT-Cts may contribute to its hypotensive action (53) (**Figure 1**).

Concerning the central nervous system, Cts plays an important role in cardiorespiratory control (14). Gaede et al. showed that Cts antagonizes both nAChR and β-adrenergic receptors involved in cardiovascular regulation using urethane-anesthetized, vagotomized rats. Cts mitigated the hypertensive effect of nicotine and prevented increased splanchnic sympathetic activity caused by isoproterenol, a non-selective β adrenergic receptor agonist (54). The results of the study indicate Cts may affect BP levels in HT (54). Further, depending on the region of the medulla, Cts exerted sympathoexcitatory or cholinergic effects (3, 55, 56). Injection of Cts into the rostral ventrolateral medulla (a key site for BP control in the brain stem) resulted in increased barosensitivity and attenuation of chemosensitivity with consequent BP elevation (3). On the other hand, injection of Cts into the caudal ventrolateral medulla (55) and the central amygdala (56) (both contain inhibitory neurons of the rostral ventrolateral medulla) of rats resulted in decreased sympathetic barosensitivity and attenuation of the peripheral chemoreflex with consequent hypotension (**Figure 1**).

Effects of Cts based on *in vitro* and *in vivo* experimental studies are presented in **Figure 1**. In summary, Cts may inhibit CA release from chromaffin cells and noradrenergic neurons (11) and induces desensitization of CAs (57); it also exhibits a potent vasodilatory effect mediated – at least in part – by histamine release (48, 49). Moreover, negative inotropic and lusitropic effects of Cts may lower BP (53). Finally, Cts plays a role in central cardiorespiratory control (3, 56). Taken together, experimental studies point to Cts as a novel regulator of BP.

Clinical Studies

On the one hand, similarly to *in vivo* studies on rodents, it was shown that infusion of Cts into the dorsal hand veins of normotensive individuals, after pharmacologic venoconstriction with phenylephrine, resulted in a dose-dependent vasodilation, which was predominantly observed in female subjects (58). On the other, clinical studies generated controversies regarding the association between Cts levels and primary HT.

O'Connor et al. showed that normotensive offspring of patients with HT had significantly lower Cts concentrations compared to normotensive participants with a negative family history of HT (1.32 ± 0.038 vs. 1.5 ± 0.076 ng/mL, $p = 0.024$). Low plasma Cts levels predicted enhanced pressor response to a sympathoadrenal stressor (59). Therefore, reduced Cts levels were postulated to predispose to the development of HT, although categorization by BP status (normotensive vs. hypertensive) did not reveal differences in plasma Cts (1.36 ± 0.03 vs. 1.26 ± 0.06 ng/mL, $p = 0.27$) (59). In line with the result, Durakoglugil et al. showed that the difference in Cts concentrations between previously untreated hypertensive patients and healthy controls was insignificant after adjusting for age, gender, height, and weight (60). However, in another study by the O'Connor group, in a larger cohort (452 normotensives, 215 primary hypertensives), Cts was significantly reduced in HT patients (1.47 ± 0.06 1.26 vs. 1.26 ± 0.08; $p = 0.036$) (61). In contrast, Meng et al. showed that Cts was significantly higher in patients with essential HT than normal controls (1.19 ± 0.74 vs. 1.53 ± 0.72 ng/mL, $p < 0.01$) but found no correlation between Cts and the degree of HT (1.56 ± 0.59 vs. 1.42 ± 0.59 vs. 1.57 ± 0.76 ng/mL, $p > 0.05$) (62). They also suggested Cts level may serve as a prognostic factor for complications of HT, as patients with left ventricular hypertrophy had lower Cts-to-norepinephrine ratios than those without (3.63 ± 1.62 vs. 2.76 ± 0.86, $p < 0.01$) (62). In order to explain the contradictory results concerning Cts levels in normo- and HT, it was hypothesized that Cts decreases in prehypertension, however, sympathetic nervous system activity increases as HT progresses, which contributes to compensatory Cts elevation. Consequently, HT develops when elevated Cts no longer inhibits CA oversecretion (62).

Cts genetic variants exert varying effects on BP (16). The Gly364Ser variant was associated with lower diastolic BP than the Gly364Gly variant (wild type) in two Caucasian hypertensive groups of European ancestry and ca. 1.8% variance of population diastolic BP was attributable to Cts single nucleotide polymorphism (63). Conversely, in Asian populations, Ser-364 allele carriers displayed elevated systolic (up to ≈8 mm Hg; $p = 0.004$) and diastolic (up to ≈6 mm Hg; $p = 0.001$) BP (Indian) (31) as well as systolic BP, pulse pressure and arterial stiffness (Japanese) (64). In line with these, in a normotensive Indian population, Ser-364 allele carriers exhibited much lower plasma CA - by about 40% (consistent with its diminished nAChR desensitization-blocking effect *in vitro*) - than Gly364Gly carriers (30), yet, BP was similar in subjects with different alleles. These contradictory results underscore the necessity of genetic association studies for ethnically different populations.

Processing of CgA to Cts by endoproteolytic enzymes may be involved in the pathogenesis of HT. O'Connor et al. showed that CgA was increased by 117% ($p < 0.001$) in HT patients, whereas Cts reduced by 15% ($p = 0.036$), which suggests diminished conversion of CgA to Cts in HT (normotensives' CgA/Cts ratio of 4.5 ± 0.2 vs. 5.9 ± 0.4 in HT patients, $p = 0.005$) (61). Fung et al. demonstrated that in women compared to men higher plasma Cts (1.30 ± 0.033 vs. 1.14 ± 0.27 nM, $p < 0.001$) combined with lower CgA precursor concentrations (3.89 ± 0.15 vs. 4.65 ± 0.33 nM, $p = 0.006$) may be associated with decreased processing of CgA to Cts in the latter (female vs. male Cts/CgA ratio: 26.3 ± 0.006 vs. 23.1 ± 0.006), which predisposes to HT (58).

Moreover, Biswas et al. showed that *CHGA* variants undergo differential processing to produce Cts in the presence of the endoproteolytic enzyme plasmin (65). Their study indicates that less efficient processing of the Pro370Leu protein (product of one of *CHGA* variants) can contribute to the prevalence of CVDs (65).

CgA variants may also impact target organ damage in HT: black people suffering from end stage renal disease exhibit a common *CHGA* variant (3′-UTR C+87T), which decreased reporter gene expression and subsequently lead to lower Cts levels in this population (2.10 ± 0.88 vs. 3.23 ± 0.29 ng/mL, $p = 0.01$) (66).

Clinical studies concerning Cts in HT are presented in **Table 1**. To summarize, Cts was shown to decrease during prehypertension (59), however, the progression of elevated BP was associated with a compensatory increase in its concentration (62). Moreover, the development of HT may well be connected with diminished conversion of CgA to Cts (58, 61, 65) and depends on different variants of Cts exerting varying effects on BP (16, 63).

CARDIAC FUNCTION OF CATESTATIN

In vitro and *in vivo* Animal Studies

Cts induces NO synthesis from endothelial cells and cardiomyocytes (43, 53). In endothelial cells, NO is acquired from both: (1) the endothelin receptor B – endothelial nitric oxide synthase (eNOS) – NO, and (2) the protein kinase B (Akt) – eNOS/neuronal nitric oxide synthase (nNOS) – NO pathways. In cardiomyocytes, NO release results from the extracellular signal-regulated kinase – eNOS – NO pathway (53) (**Figure 1**). NO reduces inotropism and lusitropism of cardiomyocytes through various pathways (52, 53, 88, 89) (**Figure 1**). It was demonstrated *in vitro* using frog (52), eel (89), and rat (90) heart preparations that higher NO generation due to Cts ameliorates the Frank-Starling response, which is a compensatory mechanism to maintain adequate heart

TABLE 1 | Clinical studies concerning catestatin.

References	Study participants	Main results
	Catestatin and hypertension	
O'Connor et al. (59)	40 normotensives with positive HT family history, 176 normotensives without HT family history, 61 patients with HT	Offspring of HT patients had lower Cts than normotensives without HT family history: 1.32 ± 0.038 vs. 1.5 ± 0.076 ($p = 0.024$) Plasma Cts was not different in normotensives vs. hypertensives (1.36 ± 0.03 vs. 1.26 ± 0.06 ($p = 0.27$)
O'Connor et al. (61)	452 normotensives, 215 patients with HT	Cts was reduced by 15% in hypertensives patients ($p = 0.036$), whereas CgA was increased by 117% ($p < 0.001$); the ratio of CgA/Cts was thus increased by 31% ($p = 0.005$), implying decreased conversion of CgA to Cts in PH.
Salem et al. (66)	Black patients with HT and ESRD ($n = 150$) and black controls ($n = 58$)	ESRD patients had lower Cts: 2.10 ± 0.88 vs. 3.23 ± 0.29 ($p = 0.01$)
Meng et al. (62)	136 HT patients (109 with and 27 without LVH 27) and 61 healthy controls	Cts was higher in hypertensives vs. controls: 1.19 ± 0.74 vs. 1.53 ± 0.72 ($p < 0.01$) There was a non-significant trend toward lower Cts in HT patients with LVH than those without: 1.55 ± 0.7 vs. 1.4 ± 0.53 ($p > 0.05$).
	Catestatin and coronary artery disease	
Wang et al. (67)	50 STEMI patients and 25 non-CAD control patients	STEMI patients had lower plasma Cts on admission than controls: 16.5 ± 5.4 vs. 21.4 ± 6.4 ($p < 0.01$), increased on day 3 to 30.7 ± 12.2 ($p < 0.01$), and on day 7 decreased to levels below than admission (13.8 ± 5.3, $p < 0.01$) in MI
Meng et al. (68)	52 healthy controls 58 STEMI patients, 31 of whom were assessed by echocardiography 3 months later to reveal 7 cases with LVR and 24 without LVR	Plasma Cts on admission higher in patients with AMI than in controls: 1.00 (0.66–1.50) vs. 0.84 (0.56–1.17) ($p < 0.05$) Mean Cts higher on day 3: 1.12 (0.76–1.70) vs. 1.00 (0.66–1.50) ($p < 0.01$) and on day 7.: 1.32 (0.81–1.73) ng/ml vs. 1.00 (0.66–1.50) ($p < 0.01$) Patients with LVR 3 months after AMI vs. those without had higher Cts: -on admission: 2.02 (1.14–5.87) vs. 0.94 (0.39–1.66) ($p = 0.001$), -on day 3: 2.47 (1.10–5.95) vs. 1.16 (0.31–1.95) ($p = 0.006$), and, -on day 7 after STEMI: 3.08 (0.41–6.77) vs. 1.20 (0.30–3.73) ($p = 0.021$)
Liu et al. (69)	30 healthy controls; 15 SAP, 47 UAP, 22 NSTEMI, and 36 STEMI patients	Plasma Cts was higher in CAD patients than controls: 0.41 ± 0.14 vs. SAP patients: 0.72 ± 0.50 ($p < 0.05$) UAP patients 0.88 ± 0.58 ($p < 0.05$) NSTEMI patients 1.05 ± 0.48 ($p < 0.05$) STEMI patients 1.31 ± 0.91 (p < 0.05)
Pei et al. (70)	STEMI patients with MA ($n = 61$) STEMI patients without MA ($n = 64$)	Plasma Cts was higher in patients with STEMI complicated by MA compared with those without MA: 0.083 ± 0.011 vs. 0.076 ± 0.007 ($p < 0.001$)
Zhu et al. (71)	30 non-CAD controls (n = 30) 100 AMI patients including 74 with adverse events on follow-up and 26 without	Cts lower in MI patients on admission vs. controls: 16.7 ± 5.4 vs. 21.8 ± 6.3 ($p <0.0001$), higher on day 3 at 30.9 ± 12.1 ($p < 0.0001$ vs. admission), and again lower on day 7: 13.9 ± 5.2 ($p = 0.0003$ vs. admission) Cts on admission and day 3 in the adverse events group (19.4 ± 6.7 ng/ml, 44.6 ± 13.0, respectively) higher than in the non-adverse events group (15.8 ± 4.5 ($p = 0.003$), 26.1 ± 7.5 ($p < 0.0001$), respectively).
Xu et al. (72)	38 patients with CTO and 38 controls	Cts higher in CTO patients than in controls 1.97 ± 1.01 vs. 1.36 ± 0.97 ($p = 0.009$)
Zhu et al. (73)	72 STEMI patients and 30 control patients without CAD on imaging	Patients with Cts level above median at day 3 (28.71 ng/ml) developed worse ventricular function during the 65 months follow-up ($p < 0.0001$).
Xu et al. (74)	46 STEMI patients, 89, 35 control patients without CAD on imaging	Cts in patients with STEMI (0.80 ± 0.62) and UAP (0.99 ± 0.63) lower than in controls (1.38 ± 0.98; $p = 0.001$).
Kojima et al. (75)	25 CAD patients: 20 with AMI and 5 with UAP; controls: 20 non-CAD patients with mild hypertension and 13 healthy volunteers	Plasma Cts levels were lower in CAD patients (2*) than in non-CAD patients (4*; $p \leq 0.05$).
Chen et al. (19)	204 healthy volunteers 224 CAD patients	CAD patients had lower serum Cts than controls: 1.14 (1.05–1.24) vs. 2.15 (1.92–2.39); $p < 0.001$, and the levels decreased in a stepwise manner with increasing number of diseased vessels: 1.95 (1.83–2.07) vs. 1.57 (1.42–1.73) vs. 1.13 (1.00–1.27), $p <0.001$ (for 1, 2, and 3 vessels, respectively)
	Catestatin and heart failure	
Zhu et al. (76)	300 moderate to severe HF patients:−108 in stage A; 76 in Stage, 116 - Stage C	Cts decreased with higher HF stages and there was a significant difference between stage A and B: 21.29 ± 7.10 vs. 14.61 ± 4.69 ($p < 0.05$).
Liu et al. (77)	172 controls 228 HF patients in NYHA class I – IV	Plasma Cts increased with higher classes, NYHA class III and class IV patients had higher Cts levels than controls: 0.848 (0.664–1.260); 1.54 (0.856–2.432), respectively vs. 0.696 (0.504–0.883) ($p < 0.05$). NYHA class I and class II patients had similar Cts to controls: 0.612 (0.52 −0.844); 0.722 (0.532–1.112), respectively vs. 0.696 (0.504–0.883) ($p > 0.05$)

(Continued)

TABLE 1 | Continued

References	Study participants	Main results
Peng et al. (78)	Cohort of 202 HF patients followed-up for a median of 52.5 months: 143 survived, 59 died – 49 for cardiac causes	Plasma Cts was higher in non-survivors both for all and cardiac causes 1.06 (0.66–1.82) and 1.18 (0.69–1.83), respectively vs. 0.75 (0.58–1.12) in survivors ($p \leq 0.005$)
Wołowiec et al. (79)	Upon a follow-up of 24 months out of 52 HFrEF patients 11 reached the composite endpoint (CE) of unplanned hospitalization and all-cause death 24 healthy volunteers served as controls	Cts lower in HFrEF patients who reached a CE than in those who did not – both before and after exertion: 14.23 (11.05–15.82) vs. 16.86 (14.25–19.46) ($p = 0.03$) and 4.81 (2.20–6.25) vs. 7.82 (5.81–63.48) ($p = 0.002$), respectively; Cts in patients similar to controls: before exertion 15.95 (13.89–18.81) vs. 16.6 (14.75–22.20) ($p = 0.12$), after: 7.04 (4.97–11.08) vs. 9.26 (6.11–140.23) ($p = 0.13$)
Borovac et al. (80)	96 HF patients hospitalized due to an acute worsening of HF, 6 did not survive	Cts was significantly higher among non-survivors than survivors: 19.8 (9.9–28) vs. 5.6 (3.4–9.8) ($p < 0.001$)
	Catestatin and other diseases affecting the cardiovascular system	
Sun et al. (81)	330 controls; 329 hemodialysis patients followed-up for 36 months – 29 died for cardiac and 28 for non-cardiac causes	Cts higher in hemodialysis patients (1.9 ± 0.3) vs. controls (1.2 ± 0.2), $p < 0.001$ Cts higher (2.2 ± 0.1) in patients who died for cardiac causes than in survivors (1.8 ± 0.2) and non-cardiac death non-survivors (1.8 ± 0.3), $p < 0.001$
Izci et al. (82)	97 controls 160 APE patients: 72 with sPESI $\geq$ 1 and 88< 1	Plasma Cts higher in APE patients than controls: 27.3 ± 5.7 vs. 17.5 ± 6.1 ($p < 0.001$); Cts higher in patients with sPESI $\geq$ 1 than with sPESI < 1: 37.3 ± 6.1 vs. 24.2 ± 5.3 ($p < 0.001$)
Tüten et al. (83)	100 women with preeclampsia 100 women with uncomplicated pregnancy as controls.	Plasma Cts was significantly increased in the preeclampsia patients compared to the controls: 0.29 ± 0.096 vs. 0.183 ± 0.072 ($p < 0.001$)
Liu et al. (84)	260 healthy workers	Plasma CgA-to-catestatin ratio correlated with effort, reward (negatively), overcommittment, and efford-reward imbalance: r = 0.218, −0.249, 0.275, and 0.279, respectively, $p < 0.001$ for all
	Catestatin and metabolic syndrome	
Simunovic et al. (85)	92 obese subjects (BMI Z score >2), age 10-18; 39 controls	Lower plasma Cts concentrations in obese subjects compared to controls: 10.03 ± 5.05 vs. 13.13 ± 6.25 ($p = 0.004$); lower Cts in the subgroup of obese patients with MS: 9.02 ± 4.3 vs. 10.54 ± 5.36 vs. 13.13 ± 6.25 ($p = 0.008$). Cts negatively correlated with DBP ($r = -0.253$, $p = 0.014$), HOMA-IR ($r = -0.215$, $p = 0.037$) and hsCRP ($r = -0.208$, $p = 0.044$).
Kim et al. (86)	85 subjects with mild OSA, 26 with moderate-to-severe OSA, 102 were controls, mean age 7.7 ± 1.4 years	Children with OSA have reduced plasma Cts levels (Log Cts in moderate-to-severe OSA: 0.12 ± 0.22 vs. mild OSA: 0.23 ± 0.20 vs. controls: 0.28 ± 0.19; differences among three groups: $p < 0.01$). Cts levels were inversely correlated with AHI ($r = -0.226$; $p < 0.01$) and with mean arterial BP level ($r = -0.184$; $p < 0.05$).
Borovac et al. (87)	78 OSA patients; 51 controls	Plasma Cts higher in OSA patients compared to controls: 2.9 ± 1.2 vs. 1.5 ± 1.1 ($p < 0.001$). In OSA patients Cts correlated with neck circumference ($r = 0.318$, $p < 0.001$; $\beta = 0.384$, $p < 0.001$) and HDL cholesterol ($r = -0.320$, $p < 0.001$; $\beta = -0.344$, $p < 0.001$).

*Catestatin concentrations are given in ng/mL and the results are shown as median (interquartile range) or mean ± standard deviation; AHA, American Heart Association; AMI, acute myocardial infarction; APE, Acute pulmonary embolism; AWHF, acute worsening of heart failure; BP, blood preassure; CAD, coronary artery disease; CD, Crohn's disease; CE, composite endpoint including unplanned hospitalization and death for all causes; CPET, Cardiopulmonary Exercise Testing (6-minut walk test); Cts, catestatin; CTO, chronic total occlusions; DBP, diastolic blood pressure; ERI, effort, reward imbalance; ESRD, end stage renal disease; HDL, high-density lipoprotein; HF, heart failure; HFrEF, Heart Failure with Reduced Ejection Fraction; HOMA-IR, homeostatic model assessment of insulin resistance; hsCRP, high sensitivity C-reactive protein; HT, hypertension; IBD, inflammatory bowel diseases; LVH, left ventricular hypertrophy; LVR, left ventricular remodeling; MA, malignant arrhythmia; MS, metabolic syndrome; NSTEMI, non-ST segment elevation myocardial infarction; NYHA, New York Heart Association; OSA, obstructive sleep apnea; SAP, stable angina pectoris; STEMI, acute ST- segment elevation myocardial infarction; sPESI, simplified PESI; UAP, unstable angina pectoris; UC, ulcerative colitis. *Mean value (standard deviation was not provided).*

function in response to changes in venous return (90). Moreover, Cts exerts counterregulatory action against β-adrenergic and endothelin-1 stimulation pointing to Cts as a novel, beneficial cardiac modulator (53) (**Figure 1**).

In line with this research, *in vitro*, Cts attenuated norepinephrine-mediated hypertrophic responses in H9c2 cardiac myoblasts and at 10-25 nM signaling was moderated primarily by β1/2-adrenoceptors (91). Interestingly, Bassino et al. observed that a low (5 nM) WT-Cts concentration reduced β-adrenergic stimulation in bovine aortic endothelial cells, although it exhibited no significant effect on myocardial contractility. Higher concentrations (10–50 nM) of Cts induced a transient positive inotropic effect followed by a negative antiadrenergic effect (43). The transient positive effect was probably related to histamine release from mast cells induced by Cts. Histamine exerts a positive inotropic effect on rat ventricular myocardium and H1 histamine receptor antagonist mepyramine blocked the positive effect induced by higher doses of WT-Cts (10 nM). In turn, WT-Cts at a minimal concentration (5 nM) reduced isoproterenol-induced enhancement of papillary muscle contractility via H1 receptor activation without altering basal contractile ability (43).

Several studies point at a cardioprotective role of Cts in ischemia/reperfusion (I/R) injury of the heart in rodent models.

It was apparent for Cts pretreatment (25 nM, 50 nM, and 100 nM infused for 15 min before ischemia) (92) and chronic administration of Cts after MI (0.25 mg/kg/12 h injection intraperitoneally 24 h after AMI for 28 days) (67). Further, infusion of a Cts dose of 75 nM for 20 min at the beginning of reperfusion significantly reduced infarct size, limited post-ischemic contracture, and improved recovery of developed left ventricular pressure (93–96). Protection due to Cts in I/R-injured myocardium was related to salvage of oxidative-stress-induced apoptosis by activation of the β2-adrenergic receptor, and PKB/Akt pathway (96). It must be highlighted that infusion of a higher dose WT-Cts (100 nM) for 120 min during reperfusion caused deleterious effects and did not activate Akt (97). Interestingly, the Gly364Ser variant of Cts at a higher dose (100 nM for 120 min) lowered infarct size, which is probably associated with lower inhibitory activity on the nicotinic cholinergic receptor of the Cts-Gly364Ser variant (97). It is worth underlining that cardioprotection achieved by ischemic preconditioning in wild-type mice was absent in Cts-KOs (21).

Modulation of cardiac glucose metabolism is another cardioprotective mechanism of Cts, in particular, improvement of glucose uptake early during post-ischemic reperfusion (18). In physiological conditions, cardiomyocyte metabolism depends mainly on fatty acid oxidation, although the heart shifts toward glucose metabolism in response to ischemia to ameliorate myocardial contractile efficiency. In isolated rat cardiomyocytes, Cts induced Akt and AS160 phosphorylation and significantly enhanced glucose uptake (18), which may be crucial for recovery of contractile function during post-ischemic reperfusion (98).

A recent study involving an *in vivo* and *in vitro* approach investigated Cts in acute pulmonary embolism (99). Its administration in mice with this pathology increased survival as well as augmented thrombus resolution by attenuating endothelial inflammation (99).

In summary, based on *in vitro* and *in vivo* animal models, it was demonstrated that Cts exhibits a potential cardioprotective effect by acting directly as a cardiodepressing peptide through multiple signaling pathways (52, 53, 88–90), it may also reduce apoptosis of cardiomyocytes induced by oxidative stress (67, 92–96), and is beneficial in acute pulmonary embolism (99). However, more studies are needed in these areas, in particular, regarding Cts in I/R injury of the heart (67, 92–97).

Clinical Studies

Coronary Artery Disease

Wang et al. (100) were the first to investigate Cts in patients with coronary artery disease (CAD) by measuring its plasma concentration in 58 acute myocardial infarction (AMI) patients on admission, day 3 and day 7, and in 25 control subjects who were admitted to the same hospital for atypical chest pain but with normal coronary arteries confirmed by coronary angiography. Compared with controls (21.4 ± 6.4 ng/ml), plasma Cts concentrations were significantly lower on admission due to AMI (16.5 ± 5.4 ng/ml, $p < 0.01$), higher on the third day (30.7 ± 12.2 ng/ml, $p < 0.01$) and again lower on day 7 (13.8 ± 5.3 ng/ml, $p < 0.01$).

Zhu et al. (71) and Xu et al. (74) obtained results consistent with the above: compared to controls, Cts was lower on admission due to AMI, it increased significantly on the third day of hospitalization, but decreased 1 week following AMI. In line with these studies, Kojima et al. showed in small samples of patients that Cts was significantly lower in patients with AMI or unstable angina pectoris than in non-CAD patients (75).

However, Meng et al. (68) and Liu et al. (69) showed that plasma Cts levels at the time of admission were significantly higher in patients with AMI compared to healthy controls. The discrepancy between studies may result from different control groups, which included patients with chest pain without CAD on imaging in studies by Wang et al. (100), Zhu et al. (71) and Xu et al. (74) vs. healthy volunteers in studies by Meng et al. (68) and Liu et al. (69).

In addition, Chen et al. demonstrated serum Cts levels were lower in stable angina pectoris (SAP) patients compared to healthy controls (median (inter-quartile range) 1.14 (1.05–1.24) ng/mL vs. 2.15 (1.92–2.39) ng/mL, $p < 0.001$). Moreover, a stepwise decrease in serum Cts was found when classifying CAD patients according to the number of diseased vessels (19). However, Liu et al. showed that SAP patients ($n = 15$) had significantly higher Cts levels compared to controls (0.41 ± 0.14 ng/mL vs. 0.72 ± 0.50 ng/mL, $p < 0.05$) (69). Divergent findings are difficult to account for, perhaps these depend on disease symptoms in patients of the latter study (Cts increases as CAs are released when pain occurs); small SAP patient sample size in the study by Liu et al. could have biased the results (69).

Further, Xu et al. demonstrated that mean plasma Cts in coronary artery chronic total occlusion patients, who underwent coronary angiography or percutaneous coronary intervention for the first time, were significantly higher than in patients with chest pain but with normal coronary arteries (1.97 ± 1.01 vs. 1.36 ± 0.97 ng/ml, $p = 0.009$) (72).

Of the 58 AMI patients mentioned above, Meng et al. studied 31 by echocardiography 3 months after AMI onset, and diagnosed 7 with left ventricular remodeling (LVR). Interestingly, these patients had significantly higher plasma Cts levels on admission, day 3, and day 7 than those without LVR (68). In line with Meng et al., Dan Zhu et al. found that AMI patients, whose Cts level exceeded the median at day 3 of hospitalization (28.71 ng/ml), exhibited worse left ventricular function in echocardiography 65 months after AMI (73).

In another study performed by Dan Zhu et al., adverse events occurred more frequently in the 65-month follow-up in patients whose Cts level on day 3 exceeded the median as well as in those whose ratios of Cts on day 7 to day 3 were below the median (71). Moreover, plasma Cts level turned out to be an independent prognostic factor for malignant arrhythmia (MA) after AMI, and was significantly higher in patients with AMI complicated by MA (70). However, Xu et al. showed no significant differences in major adverse cardiovascular events, including death from cardiovascular causes, recurrent AMI, or hospital admission for HF, or revascularization between patients with high and low Cts concentrations (72).

Heart Failure

Concerning HF, Dan Zhu et al. showed that the higher the severity stage of HF (according to the American Heart Association, AHA), the lower the Cts level, and that Cts might be a better predictive factor for stage B HF than brain natriuretic peptide, a marker commonly used in clinical practice for HF diagnosis and severity assessment (76). However, according to a study by Liu et al., plasma Cts levels were increasingly higher in patients with growing severity of the New York Heart Association (NYHA) HF classes I to IV, and NYHA class III and IV patients exhibited significantly higher plasma Cts levels than controls, NYHA class I, and class II subjects (77). Seemingly contradictory results may be a consequence of patient enrollment criteria: Zhu et al. recruited asymptomatic chronic patients with stage C HF (76), while NYHA IV patients had resting dyspnea (77). As mentioned before, symptoms that trigger CA release can cause a compensatory increase in Cts levels.

Peng et al. demonstrated that Cts was an independent risk factor for all-cause death (hazard ratio 1.84 (95% CI: 1.02–3.32, $p = 0.042$)) and cardiac death [hazard ratio 2.41 (95% CI: 1.26–4.62, $p = 0.008$)] during a median 52.5-month follow-up (78). In line with their results, in the CATSTAT-HF Study, Cts was found to be an independent and significant predictor of in-hospital death, and its level was significantly higher among non-survivors than survivors (80). However, in the study by Wołowiec et al., patients who reached the composite endpoint of unplanned hospitalization and death for all causes during a 24-month follow-up had lower Cts levels – assessed both before and after physical exertion. Firth coefficient was 6.58 (penalized 95% CI 1.66–21.78, $p = 0.003$) (79). Again, the divergent data could result from different study populations: Wołowiec et al. enrolled only hemodynamically stable patients (79), while in the CATSTAT-HF Study patients with acute decompensation of chronic HF were included (80), and more than half of the patients (55.9%) in the study by Peng et al. study were classified as NYHA III-IV (78).

Catestatin in Other Diseases Affecting the Cardiovascular System

In the scope of CVD, apart from studies on the role of Cts in HT, CAD, and HF, few other have been published.

Sun et al. studied hemodialyzed patients and demonstrated that plasma Cts levels equal to or greater than the mean (1.9 ng/ml) were associated with higher cardiac death risk (RR 6.13, 95% CI 2.54, 18.45) during a 36-month follow-up. Moreover, there was no such association for non-cardiac death (RR 1.29, 95% CI 0.70, 2.85) (81).

Izci et al. showed that plasma Cts levels were higher in patients with acute pulmonary embolism than in control subjects. Also, there was a positive correlation between Cts and right ventricular dysfunction, and between Cts and Simplified Pulmonary Embolism Severity Index(± 0.581, $p < 0.001$), a score used to estimate 30-day mortality in patients diagnosed with non-high-risk acute pulmonary embolism. Furthermore, a Cts cut-off level of 31.2 ng/ml predicted mortality with a sensitivity of 100% and specificity of 52.6% (AUC = 0.883, 95% CI: 0.689–0.921) (82).

In preeclampsia patients, plasma Cts was significantly elevated compared to controls: 0.29 ± 0.096 vs. 0.183 ± 0.072 ($p < 0.001$) (83), and correlated positively with systolic and diastolic BP, urea, creatinine and uric acid levels (83).

Stress may account for- at least in part-, the extent of sympathetic nervous activation, deleteriously affects the immune and coagulation systems, increasing the risk of CVDs. Interestingly, it was demonstrated recently that plasma CgA correlated positively with effort, overcommitment, and effort-reward imbalance ($r = 0.267$, 0.319, and 0.304, respectively, $p < 0.001$ for all three), and negatively with reward ($r = -0.237$, $p < 0.001$). Plasma CgA-to-Cts ratio was also associated with work stress in a manner similar to CgA (84).

Summary of Clinical Studies

Clinical studies regarding Cts in CAD and HF are summarized in **Table 1**. It must be highlighted that CAs release causes an increase in Cts, which changes dynamically. Low levels of Cts may play a pathogenic role in cardiac ischemia (74). Research indicates that Cts is involved in the course of CAD as well as HF, and, possibly, its concentration may be applied in monitoring.

ROLE OF CATESTATIN IN METABOLIC DISORDERS AND ATHEROSCLEROSIS

Metabolic disorders such as obesity, insulin resistance and type 2 diabetes are associated with CVDs. Obesity with an abnormal lipid profile may lead to insulin resistance and is associated with higher cardiovascular risk. In turn, insulin resistance correlates strongly with cardiovascular pathology and is a powerful predictor of future development of type 2 diabetes, an independent risk factor for CVDs (101).

Dysregulated immune system is involved in the pathogenesis of these widely prevalent metabolic disorders. The interdependence of adverse systemic metabolic conditions and immune responses gave rise to the term "immunometabolism," which is currently also used to describe pathologic reprogramming of immune cells not only in the spectrum of metabolic syndrome, but also other diseases (e.g., neoplasms and autoimmunity). Here, the former meaning of immunometabolism was adopted.

In vitro and *in vivo* Animal Studies

Based on *in vitro* and *in vivo* studies with rodent models, Cts plays a role in the crosstalk between the immune and metabolic systems (15), in particular, in the development of atherosclerosis (19). Cts may act as an anti-atherogenic and anti-inflammatory peptide that reduces leukocyte-endothelium interaction by activating angiotensin-converting enzyme-2 and suppressing tumor necrosis factor-α-elicited expression of inflammatory cytokines and adhesion molecules (19); development of atherosclerosis was attenuated by Cts treatment in apolipoprotein E knockout mice fed a high-fat diet (a mouse model of atherosclerosis) (19) (**Figure 1**). Further, after vascular injury, Cts increased the expression of *Mrc1*, a gene encoding an anti-inflammatory peptide, and

prevented macrophage-driven atherosclerosis (75). Presumably, anti-inflammatory actions of Cts partly depend on the regulation of chemotaxis (102). *In vitro* and *in vivo* (rodent models), Cts counteracted chemoattraction of monocytes and neutrophils by inflammatory chemokines (102), likely contributing to reduced immune infiltration in the heart (21, 75). Regulation of chemotaxis by Cts is complex and naturally occurring Cts variants differ in their chemotactic properties (103, 104), which may influence the propensity for CVD.

The liver plays both a critical role in metabolism, and serves as an important site of immune regulation as it contains resident immune cells as well as synthesizes a number of inflammatory proteins (105). Both tissue-resident (Kupffer cells) and recruited macrophages contribute to an inflammatory state of the liver, e.g., in obesity-induced insulin resistance and type 2 diabetes. Using a rodent model, Ying et al. showed that Cts may inhibit the function and infiltration of macrophages in the liver, which suppresses hepatic gluconeogenesis and improves insulin sensitivity (20). Moreover, the authors demonstrated that treatment of diet-induced obese mice with Cts elicited beneficial changes, including decreased plasma lipids and insulin as well as hepatic lipid content, attenuated expression of gluconeogenic and proinflammatory genes, and increased expression of anti-inflammatory genes in both Kupffer cells and recruited monocyte–derived macrophages in the liver (20) (**Figure 1**). Furter *in vivo* research on both diet-induced obese and normal chow diet mice showed that Cts decreased obesity-induced endoplasmic reticulum dilation in hepatocytes and macrophages, and enhanced insulin sensitivity in mammalian cells (106).

Latest research *in vivo* on a rodent Cts-KO model has shown that Cts directly promotes hepatic glycogen synthesis, reduces gluneogenesis and glycogenolysis, as well as enhances downstream insulin signaling (23). Positive effects of Cts were observed in Chga-KO mice too, which – despite regular chow diet - were obese, and had increased plasma CAs, leptin, adiponectin, and ketone bodies at baseline (22). Moreover, compared to WT controls, Chga-KO mice exhibited higher glucose-stimulated insulin secretion, which was likely caused by changes in secretory vesicles and mitochondria observed in CgA-deficient β-cells (107). In this model, treatment with Cts lowered circulating CAs and leptin as well as reduced adipose tissue by about 25% resulting in a lean phenotype, increased lipolysis, enhanced fatty acid oxidation and assimilation into lipids in the liver (**Figure 1**). These beneficial metabolic effects of exogenous Cts were presumed to result from reduction of CA and adiponectin resistance; the effect was mediated by the inhibition of α2 adrenergic signaling (22) (**Figure 1**). In addition, Cts improved leptin signaling (determined by phosphorylation of AMPK and Stat3 in Chga-KO mice) and peripheral leptin sensitivity in both diet-induced obese mice and in leptin-deficient *ob/ob* mice (22) (**Figure 1**). In another model with genetically modified rodents, Cts was found to restore natrium-glucose transporter 1 (SGLT1) expression and abundance as well as intestinal turnover in double knock-out leptin receptor b mice, an experimental model of obesity, type 2 diabetes with hyperleptinemia. The effect was possibly mediated by antagonistic binding of Cts to the leptin receptor a (108).

To sum up, it is increasingly clear that Cts is vital for maintaining metabolic homeostasis, which is partly achieved by affecting the immune system. Based on *in vitro* and *in vivo* experimental studies, Cts may inhibit inflammatory response and leukocyte-endothelial cell interactions (19, 21, 75, 102, 103), prevent macrophage-driven atherosclerosis (19, 75), regulate monocyte migration (103), and cytokine production and release (102–104). As a novel regulator of metabolism, in rodents, Cts was shown to help in achieving insulin sensitivity, overcoming endoplasmic reticulum stress (106), reducing adipose tissue by increasing lipolysis, enhancing oxidation of fatty acids, and their assimilation into lipids (22).

Clinical Studies

So far, only few studies have been conducted in humans on the metabolic role of Cts. Clearly, more research is required in this area.

In a study reported above, O'Connor et al. showed that plasma Cts levels correlated negatively with BMI and plasma leptin concentrations in hypertensive and normotensive subjects (59). Durakoglugil et al. reported that plasma Cts was an independent predictor of high-density lipoprotein cholesterol, and correlated inversely with plasma triglycerides (60). Interestingly, the Ser-364 allele was strongly associated with elevated plasma triglyceride and glucose levels (30).

Simunovic et al. compared plasma Cts levels in 92 obese children and adolescent with those of 39 healthy (normal weight) controls: it levels were significantly lower in the former (10.03 $\pm$ 5.05 vs. 13.13 $\pm$ 6.25 ng/mL, $p = 0.004$) (85), and, in addition, lower in the subgroup of obese patients with metabolic syndrome vs. those without and controls (9.02 $\pm$ 4.3, 10.54 $\pm$ 5.36, and 13.13 $\pm$ 6.25 ng/ml, respectively, $p = 0.008$). Moreover, Cts negatively correlated with diastolic BP ($r = -0.253$, $p = 0.014$), homeostatic model assessment of insulin resistance index ($r = -0.215$, $p = 0.037$), and high sensitivity C-reactive protein ($r = -0.208$, $p = 0.044$) (85) (**Table 1**). In another study, children with obstructive sleep apnea (OSA) had reduced plasma Cts levels (Log Cts in moderate-to-severe OSA: 0.12 $\pm$ 0.22 vs. mild OSA: 0.23 $\pm$ 0.20 vs. controls: 0.28 $\pm$ 0.19; $p < 0.01$) and Cts correlated negatively with apnea-hypopnea index ($r = -0.226$; $p < 0.01$) as well as mean arterial BP ($r = -0.184$; $p < 0.05$) (86) (**Table 1**).

Borovac et al. measured plasma Cts levels in 78 male OSA patients aged 50.3 $\pm$ 8.8 years and 51 age-, sex- and BMI-matched control subjects. They demonstrated that Cts serum levels are higher in the former (2.9 $\pm$ 1.2 vs. 1.5 $\pm$ 1.1 ng/mL, $p < 0.001$) (87). Cts significantly correlated with neck circumference ($r = 0.318$, $p < 0.001$; $\beta = 0.384$, $p < 0.001$) and high-density lipoprotein cholesterol ($r = -0.320$, $p < 0.001$; $\beta = -0.344$, $p < 0.001$), as well as apnea-hypopnea index among non-obese obstructive sleep apnea subjects ($r = 0.466$, $p = 0.016$; $\beta = 0.448$, $p = 0.026$) (87) (**Table 1**).

Evidently, reduced Cts levels seem to be associated with an adverse metabolic profile, including obesity and metabolic syndrome, abnormal lipid concentrations and insulin resistance.

CONCLUSIONS AND PERSPECTIVES

In conclusion, current data indicate that the endogenous bioactive peptide Cts is a vital regulatory factor of cardiovascular and immunometabolic homeostasis. Possibly, in future, Cts can be used in the diagnosis of CVDs and metabolic disorders as a novel biomarker, which may aid in clinical decision-making. Application of Cts in therapy might potentially mitigate detrimental sympathoexcitatory effects, which underlie cardiovascular and metabolic diseases. It should be highlighted that, so far, studies on Cts have been carried out mainly on animal models. More research is required to take advantage of beneficial effects of Cts in clinical practice.

AUTHOR CONTRIBUTIONS

EZ and PK review the literature, and EZ wrote the first draft of the manuscript. PK and EZ wrote sections of the manuscript. KS carried out critical interpretations. All authors contributed to manuscript revision, read, and approved the submitted version.

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Gdańsk, dnia 05.09.2023

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OŚWIADCZENIE

Jako współautor pracy pt."Low catestatin as a risk factor for cardiovascular disease – assessment in patients with adrenal incidentalomas" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to: opracowanie metodyki pracy, uzyskanie zgody Komisji Bioetycznej, rekrutacja grupy badanej i kontrolnej, w tym przeprowadzenie badania podmiotowego i przedmiotowego, elektrokardiografii, całodobowego pomiaru ciśnienia tętniczego metodą holter oraz zabezpieczenie materiału biologicznego celem diagnostyki laboratoryjnej; uzyskane dane opracowałam statystycznie i napisałam pierwszą wersję manuskryptu.

Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje na mój indywidualny wkład przy opracowywaniu koncepcji, wykonywaniu części eksperymentalnej, opracowaniu i interpretacji wyników tej pracy, która wchodzi w skład rozprawy doktorskiej.

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dr Piotr Kmieć
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OŚWIADCZENIE

Jako współautor pracy pt. "Low catestatin as a risk factor for cardiovascular disease – assessment in patients with adrenal incidentalomas" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to:
udział w planowaniu metodyki pracy, rekrutacji grupy badanej i opracowaniu statystycznym danych oraz korekta manuskryptu.

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek Ewę Zalewską jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek Ewy Zalewskiej przy opracowywaniu koncepcji, wykonywaniu części eksperymentalnej, opracowaniu i interpretacji wyników tej pracy.

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(podpis współautora)

Gdańsk, dnia 05.09.2023

lekarz, Jakub Sobolewski
(tytuł zawodowy, imię i nazwisko)

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Jakub Sobolewski

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(podpis współautora)

Jakub Sobolewski
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Gdańsk, dnia 05.09.2023

dr Andrzej Koprowski
(tytuł zawodowy, imię i nazwisko)

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dr med. Andrzej Koprowski
SPECJALISTA KARDIOLOG
SPECJALISTA CHORÓB WEWNĘTRZNYCH
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Andrzej Koprowski

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(podpis współautora)

Gdańsk, dnia...04.09.2023...

prof. dr hab. n. med. Krzysztof Sworczak
(tytuł zawodowy, imię i nazwisko)

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Kierownik
Katedry i Kliniki Endokrynologii
i Chorób Wewnętrznych
prof. dr hab. Krzysztof Sworczak

.....

(podpis współautora)

Gdańsk, dnia 04.09.2023

lekarz, Ewa Zalewska
(tytuł zawodowy, imię i nazwisko)

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przegląd literatury, napisanie pierwszej wersji manuskryptu oraz opracowanie rycin i tabel.

Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje na mój indywidualny wkład w powstanie artykułu poglądowego wchodzącego w skład rozprawy doktorskiej.

Ewa Zalewska
LEKARZ 3319052

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(podpis współautora)

Gdańsk, dnia 04.09.2023

dr Piotr Kmieć
(tytuł zawodowy, imię i nazwisko)

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Gdańsk, dnia 04.09.2023

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