Varijabilnost pokazatelja arterijske krutosti

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DISERTACIJA

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Istraživanje za izradu ove doktorske disertacije provedeno je u Laboratoriju za starenje krvožilja i prevenciju kardiovaskularnih bolesti Medicinskog fakulteta Sveučilišta u Splitu.

Voditelj rada: prof. dr. sc. Ana Jerončić

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1. SADRŽAJ

2. POPIS OZNAKA I KRATICA

AIx – augmentacijski indeks (engl. *augmentation index*)

AIx@HR75 - augmentacijski indeks standardiziran na 75 otkucaja srca u minuti

AHA - Američko udruženje za srce (engl. *American Heart Association*)

cAIx – centralni augmentacijski indeks (engl. *central augmentation index*)

cDBP - centralni dijastolički krvni tlak

cfPWV - karotidno-femoralna brzina pulsnog vala (engl. *carotid-femoral pulse wave velocity*)

CV - koeficijent varijacije (engl. *coefficient of variation*)

COVID-19 - Koronavirusna bolest izazvana koronavirusom SARS-CoV2 (engl. *The coronavirus disease caused by the SARS-CoV2 coronavirus*)

cSP – centralni sistolički tlak (engl. *central systolic pressure*)

DBP - dijastolički krvni tlak (engl. *diastolic blood pressure*)

ITM – indeks tjelesne mase (engl. *body mass index*)

KV – kardiovaskularni

KVB - kardiovaskularne bolesti

pAIx – periferni augmentacijski indeks (engl. *peripheral augmentation index*)

PP – tlak pulsa (engl. *pulse pressure*)

PWV – brzina pulsnog vala (engl. *pulse wave velocity*)

PWVao – aortna brzina pulsnog vala (engl. *aortic pulse wave velocity*)

3. PREGLED OBJEDINJENIH RADOVA

Ova disertacija temelji se na objedinjenju sljedećih znanstvenih radova:

1. Podrug M, Šunjić B, Bekavac A, Koren P, Đogaš V, Mudnić I, Boban M, Jerončić A. The effects of experimental, meteorological, and physiological factors on short-term repeated pulse wave velocity measurements, and measurement difficulties: A randomized crossover study with two devices. Front Cardiovasc Med. 2023 Jan 11;9:993971.
2. Podrug M, Šunjić B, Koren P, Đogaš V, Mudnić I, Boban M, Jerončić A. What Is the Smallest Change in Pulse Wave Velocity Measurements That Can Be Attributed to Clinical Changes in Arterial Stiffness with Certainty: A Randomized Cross-Over Study. Journal of Cardiovascular Development and Disease. 2023; 10(2):44.
3. Podrug M, Koren P, Dražić Maras E, Podrug J, Čulić V, Perissiou M, Bruno RM, Mudnić I, Boban M, Jerončić A. Long-Term Adverse Effects of Mild COVID-19 Disease on Arterial Stiffness, and Systemic and Central Hemodynamics: A Pre-Post Study. J Clin Med. 2023 Mar 8;12(6):2123.

3.1. UVOD

3.1.1. Stratifikacija kardiovaskularnog (KV) rizika i krutost arterija

Bolesti srca i krvnih žila su vodeći uzrok smrti u svijetu, a prognozira se da će do 2030. godine broj smrtnih slučajeva porasti s trenutnih 17,5 milijuna na 23 milijuna ljudi. Temeljem prijedloga Ujedinjenih naroda mnoge države su se na globalnoj razini obvezale na smanjenje preuranjene smrtnosti od kardiovaskularnih bolesti (KVB) za 25% do 2025. (1). Prevencija KVB usko je povezana s točnom procjenom ukupnog kardiovaskularnog (KV) rizika. Takva procjena omogućava ne samo rano otkrivanje osoba s visokim rizikom za razvoj KVB, već i utvrđivanje optimalne preventivne terapije koja se treba prilagoditi ukupnom KV riziku pojedinca (2-4). Konvencionalne metode procjene ukupnog rizika koje stratificiraju ljude u kategorije niskog, srednjeg ili visokog KV rizika pokazuju značajan jaz između predviđenih i stvarnih stopa događaja (5-7). Osobito u mladih i u

zemljama za koje jednadžbe rizika nisu dobro kalibrirane, ove metode imaju sklonost podcijeniti (u mladih) ili netočno predvidjeti (u zemljama bez dobre kalibracije rizika) rizik za fatalne KVB događaje (6, 8).

Europske smjernice o prevenciji KVB iz 2016. preporučuju korištenje dodatnih biomarkera za poboljšanje stratifikacije rizika od KVB prema tradicionalnim modelima (4). Tako je radna skupina Europskog kardiološkog društva za perifernu cirkulaciju preporučila uključivanje neinvazivnih vaskularnih biomarkera u takve modele (9). Posebice je s tog aspekta preporučena upotreba arterijske krutosti budući da se mjere arterijske krutosti kao što je to brzina pulsnog vala (PWV od engl. *pulse wave velocity*) smatraju pokazateljima kumulativnih oštećenja koja su nastala tijekom godina na arterijskom zidu, kao odgovor na učinke tradicionalnih, ali i neprepoznatih čimbenika KV rizika (10-13).

Ukrućenje arterija, koje je karakterizirano smanjenjem arterijske popustljivosti i/ili promjenama u strukturi stjenke arterije, najtipičnija je klinička značajka procesa starenja arterija (10, 14). Brojna su istraživanja dokazala da je veća krutost arterija neovisno povezana s povećanim rizikom za pojavu prve ili ponovljene teške kardiovaskularne bolesti (15-17). Također je pokazano da mjerenja krutosti arterija poboljšavaju reklasifikaciju ispitanika koji su pod srednjim rizikom od KVB, nadopunjavanjem informacija koje pružaju tradicionalni čimbenici KV rizika (15, 18-20). Uz to, arterijska krutost je povezana i s oštećenjem ciljnih organa (21-24). Nedavno su započela klinička ispitivanja koja koriste procjenu krutosti arterija kao zamjenski terapijski ishod liječenja u bolesnika s hipertenzijom (25, 26).

3.1.2. Mjere arterijske krutosti

Brzina pulsnog vala smatra se izravnim pokazateljem arterijske krutosti i predstavlja prijeđeni put pulsnog vala kroz arteriju u jedinici vremena [m/s]. Računa se mjerenjem vremena potrebnim da pulsni val prijeđe udaljenost od mjesta nastanka (korijen aorte) do mjesta mjerenja (primjerice karotidna, radijalna ili femoralna arterija). Brzina pulsnog vala različita je u različitim dijelovima arterijskog stabla, pa tako brzina u segmentu između karotidne i femoralne arterije odgovara brzini u samoj aorti. Karotidno-femoralna brzina pulsnog vala (cfPWV) klinički je najznačajnija i najsnažnije

povezana sa zdravstvenim rizicima u usporedbi s drugim segmentima na kojima se brzina pulsnog vala također mjeri (poput karotidno-radijalne, karotidno-tibijalne itd.) (19, 27). Trenutno postoji nekoliko tehnika za određivanje brzine pulsnog vala, a to su aplanacijska tonometrija, oscilometrija, ultrazvuk i magnetska rezonancija (28, 29). U konsenzusnom dokumentu Europskog društva za neinvazivna istraživanja velikih arterija (European Network for Non-invasive Investigation of Large Arteries) iz 2006. mjerenje PWV-a, posebice cfPWV-a mjerenog aplanacijskom tonometrijom, predloženo je kao zlatni standard za neinvazivnu procjenu arterijske krutosti (28).

Osim PWV-a jednako raširen, pouzdani i upotrebljavani kao pokazatelji arterijske krutosti su i augmentacijski indeks – engl. *augmentation indeks* (AIx), centralni sistolički tlak (cSP od engl. *central systolic pressure*), i tlak pulsa (PP, od engl. *pulse pressure*).

Augmentacijski indeks (AIx) označava postotak pulsnog vala kojeg čini reflektirani val. Neizravni je pokazatelj arterijske krutosti, a izravni pokazatelj valne refleksije - jednog od temeljnih hemodinamskih fenomena koji utječu na opterećenje srca u sistoli (30).

Centralni sistolički tlak (cSP) još je jedan od pokazatelja arterijske krutosti, posebice je važan u starijoj populaciji jer je u starijoj populaciji potvrđen kao čimbenik kardiovaskularnog rizika i povezanosti s hipertrofijom lijeve klijetke srca, porast centralnog sistoličkog tlaka izravno povećava tlačno opterećenje srca (31, 32).

Tlak pulsa (PP) je razlika između sistoličkog i dijastoličkog arterijskog tlaka. Može se izračunati kako iz perifernih, tako i centralnih vrijednosti arterijskog tlaka. Porast centralnog tlaka pulsa dovodi do većeg tlačnog opterećenja srca i razvoja hipertrofije lijeve klijetke, kao i kod porasta centralnog sistoličkog tlaka, što ga čini nezavisnim prediktorom morbiditeta i mortaliteta (33, 34).

Svi navedeni pokazatelji su nezavisne odrednice KV rizika, ali najznačajniji dokazi postoje za PWV (34-37).

3.1.3. Problemi s mjerenjem PWV-a u kliničkoj praksi

Unatoč značajnom potencijalu za prevenciju kardiovaskularnih bolesti i u upravljanju liječenjem hipertenzije, upotreba mjerenja PWV u kliničkoj praksi je ograničena.

Neka od ograničenja upotrebe PWV u klinici je nesigurna interpretacija rezultata zbog velike intra-individualne varijabilnosti PWV, kao i tehnički problemi s uređajem za mjerenje PWV-a koji zahtijevaju ponavljanje mjerenja i time produljuju vrijeme procjene (28, 38). U praksi, ti se problemi mogu ublažiti identificiranjem i kontroliranjem čimbenika koji utječu na njihovu pojavnost. Učinak eksperimentalnih uvjeta (različiti mjeritelji, doba dana, redni broj mjerenja) i ostalih zbunjujućih čimbenika na intra-individualnu varijabilnost PWV-a te na pojavu tehničkih problema prilikom mjerenja s određenim uređajem do sada nisu sustavno ispitani unutar iste studije, niti su studije u kojima su se čimbenici sporadično istraživali imale dovoljno snage za detekciju realnih efekta.

Prepreka koja ometa uvođenje PWV-a u kliničku praksu je i nedostatak metodološkog konsenzusa, što otežava točne usporedbe PWV-a unutar i između studija. Broj uzastopnih mjerenja korištenih u procjeni stvarne PWV vrijednosti primjer je takve nedosljednosti. Kako bi se procijenila stvarna vrijednost PWV-a, American Heart Association (AHA) preporuča usrednjavanje najmanje dva PWV mjerenja, a ako njihova razlika prelazi 0,5 m/s, potrebno je napraviti treće mjerenje te odrediti srednju vrijednost (39). Unatoč svom značaju, takva preporuka AHA temelji se na jednoj studiji (40) i klasificirana je kao preporuka temeljena na slabim dokazima (41). Neki istraživači pak koriste protokol mjerenja koji podrazumijeva ponavljanje mjerenja sve dok dvije vrijednosti ne budu unutar 0,5 m/s jedna od druge (42-45). Također, u mnogim studijama su dva ponovljena mjerenja uprosječena bez obzira na odstupanja (46-49), dok neke studije koriste samo jedno PWV mjerenje (50, 51). Niti jedna studija do sada nije procijenila učinak broja mjerenja korištenih u procjeni PWV-a na reproducibilnost PWV-a.

Nedostatak konsenzusa o očekivanoj reproducibilnosti PWV, koja se definira kao preciznost mjerenja dobivenih u različitim uvjetima tijekom kratkog razdoblja, obično nekoliko dana ili tjedana također je jedna od prepreka uvođenju PWV-a u kliničku praksu.

Takva je kvantifikacija potrebna za ispravno tumačenje rezultata longitudinalnih studija u kojima se prati promjena PWV-a u pojedinca kroz vrijeme i, posljedično, za utvrđivanje minimalne klinički važne promjene. Reproducibilnost PWV-a je u literaturi uglavnom istražena u neodgovarajućim ustrojima studije kao što su to studije validacije u kojima se novi uređaj uspoređuje s referentnim uređajem (52).

Samo je nekoliko studija koje su istraživale reproducibilnost PWV mjerenja jednog uređaja, a obično su koristile male veličine uzorka ($N \leq 21$) i procijenile ponovljivost - preciznosti mjerenja dobivenih pod istim uvjetima unutar 24 sata, a ne reproducibilnost. Također, te su studije koristile relativne mjere varijabilnosti koje se teško interpretiraju. Primjerice, često su korišteni koeficijent varijacije (CV), što je relativna mjera izražena u jedinicama standardne devijacije, ili pak koeficijent korelacije, kao što je koeficijent međuklasne korelacije (53, 54). Neke od ovih studija dodatno su izvijestile o reproducibilnosti koju su izrazile preko srednje razlike između dva PWV mjerenja uz odgovarajuće granice usklađenosti (od engl. *limits of agreement*) (54), dok su druge izvijestile o ponovljivosti - preciznosti mjerenja dobivenih pod istim uvjetima unutar 24 sata, a ne o reproducibilnosti (40, 55-58).

Za valjanu procjenu reproducibilnosti PWV-a tj. varijabilnosti koja se javlja zbog različitih eksperimentalnih uvjeta i ostalih zbunjujućih čimbenika (koji nisu klinički relevantne promjene), potrebne su studije s većim brojem ponovljenih mjerenja na određenom uređaju, odvojenih duljim razdobljem od 1-2 dana, koje reproducibilnost izražavaju rasponom – apsolutnom mjerom varijabilnosti koja se lako tumači u kontekstu određivanja minimalne klinički relevantne promjene.

3.1.4. Problemi s mjerenjem PWV-a u kliničkoj praksi u pandemijskom i post-pandemijskom razdoblju bolesti COVID-19

U prosincu 2019. u kineskom gradu Wuhanu otkriven je prvi službeni slučaj koronavirusa (bolest COVID-19). Od svog prvog izbijanja virus se brzo proširio diljem svijeta, a Svjetska zdravstvena organizacija (WHO) proglasila je pandemiju u ožujku 2020. godine. COVID-19 koji se u početku smatrao akutnom respiratornom bolešću, sada je prepoznat kao složena multsistemska bolest s opsežnim i štetnim djelovanjem na kardiovaskularni sustav (59, 60). Uz izravne posljedice i komplikacije koje izazove COVID-19, recentna studija je pokazala da 25% ispitanika koji su bili naizgled zdravi prije infekcije COVID-om 19 još uvijek ima simptome bolesti 12 mjeseci nakon (61).

COVID-19 može utjecati na arterijsku krutost preko nekoliko mehanizama: aktiviranjem upalnog odgovora, kao i aktiviranjem endotelne disfunkcije te disfunkcije autonomnog živčanog sustava. Stoga postoji mogućnost da nekoliko mjeseci nakon infekcije parametri arterijske elastičnosti budu značajno povišeni što ometa interpretaciju rezultata u longitudinalnim studijama kao i stratifikaciju rizika (62, 63).

Do sada nije provedena niti jedna prije-poslije studija koja uspoređuje arterijsku krutost prije i par mjeseci nakon infekcije COVID-om 19 i koja omogućava kvantifikaciju učinka COVID-a 19 na krutost arterija.

3.1.5. Problemi s mjerenjem PWV-a u kliničkoj praksi – sažetak

Usprkos velikom potencijalu pokazatelja arterijske krutosti kao što je to PWV u klinici, postoji niz čimbenika koji otežavaju njihovo uvođenje u kliničku praksu. Veliki broj uređaja s različitim principima mjerenja, velika intra-individualna varijabilnost mjerenja koja otežava interpretaciju longitudinalnih mjerenja i smanjuje preciznost stratifikacije rizika od KVB, česti tehnički problemi s uređajima koji zahtijevaju ponavljanje mjerenja, kao i nedostatak konsenzusa – metodološkog konsenzusa oko protokola procjene vrijednosti PWV-a te konsenzusa o očekivanoj reproducibilnosti PWV neki su od otežavajućih čimbenika za uvođenje PWV-a u kliničku praksu. Uz to, široka rasprostranjenost infekcije COVID-a 19 diljem svijeta uz pretpostavljenu

povezanost te infekcije s povećanjem krutosti arterija zahtijeva da se razluči dugoročan učinak COVID-a 19 – povećava li se arterijska krutost u inficiranih osoba dugoročno – trajno povećavajući rizik za KV događaje, ili se pak povećava na određeni duži period nakon infekcije – što je privremeni učinak koji ometa procjenu arterijske elastičnosti, ili pak nije pod dugoročnim utjecajem COVID-a 19.

3.1.6. Cilj objedinjenih radova

Cilj objedinjenih radova je osigurati dokaze na temelju kojih se može ublažiti utjecaj otežavajućih čimbenika i time olakšati uvođenje mjerenja PWV u kliničku praksu.

Specifični ciljevi su:

1. Identificirati eksperimentalne uvjete (različiti mjeritelji, različit period dana i broj posjeta) i ostale zbunjujuće čimbenike (vanjska temperatura, atmosferski tlak i vlažnost, sistolički, dijastolički i srednji arterijski tlak: MAP od engl. *mean arterial pressure*, srčani otkucaji, dob, spol, indeks tjelesne mase) koji utječu na izmjerene vrijednosti PWV-a a nisu kontrolirani u standardnom protokolu mjerenja (kao što su to primjerice obroci, alkohol, pušenje, tjelovježba), te kvantificirati njihove učinke.
2. Utvrditi reproducibilnost tj. količinu varijabilnosti PWV-a unutar ispitanika koja se može pripisati uvjetima/čimbenicima koji nisu klinički relevantna promjena, praćenjem PWV-a tijekom 2 tjedna u širokom rasponu sudionika i pod različitim eksperimentalnim uvjetima sličnima onima u kliničkoj praksi.
3. Utvrditi kako broj ponovljenih mjerenja korištenih u protokolu procjene PWV-a utječe na reproducibilnost PWV-a i koji to čimbenici pridonose velikim odstupanjima (>0.5 m/s) između uzastopnih mjerenja PWV-a.
4. Odrediti čimbenike koji utječu na poteškoće mjerenja PWV-a dvaju različitih uređaja (potreba za ponavljanjem mjerenja PWV-a ili za ručnim odabirom signala za analizu) te kvantificirati njihove učinke.
5. Utvrditi dugoročne učinke blage infekcije COVID-om 19 na arterijsku krutost.

3.2. PREGLED METODOLOGIJE OBJEDINJENIH RADOVA

Istraživanja su provedena u Laboratoriju za starenje krvožilja i prevenciju kardiovaskularnih bolesti Medicinskog fakulteta Sveučilišta u Splitu u periodu od listopada 2019. do travnja 2022. godine.

3.2.1. Mjerenje PWV-a

Uređaji za mjerenje PWV-a koji su korišteni u studijama su SphygmoCor CvMS (Atcor Medical, Sydney Australia) koji radi na principu aplanacijske tonometrije i Arteriograph (TensioMed, Budapest, Hungary) koji je oscilometrijski uređaj. Uređaj SphygmoCor mjeri cfPWV – brzinu pulsnog vala između karotidnog i femoralnog mjesta dok Arteriograph procjenjuje aortnu PWV (PWVao, od engl. *aortic pulse wave velocity*) iz mjerenja provedenih na jednom mjestu na brahijalnoj arteriji. Za PWVao se smatra da je točna aproksimacija cfPWV. Iznimno, u studiji III provedenoj na oboljelima od COVID-a 19 dio ispitanika je snimljen i uređajem SphygmoCor XCEL (Atcor Medical, Sydney Australia) pri čemu su ponavljajuća mjerenja za svakog ispitanika provedena uvijek istim uređajem.

Protokol za procjenu PWV-a bio je jednak za sve tri studije. Za oba uređaja, mjerenja su obavljena u skladu s preporukama American Heart Association's (AHA) za poboljšanje i standardizaciju vaskularnog istraživanja krutosti arterija (39). Oba mjeritelja su prije početka mjerenja prošla intezivan trening u trajanju od 7 dana, tijekom kojeg su pod nadzorom obavili približno 50 visokokvalitetnih mjerenja.

Mjerenja su provedena u tihoj prostoriji s kontroliranom sobnom temperaturom od ugodnih 21–23 °C. Ispitanici su dobili uputstva da se suzdrže od napornog vježbanja i alkohola 24 sata prije snimanja (jutarnjeg i poslijepodnevnog). Također, bilo im je zabranjeno jesti ili piti bilo što osim vode, te pušiti najmanje 3 sata prije svakog snimanja. Onima koji su uzimali vazoaktivne lijekove savjetovano je da ih nastave uzimati kao i obično i da ne mijenjaju dozu tijekom studije. Kako bi se osigurala hemodinamska stabilnost, sudionici su se odmarali u ležećem položaju 10 minuta prije prvog mjerenja PWV. Nakon završetka niza mjerenja pomoću jednog uređaja, sudionici bi se ustali,

prošetali prostorijom, a potom ponovno legli 10 minuta kako bi se hemodinamski pripremili za mjerenja pomoću drugog uređaja. Ovaj je postupak bio neophodan kako bi se spriječilo da sudionici zaspu dok leže na leđima kroz dulje razdoblje, posebno tijekom jutarnjih mjerenja. Sudionici nisu smjeli razgovarati niti spavati tijekom mjerenja. Sva mjerenja su obavljena na desnoj ruci (Arteriograph), te desnoj karotidnoj i femoralnoj arteriji (SphygmoCor).

Za kalibraciju signala pulsnih valova snimljenih SphygmoCorom, koristili smo mjerenja brahijalnog arterijskog krvnog tlaka učinjena validiranim oscilometrijskim tlakomjerom (Welch Allyn Connex ProBP 3400 digitalni monitor krvnog tlaka sa SureBP tehnologijom).

3.2.2. Mjerenja udaljenosti između karotidnog i femoralnog mjernog mjesta

Prema preporuci AHA smjernice za mjerenje arterijske krutosti (39), za izračun udaljenosti putovanja pulsnog vala korištena je metoda oduzete udaljenosti (od engl. *substraction method*). Prateći preporuke navedenih AHA smjernica, mjerenje udaljenosti između vrha drške prsne kosti (*incisura jugularis*) i mjesta palpiranja femoralne arterije učinili smo koristeći veliki školski šestar umjesto metarske vrpce, a zatim smo od te udaljenosti oduzeli udaljenost između mjesta palpiranja karotide arterije i vrha drška prsne kosti (*incisura jugularis*).

Naime, školskim šestarom smo dobili pravocrtnu udaljenost koja je neovisna o oblicima tijela kao što su veliki trbuh i/ili grudi kod pretilih osoba (64). Slično se dobije i korištenjem kliznog kalipera koji se preporučuje za mjerenje udaljenosti kada je pravocrtno mjerenje metarskom vrpcom otežano (39, 65, 66). Međutim, za razliku od kliznog kalipnera čije klizne lopatice ili hvatište još uvijek može ometati oblik tijela, kod školskog šestara to nije problem zbog njegovih dugih krakova.

Udaljenost između karotidnog i femoralnog mjesta izmjerena je samo jednom - tijekom prvog dolaska ispitanika.

Kako bismo opisali meteorološke (vanjske) uvjete u kojima su mjerenja obavljena u studiji opisanoj u radovima I i II (64, 67), podatke o vanjskoj temperaturi, tlaku i vlažnosti zraka dobili smo od Državnog hidrometeorološkog zavoda Republike Hrvatske

te smo ih koristili za procjenu vremenskih uvjeta tijekom svakog snimanja. Tijekom istraživanja temperatura se kretala od 4,5 do 23,3 ◦C, tlak zraka od 972 do 1011 hPa, a vlažnost zraka od 32 do 92%.

3.2.3. Ustroji studija

3.2.3.1. Ustroj studije korišten u radovima I i II

Radovi I i II (64, 67) temelje se na jedno-zaslijepljenoj blok-randomiziranoj ukriženoj longitudinalnoj studiji. Kako bi se osiguralo iste uvjete snimanja za svaki od uređaja, studija je randomizirana s obzirom na uređaj i mjeritelja korištenjem veličine bloka od 4. Dva su mjeritelja bila zaslijepljena za očitanja drugoga. Mjerenja očitana na svakom od uređaja analizirana su zasebno.

Studija je obuhvatila 36 ispitanika u dobi od 20 do 60 godina. Ispitanici su namjerno uzorkovani (od. engl. *purposive sampling*) prema dobi, spolu, hipertenzijskom statusu (normotenzivni ili hipertenzivni) i indeksu tjelesne mase (u rasponu od normalne težine do pretilih) kako bi se osigurala ravnomjerna distribucija po kriterijima uključivanja.

Hipertenzivni status ispitanici su naveli prilikom davanja anamnestičkih podataka, pri čemu su svi ispitanici s hipertenzijom naveli i lijekove koje inače primaju za tu bolest. Kategorija indeksa tjelesne mase određivala se prema klasifikaciji Centra za kontrolu i prevenciju bolesti za odrasle.

Kriteriji isključenja uključivali su aritmije koje su ispitanicima bile poznate od prije ili su evidentirane tijekom prvog dolaska: cerebrovaskularne bolesti, trudnoću, kiruršku amputaciju, onkološke bolesti, psihijatrijske bolesti i infekcije tijekom trajanja ispitivanja.

Svaki je ispitanik snimljen ukupno 12 puta tijekom 2 tjedna, četiri puta tijekom svakog od tri dana posjeta, a svaki je posjet bio odvojen jednim tjednom. Tijekom svakog posjeta dva mjeritelja su ispitanika snimala ujutro (7 - 10 h) i poslijepodne (16 - 18 h).

3.2.3.2. Ustroj studije korišten u radu III

Rad III (68) temelji se na prije-poslije ustroju studije u kojoj su mjere arterijske krutosti i središnje hemodinamike zabilježene u skupini sudionika prije i nakon infekcije COVID-19. Za procjenu arterijske krutosti i središnjih hemodinamskih parametara prije infekcije upotrijebili smo podatke ispitanika koji su bili dostupni iz prethodnih studija u Laboratoriju za starenje krvožilja i prevenciju kardiovaskularnih bolesti, a isti protokol mjerenja PWV-a je korišten nakon preboljenja infekcije. Za sve ispitanike koji su bili pozvani u studiju, dijagnoza COVID-19 postavljena je molekularnim testom lančane reakcije polimeraze reverznom transkriptazom (RT-PCR test).

Mjerenje nakon infekcije provedeno je između 8. i 12. tjedna nakon prestanka infekcije COVID-19, a maksimalni vremenski period između dva mjerenja bio je 24 mjeseca. Ukupno smo pozvali 36 ispitanika od kojih nam se 32 (89%) odazvalo u studiju, a svi su ispitanici prijavili blagi oblik bolesti COVID-19.

3.2.4. Statističke metode

Prikupljeni podaci su kodirani, sortirani i pripremljeni za analizu pomoću softverskog paketa (SPSS 25.0, IBM Corp., 2017). Deskriptivna statistika je korištena za opis razdiobi kvantitativnih i kvalitativnih varijabli, pri čemu je normalnost razdiobe kvantitativnih varijabli ispitana D'Agostino-Pearsonovim testom.

Za analizu ponovljenih mjerenja koristili su se višerazinski regresijski modeli s mješovitim učincima (od. engl. *multilevel mixed-effects regression models*). Ovisno o vrsti ovisne varijable korišteni su: višerazinski generalizirani linearni modeli s mješovitim učincima, višerazinski logistički regresijski model s mješovitim učincima, ili pak višerazinski Poissonov regresijski model s mješovitim učinkom. Analiza osjetljivosti učinjena je metodom najveće vjerojatnosti (od engl. *maximum likelihood method*). Za analizu promjena u odnosu na početnu vrijednost parametra (vrijednost prije bolesti COVID-19) korišteni su jednostavni, te višestruki linearni regresijski modeli.

Neovisne varijable u modelima iz radova I i II bile su: eksperimentalni uvjeti – broj posjete, mjeritelj, doba dana (jutro/poslijepodne), te prvi uređaj korišten u seriji snimanja;

zatim meteorološke varijable: vanjska temperatura (°C), tlak zraka (Pa) i relativna vlažnost (%); fiziološke varijable: sistolički, dijastolički i srednji tlak (u mmHg), te srčana frekvencija (otkucaji/po minuti); i karakteristike sudionika – dob, spol, indeks tjelesne mase (ITM), i dijagnoza hipertenzije (normotenzivni/hipertenzivni).

Neovisne varijable u modelima iz rada III bile su: dob, spol, vrijeme koje je prošlo od početka bolesti COVID-19 do drugog mjerenja, vrijeme koje je prošlo između dva mjerenja (prije i poslije bolesti COVID-19), početne vrijednosti modeliranog parametra (vrijednosti prije bolesti COVID-19) i vrsta uređaja koja se koristila za procjenu pojedinca.

Procjena minimalno potrebne veličine uzorka za navedene analize može se naći u izvornim radovima.

3.2.5. Etička načela

Opažajne studije iz ovog doktorskog rada provedene su u skladu s Helsinškom deklaracijom i odobrene su od strane Etičkog povjerenstva Medicinskog fakulteta Sveučilišta u Splitu (br. 2181-198-03-04-20-0015 za rad I i II, te br. 2181-198-03-04-20-0096 za rad III). Svi su ispitanici dali pismeni informirani pristanak za sudjelovanje.

3.3. Sažeti pregled rezultata objedinjenih radova

Sustavno su istraženi eksperimentalni uvjeti, kao i ostali potencijalni zbunjujući čimbenici kao što su meteorološki uvjeti, fiziološki čimbenici i obilježja sudionika, kako bi se utvrdilo jesu li i u kojoj mjeri utjecali na: a) vrijednosti PWV-a opažene između i unutar pojedinog ispitanika i izmjerene sa svakim uređajem zasebno, te b) na poteškoće mjerenja PWV-a s pojedinim uređajem.

Uz dob i MAP koji su očekivano snažno utjecali na vrijednosti PWV-a izmjerene s oba uređaja (varijabilnost PWV-a između ispitanika koja je pripisana razlici u ovim čimbenicima iznosila je 1,4-1,6 m/s za SphygmoCor te 2,3-3,2 m/s za Arteriograph), ostali značajni čimbenici razlikovali su se između uređaja. Tako je cfPWV izmjerena

SphygmoCorom, kao direktna mjera krutosti arterija, dodatno ovisila o dijagnozi hipertenzije, vanjskoj temperaturi, redoslijedu posjeta, ali i o interakciji MAP-a s vanjskom temperaturom. Intra-individualna varijabilnost MAP-a doprinijela je u prosjeku 0,27 m/s razlici u ponovljenim mjerenjima PWV-a na 5°C vanjske temperature te tek 0,004 m/s pri 25°C. S druge strane, mjerenja PWVao učinjena Arteriografom bila su u većoj mjeri ovisna o dobi nego mjerenja učinjena SphygmoCorom, a dodatno su ovisila i o karakteristikama ispitanika kao što su visina i spol, te broj otkucaja srca. Pri tome je intra-individualna varijabilnost MAP-a doprinijela u prosjeku 0,23 m/s razlici u ponovljenim mjerenjima PWV-a. Također, ženski spol je u prosjeku povećavao izmjerene vrijednosti PWVao za 1,56 m/s (95% CI 0,7-2,4). Doba dana (ujutro/poslijepodne), različiti mjeritelji koji su prošli istu količinu treninga, te vrsta uređaja koja je korištena prva u seriji snimanja nisu utjecali na izmjerene vrijednosti PWV-a.

Međutim, poteškoće s mjerenjem na oba uređaja javljale su se znatno rjeđe u poslijepodnevnim satima: omjer stopa incidencije za ponovljena mjerenja SphygmoCorom tijekom poslijepodneva (IRR, od engl. *incidence rate ratio*) iznosio je 0,88, 95% CI 0,79-0,97), dok je omjer izgleda (OR, od engl. *odds ratio*) za ručni odabir signala za analizu u poslijepodnevnim satima bio 0,23 (95% CI 0,10-0,54). Također, za oba smo uređaja utvrdili povezanost između tehničkih problema tijekom mjerenja i ženskog spola. Dok je broj ponovnih mjerenja s SphygmoCorom bio umjereno povećan u žena (IRR 1.23, 95% CI 1.01-1.50), ručni odabir signala s uređajem Arteriograph bio je mnogo češći u žena nego u muškaraca (OR 51.4, 95% CI 6.42-412.2).

Konačno, na oba uređaja otkrili smo da povećanje dobi povećava ne samo vrijednosti PWV-a, već i varijabilnost njihovih ponovljenih mjerenja, što sugerira da bi se u starijih osoba trebala povećati preciznost mjerenja, po mogućnosti korištenjem 3 - 4 mjerenja u nizu umjesto samo 2.

Vezano uz procjenu reproducibilnosti PWV u periodu od 2 tjedna utvrdili smo da je reproducibilnost oba uređaja problematična jer je intra-individualni raspon izmjerenih vrijednosti PWV-a širi od 1m/s za većinu ispitanika. Razliku od 1 m/s neki stručnjaci smatraju minimalnom klinički značajnom razlikom u PWV iako se takvo zapažanje ne temelji na dokazima.

Broj uzastopnih mjerenja u protokolu procjene PWV-a značajno utječe na reproducibilnost pri čemu smo najbolju reproducibilnost dobili za medijan 4 uzastopna

mjerenja i gornju granicu za prihvatljivu varijabilnost (varijabilnost uzrokovanu drugim izvorima osim klinički značajne promjene) od 1,1 m/s umjesto 1,0 m/s.

Također je utvrđeno da relativne mjere varijabilnosti kao što je koeficijent varijacije (CV od engl. *coefficient of variation*) nisu primjerene za usporedbu reproducibilnosti i ponovljivosti koje se često u literaturi koriste kao istoznačnice. Dok su vrijednosti CV pridružene reproducibilnosti i ponovljivosti slične, njihovi rasponi se klinički značajno razlikuju.

Čimbenici koji utječu na velika odstupanja u PWV (>0.5 m/s) između susjednih mjerenja su istovjetni onima koji utječu na vrijednosti PWV-a općenito, identificiranima u radu I. Velika odstupanja nisu povezana s karakteristikama ispitanika.

Konačno, u radu na ispitanicima koji su preboljeli COVID-19 utvrđeno je postojanje odgovora na blagi oblik bolesti u različitim parametrima arterijske krutosti i središnje hemodinamike: cfPWV, augmentacijskom indeksu standardiziranom na 75 otkucaja srca u minuti (AIx@HR75), centralnom dijastoličkom krvnom tlaku (cDBP); kao i dijastoličkom krvnom tlaku (DBP) i MAP.

Veličina ovih odgovora ovisila je o vremenu koje je prošlo od početka bolesti COVID-19, kao i o dobi ispitanika ($p_{\text{regresijski koeficijenti}} \leq 0.013$), a osim za parametar AIx@HR75 ($p = 0.003$), za sve je ostale parametre veličina odgovora bila neovisna o početnim vrijednostima parametara. Zapravo, modeli mješovitog učinka pokazali su na razini grupe klinički značajnu progresiju vaskularnog oštećenja unutar razdoblja od 2 - 3 mjeseca nakon infekcije: (srednja promjena na razini grupe nakon infekcije +1.4 m/s za cfPWV, +15% za AIx@HR75, +8 mmHg za DBP, cDBP i MAP).

3.4. Rasprava

Po prvi puta su sustavno, unutar jedne studije, istraženi brojni eksperimentalni i meteorološki uvjeti kao i fiziološki čimbenici te karakteristike sudionika kako bi se utvrdilo utječu li, i ako da, do koje razine, na vrijednosti PWV-a opažene između i unutar ispitanika te na pojavu tehničkih poteškoća u mjerenju PWV-a. Vrijednosti PWV-a više su puta prikupljene od ispitanika tijekom 2 tjedna – u vremenskom razdoblju u kojem se ne očekuju klinički značajne promjene u ispitanika. Nadalje, analize su učinjene odvojeno

za mjerenja dobivena s različitim validiranim uređajima: cfPWV mjerenja dobivena uređajem za aplanacijsku tonometriju SphygmoCor CvMS i PWVao mjerenja dobivena uređajem za oscilometriju Arteriograph.

Kako bi se odgovorilo na postavljena istraživačka pitanja upotrijebljen je ustroj studije koji osigurava snažne dokaze. Provedena je jedno-zaslijepljena blok-randomizirana ukrižena longitudinalna studija s čak 12 ponovljenih mjerenja po ispitaniku sa svakim od uređaja. Nadalje, naš validacijski uzorak pokazao je prilično širok raspon PWV-a, dobi, ITM-a i brahijalnih krvnih tlakova te ujednačenu distribuciju prema dobi, spolu, ITM-u i statusu hipertenzije. S obzirom na to da uniformna distribucija stavlja manji naglasak na središte distribucije, a više na njezine ekstreme, ovakav uzorak osigurava preciznije validacijske procjene od uzorka koji je reprezentativan za ciljanu populaciju.

Nema mnogo studija koje su istraživale reproducibilnost PWV-a, a one koje jesu uglavnom su provele od 2 do 6 ponovljenih mjerenja po sudioniku u periodu od jednog do dva dana (57, 69-72). Unatoč činjenici da smo zabilježili najviše ponovljenih PWV mjerenja po sudioniku (N = 12), te da je naš uzorak imao relativno širok raspon PWV-a i PWV determinanti, slaganje ponovljenih PWV mjerenja procijenjeno u našem istraživanju bilo je usporedivo s onim što je objavljeno u literaturi (57, 73, 74).

3.4.1. Čimbenici koji utječu na izmjerene vrijednosti PWV-a

3.4.1.1. Životna dob

Očekivano, životna dob je bila čimbenik s najvećim učinkom na vrijednosti cfPWV i PWVao, dok je MAP slijedio kao drugi najutjecajniji čimbenik. Ovi su podaci u skladu s podacima iz drugih studija koje također pokazuju jaku povezanost PWV-a s životnom dobi i MAP-om (39, 75-77). U prosjeku, dob je bila odgovorna za približno 2 - 3 m/s razlike u PWV-u između ispitanika, pri čemu je njen utjecaj na vrijednosti PWVao bio snažniji negoli na vrijednosti cfPWV. Naime, za svakih 10 godina života cfPWV je porastao za 0,4 m/s (95% CI 0,2-0,6), a PWVao za 0,8 m/s (95% CI za 0,6–1,0). Druge studije koje su koristile uređaj SphygmoCor CvMS izvijestile su o usporedivim učincima

dobi na cfPWV mjerenja u rasponu od 0,2 m/s za 10 godina (78), preko 0,3–0,4 m/s (79), do nešto većih učinaka od oko 0,7-0,9 m/s za 10 godina (75, 80), o kojima je izvijestila ista skupina autora. Iako je dob također identificirana kao najjača determinanta vrijednosti PWVao (81), studije koje su istraživale čimbenike koji utječu na Arteriographova mjerenja PWVao nisu izvijestile o usporedivim, nestandardiziranim regresijskim metrikama. Međutim, studija koja je istraživala PWVao mjereno scilometrijskim Vicoder uređajem izvijestila je o usporedivom učinku od 0,4–1,0 m/s za 10 godina (82).

Iako je dob ostala konstantna tijekom 2 tjedna studije i stoga nije pridonijela apsolutnoj promjeni u ponovljenim mjerenjima osobe, također je utvrđeno da je starija dob povećala varijabilnost ponovljenih mjerenja i za cfPWV i za PWVao, povećavajući grešku mjerenja. Grillo i suradnici također su otkrili da pacijenti s povećanom krutošću arterija imaju veću varijabilnost u ponovljenim mjerenjima PWV-a dobivenim različitim uređajima za procjenu cfPWV-a (57). Smatra se da je ovaj fenomen posljedica činjenice da se PWV definira kao omjer prijeđene udaljenosti i vremena prolaska pulsnog vala (PWTT, od engl. *pulse wave transit time*). U tom slučaju mala razlika u PWTT može uzrokovati relativno veliku razliku u PWV-u u ispitanika s povećanom arterijskom krutošću, dok je ta razlika zanemariva u ispitanika s normalnom krutošću arterija. Sve navedeno sugerira da bi, kada se u osoba starije životne dobi prate promjene PWV-a s vremenom, bilo korisno povećati broj mjerenja tijekom jednog posjeta s 2 na 3 - 4 kako bi se poboljšala preciznost procjene.

3.4.1.2. MAP i vanjski meteorološki čimbenici

Drugi najjači učinak na izmjerene vrijednosti PWV-a imao je MAP, koji se smatra najznačajnijom fiziološkom varijablom koja utječe na krutost arterija (83-85). S povećanjem MAP-a, krvne žile se ukrućuju, što znači da svaki put kada se mjerenja provode pod različitim vrijednostima krvnoga tlaka treba uzeti u obzir i učinak MAP-a na ponovljena mjerenja PWV-a.

U našoj studiji, MAP je u prosjeku bio odgovoran za približno 1-2 m/s razlike u PWV-u između ispitanika, pri čemu su se vrijednosti PWVao povećavale za 0,2 m/s (95% CI 0,10-0,25) za svakih 5 mmHg porasta MAP-a. Takvi su učinci usporedivi s procjenom

oscilometrijskog uređaja Vicordera: 0,05–0,20 m/s za svakih 5 mmHg (82). Nadalje, uzimajući u obzir varijabilnost MAP-a unutar ispitanika, u prosjeku 0,23 m/s (od 0,10 do 0,33 kada se uzme u obzir nesigurnost u procjenama) razlike u ponovljenim PWVao mjerenjima može se pripisati promjenama MAP-a.

Za razliku od PWVao, očekivane promjene u cfPWV-u zbog promjena u MAP-u ili promjena vanjske temperature ne mogu se jednostavno interpretirati. Razlog je značajna interakcija između vanjske temperature i MAP-a, koja sugerira da vanjska temperatura moderira odnos između cfPWV-a i MAP-a. Tako je očekivana razlika između predviđenog cfPWV-a dodijeljenog najnižem i najvišem opaženom MAP-u i prilagođenog za druge čimbenike bila 2,62 m/s na vanjskoj temperaturi zraka od 5◦C, ali samo 0,04 m/s na temperaturi zraka od 25◦C. Nadalje, intra-individualne razlike u ponovljenim mjerenjima PWV-a uzrokovane varijacijama MAP-a predviđene su na 0,27 m/s pri 5◦C i 0,004 m/s m/s pri 25◦C. Zanimljivo je da za PWVao mjerenja nije utvrđen značajan utjecaj vanjske temperature ili značajna interakcija temperature s MAP-om.

Dok je negativan odnos između vanjske temperature zraka i očitanja arterijskog krvnog tlaka uočen u mnogim studijama (86-90), sličan je odnos s vanjskom temperaturom pretpostavljen i za krutost arterija, ali postojeće studije imaju nedosljedne rezultate. Di Pilla i suradnici izvijestili su da je u neprilagođenoj regresijskoj analizi cfPWV zabilježen pomoću SphygmoCora bio slabo i obrnuto proporcionalno povezan s vanjskom temperaturom, ali ne i u višestrukoj regresijskoj analizi u kojoj se dodatno kontrolirao utjecaj dobi, ITM, vrijednosti krvnoga tlaka, doba dana, te razina plinova O_3, CO i N_2O (91). U ANOVA analizi ponovljenih mjerenja, Kita i suradnici pokazali su značajnu promjenu indeksa arterijske krutosti tijekom sezona pri čemu je veća krutost uočena tijekom ljeta (92). Iako je mehanizam na kojem se temelji interakcija temperature i MAP-a izvan opsega naše studije, simpatička aktivacija izazvana hladnoćom mogla bi objasniti ovisnost izmjerenih vrijednosti cfPWV o vanjskoj temperaturi u uvjetima u kojima je sobna temperatura konstantna (91, 93). U tom slučaju, kod nižih vanjskih temperatura, vrijeme odmora u ležećem položaju u prostoriji s kontroliranom temperaturom moglo bi se produžiti za više od 10 minuta kako bi se omogućila prilagodba tijela na temperaturu. Alternativno, mjerenje bi se moglo provesti u periodu kada su vanjske temperature više, poput onih oko 25 °C.

3.4.1.3. Brzina otkucaja srca

Pored utjecaja vanjske temperature na mjerenja PWV-a, također je uočena i razlika u utjecaju brzine otkucaja srca na vrijednosti cfPWV i PWVao. Ovaj fiziološki čimbenik imao je umjereni utjecaj na mjerenja PWVao i bio je odgovoran za približno 1,5 m/s razlike u PWVao između ispitanika povećavajući PWVao za 0,2 m/s (95% CI 0,02– 0,40) za svakih 10 otkucaja srca u minuti. Učinak je sličan onome o kojem su izvijestili Tan i suradnici koji su koristili hibridni uređaj za aplanacijsku tonometriju i oscilometriju SphygmoCor XCEL i otkrili povećanje cfPWV od 0,11–0,28 m/s za svakih 10 otkucaja srca u minuti (94). Osim toga, individualna varijabilnost u srčanim otkucajima u našoj studiji je u prosjeku uzrokovala odstupanje od 0,12 m/s (nesigurnost, 0,01–0,27) u ponovljenim mjerenjima PWVao. S obzirom na te činjenice, može se pretpostaviti da će male promjene u broju otkucaja srca imati minimalno fiziološki značajne promjene na PWVao. Međutim, za veće promjene u broju otkucaja srca, očekuje se da će dovesti do značajnih razlika u PWVao. Za razliku od PWVao, nismo uočili značajan utjecaj brzine otkucaja srca na mjerenja cfPWV-a dobivena aplanacijskom tonometrijom.

3.4.1.4. Eksperimentalni uvjeti

Od svih testiranih eksperimentalnih uvjeta samo je redoslijed posjeta utjecao na mjerenja PWV. Točnije, dok redoslijed posjeta nije imao utjecaja na mjerenja PWVao, mjerenja cfPWV snimljena tijekom drugog posjeta bila su u prosjeku 0,23 m/s (95% CI 0,03–0,43) niža od onih u prvom posjetu. Međutim, nije se utvrdila značajna razlika kada su mjerenja cfPWV iz trećeg posjeta uspoređeni s onima iz prvog. Jedno od mogućih objašnjenja za nedostatak trenda u trećoj posjeti je utjecaj vanjske temperature. Budući da je vanjska temperatura prepoznata kao važan čimbenik koji utječe na mjerenja cfPWV, izraženije meteorološke promjene pri trećem posjetu vjerojatno su prikrile učinak redoslijeda posjeta. Naime, za vrijeme snimanja nekih ispitanika, vanjska temperatura zraka varirala je za 8 - 10◦C između trećeg i druga dva posjeta.

Otkriće viših vrijednosti cfPWV tijekom prvog posjeta može sugerirati da, unatoč tome što su mjeritelji prošli obuku koju preporučuju trenutne smjernice (39), možda tijekom prvog posjeta nisu bili dovoljno utrenirani za korištenje SphygmoCor CvMS

uređaja. Međutim, kada bi to bio slučaj, očekivali bi i da se varijabilnost mjerenja cfPWV koja je opažena unutar ispitanika smanjuje s povećanjem redoslijeda posjeta što nije bio slučaj. Nadalje, Grillo i suradnici su pokazali da je dvotjedno razdoblje obuke bilo dovoljno za postizanje prihvatljive do izvrsne usklađenosti ponavljajućih mjerenja PWV-a za različite uređaje uključujući i SphygmoCor CvMS (57), a kao što je prethodno navedeno, reproducibilnost ponovljenih mjerenja cfPWV u našoj je studiji bila usporediva s onom o kojoj su izvijestili Grillo i suradnici. Elliot i suradnici su također proučavali utjecaj treninga na ponovljivost mjerenja cfPWV, no u studiji su koristili hibridni SphygmoCor XCEL uređaj s drugačijim načinom rada od uređaja SphygmoCor CvMS (95).

Alternativno, više vrijednosti cfPWV tijekom prvog posjeta mogu ukazivati na učinak bijele kute na arterijsku krutost (96, 97) – učinak koji opisuje privremenu ili trajnu reakciju pacijenta na sam proces mjerenja krvnih tlakova. Doista, prvo mjerenje cfPWV-a u našoj studiji bilo je znatno više od drugog mjerenja učinjenog u seriji mjerenja tijekom prvog posjeta (medijan razlike 0,35 m/s, 95% CI 0,05–0,65), a također je bilo više i od prvog mjerenja učinjenog u seriji mjerenja tijekom drugog posjeta (0,60 m/s, 95% CI 0,20–1,00). Međutim, Barochiner i suradnici su izvijestili o značajno većem učinku bijele kute na vrijednosti cfPWV s medijanom razlike prvog i drugog mjerenja od 1,2 m/s (97). Nadalje, analiza sistoličkog i dijastoličkog krvnog tlaka izmjerenih u našoj studiji nije pokazala klinički značajan učinak bijele kute, koji je definiran kao vrijednost krvnog tlaka izmjerenog u ordinaciji ili klinici koja prelazi vrijednosti dnevnog ambulantnog mjerenja krvnog tlaka za 20 mmHg za sistolički ili 10 mmHg za dijastolički krvni tlak, bez obzira na primjenu anti-hipertenzivnih lijekova (98). Stoga, iako je u našem uzorku moguće bio prisutan učinak bijele kute, veličina tog učinka je bila nedovoljna da bi se opravdalo odbacivanje prvog mjerenja.

Nije utvrđeno da doba dana ili različiti ispitivači utječu na izmjerene vrijednosti cfPWV ili PWVao iako trenutne smjernice preporučuju da se ponovljena mjerenja trebaju provoditi u isto doba dana, po mogućnosti s istim ispitivačem (39). Nekoliko je studija provedenih na različitim populacijama: mladim zdravim dobrovoljcima (99), ženama s urednom trudnoćom (100) ili u zdravih pojedinaca različite životne dobi te u bolesnika sa srčanim bolestima (73), potvrdilo naš rezultat o izostanku cirkadijalne varijacije u mjerenjima PWV-a. Jedna je pak studija, koja je izvijestila o povećanju cfPWV-a s

dobom dana otkrila da su dnevne promjene PWV-a izgubile značaj nakon prilagodbe za krvne tlakove u regresijskom modelu, što sugerira da su uočene promjene u arterijskoj krutosti primarno određene promjenama u BP-u (101). Temeljem ovih nalaza, protokoli za mjerenje PWV-a u budućim longitudinalnim istraživanjima mogu biti pojednostavljeni, jer nije potrebno mjeriti PWV u isto vrijeme dana ili s istim mjeriteljem, pod uvjetom da je količina obuke za sve mjeritelje bila usporediva.

3.4.1.5. Obilježja ispitanika: spol, visina i hipertenzivni status

Uočene razlike u modelima PWV-a dvaju uređaja uključivale su i obilježja ispitanika poput spola i visine koja su imale značajan učinak na PWVao, ali ne i na cfPWV mjerenja; te hipertenzivni status, koji je u prosjeku povećao cfPWV u ispitanika s hipertenzijom za 0,44 m/s, ali nije utjecao na mjerenja PWVao.

Ženski spol je u prosjeku povećao PWVao pojedinca za 1,56 m/s, dok je jedan cm visine povećao PWVao za 0,06 m/s (95% CI 0,02–0,09).

Spol, s višim vrijednostima PWVao u žena u usporedbi s muškarcima, također je utvrđen kao značajan čimbenik u drugoj studiji koja je istraživala mjerenja PWVao Arteriograph-a (81). Međutim, dok su izvješća o odnosu između spola i PWV-a izmjerenog drugim uređajima osim Arteriographa bila nekonzistenta, sve studije, uključujući one koje su koristile oscilometrijske uređaje, prijavile su veće vrijednosti PWV-a u muškaraca (75, 79, 80, 82, 102-104) ili pak nisu utvrdile povezanost sa spolom (105-109). Stoga se čini da su veće vrijednosti mjerenja PWVao u žena specifične za Arteriograph uređaj.

Za razliku od mjerenja PWVao, nije utvrđeno da su mjerenja cfPWV povezana sa spolom. Vermeersch i suradnici su procijenili perifernu i središnju PWV-a koristeći aplanacijsku tonometriju na velikom uzorku zdrave populacije srednjih godina života i zaključili da dok su periferna i karotidna mjerenja PWV-a bila povezana sa spolom, to nije bio slučaj s parametrima središnje arterijske krutosti kao što je cfPWV (106). Također, velika studija u kojoj su se procjenjivale referentne vrijednosti za arterijsku krutost nije pronašla značajne razlike između spolova u cfPWV-u nakon prilagodbe za dob i MAP (77). Naime, iako je cfPWV bio izrazito viši u muškaraca, prisutnost muškog

spola također je bila popraćena izrazitim razlikama u dobi i krvnom tlaku. Nakon prilagodbe za dob i MAP, autori su pronašli zanemariv utjecaj spola na cfPWV (razlika između muškaraca i žena od 0,1 m/s) i nastavili s definicijom referentne vrijednosti populacije uključivanjem ispitanika bez obzira na spol. Konačno, spol nije utvrđen kao značajan prediktor cfPWV-a u višestrukim modelima linearne regresije koji su kontrolirani za dob, MAP, HR i temeljeni na naizgled zdravoj kohorti nepušača bez kardiovaskularnih bolesti, dijabetesa i pretilosti uzorkovanih iz studije Framingham Heart Study (110).

Nismo otkrili značajnu interakciju između spola i dobi ni za jedan uređaj. Što se tiče učinka visine, slično Jatoiju i suradnicima koji su također istraživali Arteriographova mjerenja PWVao, pronašli smo obrnutu korelaciju između PWVao i visine (podaci nisu prikazani). Međutim, dok je u našem višestrukom regresijskom modelu PWVao rastao s povećanjem visine, u Jatoijevom modelu ta povezanost nakon prilagodbe za ostale prediktore nije dosegla statistički značaj (81). Nadalje, povezanost visine i PWVao utvrđena u studiji iz ove doktorske disertacije bila je pozitivna; slično procjenama jednostavnog regresijskog modela koji su razvili Mellin i suradnici na Vircorderovim mjerenjima (82) i višestrukog regresijskog modela razvijenog na PulsePen mjerenjima u djece (111).

3.4.1.6. Općenito o modelima PWV-a dvaju uređaja

Kada se dobiveni rezultati sagledaju zajedno može se zaključiti da su vrijednosti cfPWV izmjerene SphygmoCorom osjetljivije na stanje arterijskog stabla jer, osim o dobi i MAP-u, također ovise o statusu hipertenzije, interakciji između MAP-a i vanjske temperature, vanjskoj temperaturi i redoslijedu posjeta; dok PWVao vrijednosti izmjerene Arteriographom više ovise o obilježjima pojedinca kao što su spol, visina, ali i dob budući da je dob značajno snažniji prediktor PWVao-a negoli cfPWV-a.

3.4.2. Čimbenici koji utječu na poteškoće mjerenja

Poteškoće s mjerenjem pojavljivale su se relativno često: od 19% slučajeva u kojima je Arteriographov oscilometrijski signal morao biti ručno odabran za analizu, do preko 24% slučajeva u kojima je SphygmoCorov signal pulsnog vala bio marginalne, ali prihvatljive kvalitete, do 32% slučajeva u kojima su se morala ponoviti snimanja SphygmoCora. Uz činjenicu da sve ove poteškoće produžuju vrijeme procjene PWV-a što otežava upotrebu takvih uređaja u klinici, također smo otkrili da jedna od tih poteškoća — marginalna kvaliteta SphygmoCorovog signala, utječe na točnost procijenjenih vrijednosti cfPWV na način da povećava te vrijednosti u prosjeku za 0,37 m/s (95% CI 0,16–0,58 m /s). S druge strane, nismo utvrdili da poteškoće vezane uz ručni odabir signala Arteriographa, za koje se može očekivati da povećavaju nesigurnost procjene PWVao-a i utječu na točnost očitanja, povezuju s promjenom vrijednosti PWVao.

Pojavnost svih gore navedenih poteškoća s mjerenjem PWV-a smanjena je kada su mjerenja obavljena poslijepodne (16 - 18 sati) u usporedbi s jutarnjim terminima (7 - 10 sati). Učinak doba dana na potrebu za ručnim odabirom signala Arteriographa bio je snažan. Poslijepodnevna snimanja smanjila su vjerojatnost ručnog odabira s 23% tijekom jutarnjih termina na 6% poslijepodnevnih snimanja. Doba dana je također imalo umjeren utjecaj na potrebu za ponavljanjem mjerenja SphygmoCorom smanjujući IRR za takav događaj u poslijepodnevnim mjerenjima u prosjeku za 12%. Konačno, vjerojatnost za pojavnost signala SphygmoCora granične kvalitete smanjila se s 26% tijekom jutra na 19% poslijepodne. Kako doba dana nije pokazalo značajan utjecaj na apsolutne vrijednosti cfPWV ni PWVao, provođenjem mjerenja PWV-a u poslijepodnevnim satima može se značajno smanjiti mogućnost tehničkih poteškoća s mjerenjem.

Spol je bio još jedan čimbenik koji je utjecao na poteškoće mjerenja na oba uređaja. Snažno je povećao OR za ručni odabir signala Arteriographa za 51 put, a također je povećao IRR za ponovljena mjerenja SphygmoCorom za 1,23 puta. Poteškoće koje smo uočili u procjeni PWV vrijednosti ženskih ispitanica na oba uređaja mogli bi se pripisati činjenici da su muškarci češći prototipni ispitanici. Naime, zbog utjecaja menstrualnog ciklusa na mjerenja PWV-a u žena, moguće je da se izbjegava uključiti žene u prototipna istraživanja što bi moglo rezultirati suboptimalnim prikupljanjem i obradom signala u žena. No, kako u literaturi nema radova koji opisuju uzorak za razvoj prototipa, nedostaje

dokaza kojima bi potvrdili ili opovrgli ovu pretpostavku. Također je utvrđeno da je redoslijed posjeta, koji je utjecao na vrijednosti ponovljenih mjerenja cfPWV, također utjecao i na pojavu poteškoća s mjerenjem sa SphygmoCorom, na način da su pojavnosti potrebe za ponovljenim mjerenjem i signala s marginalnom kvalitetom značajno smanjeni tijekom trećeg posjeta u usporedbi s prvim posjetom ispitanika, dok je pojavnost tijekom drugog posjeta bila usporediva s onom tijekom prvog posjeta. Dakle, čini se da u smislu poteškoća s mjerenjem sa SphygmoCorom još uvijek postoji krivulja učenja tijekom prva 2 tjedna.

3.4.2.1. Broj mjerenja korištenih u protokolu za procjenu PWV-a

Kada govorimo o primjeni cfPWV-a u kliničkoj praksi, pitanje broja uzastopnih mjerenja koje se koriste u procjeni PWV-a je iznimno važno, ne samo u kontekstu željene preciznosti te procjene već i vremena koje je potrebno za snimanje. Dokazi koji podupiru postojeći odabir broja mjerenja korištenih u procjeni PWV-a, bez obzira na njihovu važnost, oskudni su i trenutno se oslanjaju na jednu studiju (40). Iako su autori studije tvrdili da su ispitali učinak broja uzastopnih mjerenja PWV-a na njenu reproducibilnost, u studiji koja je uključivala 80% muškaraca, autori su proveli tri uzastopna mjerenja PWV-a uređajem Compilor učinjenih u razmaku od približno 1 minute i u stvarnosti su procijenili učinak broja mjerenja na ponovljivost PWV-a. Osim toga, Souze i suradnici procijenili su utjecaj broja mjerenja na ponovljivost SphygmoCorovih mjerenja PWV-a u starijih osoba (112). Istraživanje opisano u ovoj doktorskoj disertaciji prva je studija koja istražuje učinak broja mjerenja korištenih u protokolu za procjenu PWV-a na reproducibilnost PWV-a, koji je ovdje procijenjen pod različitim uvjetima i u širokom rasponu ispitanika.

Na temelju opaženog učinka broja mjerenja na reproducibilnost PWV-a, ne može se preporučiti korištenje jednog ili dva mjerenja prilikom procjene PWV-a. To uključuje i postupak predložen trenutnim smjernicama za PWV (38, 113) za procjenu PWV-a u kojem se sporadično može koristiti i treće mjerenje ukoliko je razlika prva dva mjerenja veća od 0,5 m/s. Optimalna reproducibilnost je u ovom istraživanju opažena tek s četiri mjerenja, i to kada se prag pogreške koja se može tolerirati povećao s 1,0 na 1,1 m/s.

3.4.2.2. PWV Ponovljivost i Reproducibilnost: Postoji li razlika?

Osim kliničke promjene arterijske krutosti, procjena reproducibilnosti mjerenja PWV-a trebala bi uzeti u obzir svaki izvor varijabilnosti PWV-a za koji se može realno očekivati da će se susresti u kliničkom kontekstu. Stoga je u ovom istraživanju ispitan i utjecaj različitih mjeritelja, doba dana i broja posjeta, ali i različitih vanjskih meteoroloških prilika na takvu reproducibilnost.

Kada je ponovljivost PWV-a utvrđena u istraživanju iz ove doktorske disertacije izražena preko CV, ona je bila u skladu s prethodno objavljenim procjenama za ponovljivosti SphygmoCorovih (57) i Arteriographovih (Li, Cordes et al. 2014; Ring, Eriksson et al. 2014) mjerenja PWV-a, uključujući i studiju od Li Y et al., u kojoj su mjerenja snimljena u različito doba dana tijekom 24 sata. Nadalje, naši su rezultati, kao i nalazi drugih studija koje su izvještavale o ponovljivosti PWV-a koristeći CV, bili u skladu s procjenama reproducibilnosti PWV-a iz tri randomizirane kontrolirane studije koje su pratile promjene PWV-a tijekom vremena (58). Dosljednost svih ovih CV-ova ilustrira poteškoće u interpretaciji rezultata kada se za procjenu preciznosti koristi relativna mjera varijabilnosti (CV, ali i ICC, Pearsonov koeficijent korelacije i drugi) i ukazuje da bi upotreba relativnih mjera varijabilnosti mogla biti jedan od čimbenika zbog kojeg se često zamjenjuju pojmovi ponovljivosti i reproduktivnosti u studijama (40, 55-58). Iako je u nekim od navedenih studija korišteno više promatrača, što bi se moglo smatrati razlogom za korištenje termina „reproducibilnost", njihova definicija ne odgovara definiciji reproducibilnosti PWV-a koju propisuje AHA za mjerenja PWV-a koja se obavljaju s razmakom većim od 24 sata. Za razliku od usporedbi CV, kada smo uspoređivali ponovljivost i reproducibilnost PWV-a koristeći raspon vrijednosti iz dva uzastopna mjerenja, kao što se trenutno preporučuje od strane AHA, naši rezultati su pokazali klinički značajnu razliku od 0,7 m/s između ponovljivosti i reproducibilnost PWV-a. Za razliku od reproducibilnosti, ponovljivost PWV-a bila je prihvatljiva za oba uređaja, budući da je raspon vrijednosti PWV-a veći od 1 m/s uočen za samo 14% (SphygmoCor) i 13% (Arteriograph) dana snimanja.

3.4.2.3. Odstupanja između uzastopnih PWV mjerenja od ≥ 0.5 m/s

U ovom je istraživanju po prvi puta detaljnije istražena pojavnost velikih odstupanja (>0,5 m/s) između uzastopnih mjerenja PWV-a i čimbenici koji utječu na veličinu takvih odstupanja. Rezultati u cjelini ukazuju na važne razlike između uređaja.

Pokazano je da se izgledi za pojavu jednog para mjerenja s velikim odstupanjem povećavaju s medijanom ispitanikovog MAP-a ili DBP-a za SphygmoCor ili medijanom HR-a za Arteriograph. Međutim, na pojavnost takvog para mjerenja ne utječu eksperimentalni niti vanjski meteorološki uvjeti koje smo testirali, niti obilježja ispitanika kao što su dob, spol, ITM ili hipertenzivni status. Barem dio učinka ovih fizioloških varijabli na pojavnost para mjerenja PWV-a s velikim odstupanjem je posljedica njihove prirodne varijacije unutar pojedinca. Varijabilnost krvnog tlaka raste s prosječnom vrijednošću krvnog tlaka i pozitivno je povezana s težinom oštećenja organa i kardiovaskularnim morbiditetom i mortalitetom u bolesnika s hipertenzijom i u općoj populaciji (114). Varijabilnost brzine otkucaja srca, s druge strane, rezultat je složenih, nelinearnih interakcija različitih fizioloških sustava (115). Razlika između uređaja, u smislu identificiranih fizioloških varijabli koje su značajni prediktori velikog odstupanja između parova mjerenja, najvjerojatnije je posljedica njihovih različitih načina rada, pri čemu cfPWV aplanacijska tonometrija naizgled pruža izravniju procjenu arterijske krutosti zbog povezanosti s krvnim tlakom (83, 84, 116).

U skladu s gore navedenim rezultatima identificirali smo i čimbenike koji utječu na veličinu odstupanja između uzastopnih PWV mjerenja, i ponovno smo pronašli jasnu razliku u značajnim prediktorima između uređaja. Nalazi SphygmoCora pokazuju da su odstupanja veća u starijih osoba s krućim arterijama. Nadalje, otkrili smo značajnu interakciju između ispitanikovog MAP-a i vanjske temperature, što ukazuje da vanjska temperatura ima moderacijski učinak na odnos između MAP-a i veličine odstupanja. Osobne karakteristike kao što su ITM, spol i status hipertenzije utječu samo na veličinu odstupanja između uzastopnih PWV mjerenja izvršenih pomoću Arteriographa. Međutim, za razliku od SphygmoCora čiji prediktori velikih odstupanja uključuju dob, MAP i temperaturu, prediktori velikih odstupanja za Arteriograph su manje direktno povezani s krutošću arterija.

3.4.2.4. O varijabilnosti PWV-a općenito

Iako varijabilnost PWV-a može ometati procjenu stvarne vrijednosti PWV-a te utječe na mogućnost razlučivanja klinički važnih promjena u PWV-u, osjetljivost mjerenja PWV-a na trenutno stanje arterijskog stabla nužna je ako se žele pratiti kliničke promjene PWV-a tijekom vremena. U tom kontekstu, dok se neki uređaji mogu činiti superiornijima u smislu ponovljivosti i reproduktivnosti PWV-a (57), upitno je koliko je način rada tih uređaja osjetljiv na patološke promjene. Samo klinička procjena ovih uređaja u smislu usporedbe učinkovitosti terapija vođenih PWV-om ili s PWV-om kao terapijskim ciljem može odrediti koji je uređaj najbolji za upotrebu u kliničkom kontekstu.

3.4.3. Dugoročni štetni učinci blage bolesti COVID-19 na krutost arterija

Dio ovog doktorata čini i jedina studija do sada koja uspoređuje vrijednosti prije i nakon infekcije COVID-19 u istoj skupini sudionika u velikom broju parametara arterijske krutosti te centralne i središnje hemodinamike. Studijom se željelo ispitati može li se takva infekcija smatrati zbunjujućim čimbenikom prilikom procjena vrijednosti PWV-a u longitudinalnim studijama u smislu da ona privremeno ili stalno povećava arterijsku krutost.

Studija je otkrila da odgovori krvožilnog sustava na blagu bolest COVID-19 koju su imali svi ispitanici iz studije, a koji su definirani ovdje kao sustavne, individualne razlike prije i poslije u ispitivanim parametrima, nisu jednostavni u smislu da COVID-19 povećava ili smanjuje parametar u zaraženih bolesnika za određeni iznos. Zapravo, osim neznačajnog trenda za cfPWV, nismo uspjeli otkriti niti jedan parametar čija se srednja promjena prije i nakon COVID-19 razlikuje od 0.

Umjesto toga, odgovori na infekciju COVID-19 su dinamične i ovise o vremenu proteklom od pojave same infekcije. Takvi odgovori su utvrđeni u parametrima arterijske krutosti - cfPWV i AIx@HR75, centralnom hemodinamičkom parametru - cDBP, te sistemskim hemodinamičkim parametrima - DBP i MAP. Za te je parametre pokazano da njihove vrijednosti rastu s vremenom proteklim od pojave infekcije COVID-19, i to neovisno o dobi ili drugim zbunjujućim čimbenicima kao što je protok vremena između

dva mjerenja. Vaskularno oštećenje koje je utvrđeno u regresijskim modelima za opservacijski period od dva do tri mjeseca nakon infekcije je klinički značajno, što se može vidjeti kroz povećanje cfPWV od +1.4 m/s, +15% u AIx@HR75, +8 mmHg u DBP, i +7.6 mmHg u cDBP i MAP. Otkriće kako duži period od infekcije COVID-19 rezultira većim vaskularnim oštećenjem je iznenađujuće, s obzirom na to da smo očekivali smanjenje upalnog odgovora povezanog s COVID-19 protekom vremena.

Iako možemo samo nagađati o uzrocima ovog fenomena, novi dokazi sugeriraju kako je posrijedi nemogućnost rješavanja autoantitijela opaženih tijekom akutne faze bolesti (117-119), ili, da je posrijedi pojava *de novo* patogenih autoimunih reakcija nakon oporavka (120-122). U tom kontekstu, ono što smo uočili na razini skupine, 2 - 3 mjeseca nakon infekcije, moglo bi biti povezano s arterijskim ukrućivanjem prouzročenim upalom u nekih pojedinaca (123), pri čemu je uzrok upale autoimuna reakcije ili kronična upala koja joj prethodi (124). Nedavna je studija pokazala kako su u pacijenata koji su imali COVID-19, a nisu imali dijagnosticirane autoimune bolesti u vrijeme infekcije, opažena veća razina cirkulirajućih anti-/ekstraktibilnih nuklearnih autoantitijela (ANA/ENA) 3 mjeseca nakon oporavka u usporedbi sa zdravim ispitanicima te ispitanicima koji nisu imali COVID-19 (61). Visoki titar ANA/ENA antitijela, koji je bio u korelaciji sa simptomima dugog COVID-a, održao se i 6 mjeseci nakon oporavka. Čak i 12 mjeseci nakon preboljenja, nekoliko je patogenih ANA/ENAs utvrđeno u oko 30% ispitanika koji su preboljeli bolest COVID-19 (125). Nadalje, retrospektivna studija provedena na 4 milijuna ispitanika otkrila je povećani rizik od autoimunih bolesti u bolesnika s COVID-19, pri čemu je prilagođeni omjer hazarda za različite autoimune bolesti varirao od 1,78 do čak 3,21 (125).

Na sve vremenski ovisne odgovore na infekciju COVID-19 utjecala je i dob na način da svaka dodatna godina života pojačava vaskularno oštećenje nakon infekcije. Utjecaj dobi nije bio posljedica fiziološkog starenja arterija između dvaju mjerenja budući da je ta zbunjujuća varijabla kontrolirana u našim analizama. Moguće je da dob djeluje kao moderator odgovora krvožilja na blagu bolest COVID-19 u različitim dobnim skupinama. Prethodne studije sugeriraju da dob moguće moderira vaskularni odgovor na različite okidače (126-128). Iako naši rezultati sugeriraju ulogu dobi kao moderatora odgovora krvožilja na blagi oblik bolesti COVID-19, takva uloga bi trebala biti dodatno ispitana u istraživanjima s većim uzorkom.

Odgovor na bolest COVID-19 bolest utvrdili smo u raznim parametrima arterijske krutosti i u njihovim hemodinamičkim posljedicama uključujući: direktne (cfPWV) i indirektne (augmentacijski indeks) mjere arterijske krutosti, te parametre centralne hemodinamike (cDBP). Svaki od ova tri parametra predstavlja različiti aspekt aterosklerotskog procesa, koji uključuje morfološke i/ili funkcionalne promjene na zidu krvne žile (129). Stoga, istovremeno otkrivanje odgovora na COVID-19 u različitim parametrima vaskularne strukture i funkcije ukazuje na postojanje rasprostranjenog i dugotrajnog patološkog procesa u krvožilju nakon završetka infekcije (130).

Samo je manji broj studija do sada istraživao utjecaj infekcije COVID-19 na arterijsku krutost i centralnu hemodinamiku. Ustroji većine tih studija bili su istraživanja parova s malim uzorcima (10 - 22 ispitanika po skupini) u kojima su bolesnici koji se oporavljaju od COVID-a 19 uspoređeni s kontrolama (62, 63, 131). Unatoč potencijalno ograničenoj snazi tih istraživanja, njihovi rezultati podupiru naše zaključke o postojanju vaskularnog oštećenja nakon COVID-a 19. Povišeni cfPWV u ispitanika nakon infekcije COVID-19 u usporedbi s kontrolama je otkriven u više studija na: mladim zdravim pacijentima i njihovim kontrolama i to 3 - 4 tjedna nakon početka infekcije (povećanje od 0.7 m/s) (62), u akutno bolesnih starijih pacijenata (povećanje od 3.3 m/s) (131), kao i u pacijenata srednje životne dobi koji su uspoređeni s kontrolama 4 (povećanje od 2.05 m/s) (132) i 12 mjeseci (povećanje od 1.15 m/s) nakon početka COVID-19 infekcije (133).

Otkrili smo kako je AIx, poput cfPWV, bio viši u ispitanika s COVID-19 infekcijom naspram kontrola. Porast od 10% u augmentacijskim indeksima AIX AP/PP i AIx@HR75 pronađen je u ispitanika s COVID-19 u studiji u kojoj je 15 mlađih odraslih osoba uspoređeno 3 - 4 tjedna poslije pozitivnog COVID-19 testa sa zdravim mladim kontrolama (63).

Konačno, glede cSBP, pretrage koje su provedene 4 i 12 mjeseci nakon početka infekcije pokazale su da su pacijenti koji su imali COVID-19 imali postojani porast od 10 mmHg cSBP-a u usporedbi s kontrolama (132, 133). Nadalje, Akpek i suradnici (134) su utvrdili porast parametara sistemske hemodinamike u pacijenata s dijagnozom COVID-19 tijekom kratkoročnog praćenja pacijenata.

Samo jedno istraživanje parova nije pronašlo značajne razlike u parametrima arterijske krutosti - PWV i AIx75, 4 tjedna nakon infekcije kada su mlađi odrasli ispitanici

koji su preboljeli bolest uspoređeni s kontrolama (135). Nadalje, dvije manje longitudinalne studije su izvijestile o rezultatima koji su u suprotnosti s rezultatima studije iz ovog doktorata. U prvoj od tih studija, koja je pratila 14 mlađih ispitanika između prvog i šestog mjeseca nakon infekcije, autori su izvijestili o prosječnom smanjenju cfPWV (za 0.82 m/s), SBP (za 11 mmHg), i MAP (za 11 mmHg) tijekom perioda promatranja; dok nikakva promjena s vremena nije utvrđena za AIx@HR75 (136). Druga je studija pratila 10 mlađih odraslih osoba tijekom 6 mjeseci nakon COVID-19 infekcije i otkrila kako su se SBP i DBP smanjivali tijekom trajanja istraživanja za 10-15 mmHg (137). Uzimajući u obzir da su obje navedene longitudinalne studije navele osipanje ispitanika unutar veoma malih uzoraka, koristile neprikladnu statistiku kako bi opisale distribuciju vrlo ograničenog skupa podataka (srednja vrijednost i standardna devijacija), i uklonile vrijednosti koje odstupaju iz vrlo malog uzorka (136), postoji mogućnost da su njihovi rezultati posljedica metodoloških problema. S druge strane, takvi rezultati su moguća posljedica i heterogenih individualnih odgovora na COVID-19 u istraživanim parametrima vaskularne strukture i funkcije, koje smo uočili u ovom radu i koji su moguća posljedica toga da se autoimuni odgovor, ukoliko je prisutan, neće aktivirati u svih ispitanika.

3.5. Zaključci

Sustavno je procijenjen velik broj eksperimentalnih, meteoroloških, i fizioloških čimbenika te karakteristike ispitanika kako bi se utvrdilo koji od njih utječu na mjerenja PWV-a i doprinose neželjenoj varijabilnosti kratkoročnih ponovljenih mjerenja. Kvantificirali smo te učinke zasebno za dva uređaja koji koriste različite mjerne tehnike, koristeći ustroj studije koji pruža snažne dokaze.

Otkrili smo da se s dobi povećavaju ne samo vrijednosti cfPWV i PWVao, već i varijabilnost njihovih ponovljenih mjerenja, što ukazuje na to da bi u starijih osoba trebalo povećati preciznost mjerenja, moguće s korištenjem 3 - 4 mjerenja u seriji umjesto samo 2.

Za SphygmoCorova mjerenja cfPWV-a utvrđena je značajna interakcija između MAP-a i vanjske temperature, kao i značajan prosječni učinak temperature, što može

dovesti do većih odstupanja u kratkoročnim ponovljenim mjerenjima unutar osobe. Snimanje cfPWV-a tijekom sezone s višom vanjskom temperaturom (npr. 25°C), kada razlike u MAP-u dovode do manjih razlika u cfPWV-u, može smanjiti neželjenu intra-individualnu varijabilnost cfPWV-a. Također bi trebalo dalje istražiti može li produljenje vremena odmora tijekom razdoblja s niskom vanjskom temperaturom pomoći u smanjenju te varijabilnosti.

Doba dana ili različiti promatrači koji su primili istu količinu prethodne obuke nisu utjecali na mjerenja cfPWV ili PWVao. Međutim, tehničke poteškoće pri mjerenju s oba uređaja su se značajno rjeđe javljale poslijepodne. Kako bi se olakšale buduće longitudinalne studije, preporučljivo je da se PWV mjeri poslijepodne kad god je to moguće, ne nužno s istim mjeriteljem.

Za oba uređaja utvrđena je povezanost tehničkih poteškoća pri mjerenju PWV-a i ženskog spola. Dok se broj ponovljenih SphygmoCorovih mjerenja povećao umjereno u žena, povezanost sa spolom bila je vrlo jaka za ručni odabir signala kod uređaja Arteriographa koji je bio puno češći u žena negoli muškaraca.

Razlike u čimbenicima koji utječu na mjerenja PWV-a dvaju uređaja najvjerojatnije odražavaju razlike u njihovim tehnikama mjerenja. Osim dobi i MAP-a, mjerenja cfPWV-a koja se smatraju izravnom mjerom arterijske krutosti, također su bila pod utjecajem statusa hipertenzije, vanjske temperature, redoslijed posjeta, ali i pod utjecajem interakcije MAP-a s temperaturom vanjskog okoliša. Vrijednosti PWVao s druge strane, bile su dodatno pod utjecajem obilježja pacijenta poput spola, visine i HR-a.

Vezano uz istraživanja reproducibilnosti PWV-a pokazali smo da su trenutne AHA smjernice za procjenu PWV-a suboptimalne budući da je u većine ispitanika intra-individualni raspon PWV-a uočen tijekom 2 tjedna bio izvan praga od 1 m/s, što je vrijednost koja se predlaže kao minimalna klinički važna razlika.

Najbolju reproducibilnost PWV-a postigli smo korištenjem medijana 4 uzastopna mjerenja uz korištenje praga od 1,1 m/s.

Čimbenici koji utječu na veličinu odstupanja između susjednih mjerenja PWV-a (>0,5 m/s) su isti oni koji utječu na izmjerene vrijednosti PWV-a. Dob, MAP, vanjska temperatura i interakcija između vanjske temperature i MAP-a bili su prediktori veličine odstupanja SphygmoCorovih mjerenja dok su prediktori za Arteriograph uključivali

osobne karakteristike kao što su ITM, spol i status hipertenzije i generalno bili manje izravno povezani s krutošću arterija nego što su prediktori SphygmoCora.

Po prvi je puta bilo moguće usporediti promjene arterijske krutosti i centralne hemodinamike prije i poslije infekcije COVID-19. Rezultati pokazuju da se vrijednosti tih parametara nastavljaju pogoršavati u periodu između 2. i 3. mjeseca nakon infekcije sugerirajući da nakon COVID-a 19 moguće dolazi do trajnog pogoršanja arterijske krutosti, čak i u dominantno mladih, zdravih osoba s blagim oblikom bolesti, kao što su oni opisani u ovoj studiji.

3.6. Sažetak

Cilj doktorske disertacije je bio istražiti čimbenike i korake u protokolu procjene PWV-a – zlatnog standarda za mjerenja arterijske krutosti, koji mogu utjecati na smanjenje neželjene varijabilnosti PWV-a unutar ispitanika i tako olakšati uvođenje PWV-a u kliničku praksu. Nadalje, istraženi su i identificirani i čimbenici koji mogu dovesti do tehničkih poteškoća u mjerenju PWV-a, a istraživala se i mogućnost utjecaja nedavne infekcije COVID-19 na stanje krvožilja nekoliko mjeseci nakon izlječenja i njezinog ometajućeg djelovanja u longitudinalnim studijama.

Analize su provedene odvojeno za svaki od dva validirana uređaja - aplanacijski tonometar SphygmoCor i oscilometar Arteriograph - koji koriste različite tehnike za neinvazivno mjerenje PWV-a, osim za studiju s ispitanicima koji su nedavno preboljeli COVID-19 u kojoj su mjerenja učinjena samo sa SphygmoCorom. Rezultati se temelje na dva jedinstvena ustroja studija u području.

U studiji u kojoj se željelo identificirati eksperimentalne uvjete (različiti mjeritelji, doba dana i broj posjeta) i ostale zbunjujuće čimbenike (vanjski meteorološki uvjeti, fiziološki parametri – arterijski krvni tlakovi i brzina otkucaja srca, te obilježja ispitanika – dob, spol, status hipertenzije, ITM) koji utječu na izmjerene vrijednosti PWV-a i/ili uzrokuju tehničke poteškoće s mjerenjem PWV-a, a koji nisu kontrolirani u standardnom protokolu mjerenja, po prvi je puta sustavno ispitana kratkoročna varijabilnost PWV-a unutar ispitanika. Kako se unutar kratkog perioda promatranja od 2 tjedna ne očekuju klinički značajne promjene arterijske krutosti u pojedinca, intra-individualna varijabilnost PWV-a koja je opažena u studiji predstavlja grešku u longitudinalnim mjerenjima PWV-a koja se može očekivati u klinici. Ustroj koji je korišten – jedno-zaslijepljena ukrižena studija s dva različita uređaja, randomizirana s obzirom na dva različita mjeritelja i dva različita doba dana (ujutro/poslijepodne) s 12 ponovljenih mjerenja po ispitaniku te ispitanicima koji su jednoliko i široko raspodijeljeni prema dobi, ITM-u, spolu i dijagnozi hipertenzije (normotenzivni/hipertenzivni) osigurava visoku razinu dokaza. Temeljem rezultata ove studije, predložen je niz preporuka koje bi mogle smanjiti neželjenu intra-individualnu varijabilnost prilikom procjene PWV-a te olakšati korištenje PWV-a u kliničkoj praksi.

Druga studija je jedina studija do sada u kojoj su se promjene arterijske krutosti i centralne hemodinamike usporedile prije i poslije infekcije COVID-19. Rezultati pokazuju da se vrijednosti parametara nastavljaju pogoršavati u periodu između 2. i 3. mjeseca nakon završetka infekcije sugerirajući da je moguće da infekcija COVID-19 dolazi do trajnog pogoršanja arterijske krutosti. To posljedično znači i povećanje rizika za razvoj KVB, čak i u dominantno mladih, zdravih osoba s blagim oblikom bolesti, kao što su oni opisani u ovoj studiji.

Rezultati ovog doktorskog rada, koji uključuju niz preporuka za smanjenje neželjene intra-individualne varijabilnosti PWV-a u pojedinca, kao i smanjenje pojavnosti tehničkih poteškoća prilikom mjerenja te identificiraju nove zbunjujuće čimbenike poput COVID-19, mogu olakšati uvođenje mjerenja PWV-a u kliničku praksu i interpretaciju longitudinalnih studija.

Ključne riječi: arterijska krutost, brzina pulsnog vala, intra-individualna varijabilnost, prediktori, COVID19

3.7. Summary

Dissertation title: Variability of arterial stiffness indicators

The goal of the doctoral dissertation was to investigate the factors and steps in the PWV assessment protocol - the gold standard for arterial stiffness measurements, which can affect the reduction of unwanted PWV variability within subjects and thus facilitate the introduction of PWV into clinical practice. Furthermore, the factors that can lead to technical difficulties in measuring PWV were investigated and identified, and the possibility of the impact of a recent infection of COVID-19 on the state of blood vessels several months after recovery and its disruptive effect in longitudinal studies was also investigated.

Analyses were performed separately for each of the two validated devices - the SphygmoCor applanation tonometer and the Arteriograph oscillometer - which use different techniques for non-invasive PWV measurement, except for the study with subjects who had recently recovered from COVID-19 in which measurements were made only with the SphygmoCor. The results are based on two unique institutions of study in the area.

In a study in which the aim was to identify the experimental conditions (different measuring devices, time of day and number of visits) and other confounding factors (external meteorological conditions, physiological parameters - arterial blood pressure and heart rate, and characteristics of the subjects - age, gender, hypertension status, BMI) that affect the measured PWV values and/or cause technical difficulties with PWV measurement, which are not controlled in the standard measurement protocol, for the first time the short-term variability of PWV within subjects was systematically examined. As clinically significant changes in arterial stiffness in an individual are not expected within a short observation period of 2 weeks, the intra-individual variability of PWV observed in the study represents an error in longitudinal measurements of PWV that can be expected in the clinic. The design used – a single-blind cross-over study with two different devices, randomized with regard to two different measuring devices and two different times of day (morning/afternoon) with 12 repeated measurements per subject and subjects uniformly and widely distributed by age, BMI -in, gender and diagnosis of hypertension

(normotensive/hypertensive) provides a high level of evidence. Based on the results of this study, a number of recommendations were proposed that could reduce unwanted intra-individual variability when assessing PWV and facilitate the use of PWV in clinical practice.

The second study is the only study to date that compared changes in arterial stiffness and central hemodynamics before and after infection with COVID-19. The results show that the values of the parameters continue to deteriorate in the period between 2 and 3 months after the end of the infection, suggesting that it is possible that the COVID-19 infection leads to a permanent deterioration of arterial stiffness. This consequently means an increase in the risk for the development of CVD, even in predominantly young, healthy individuals with a mild form of the disease, such as those described in this study.

The results of this doctoral thesis, which include a number of recommendations for reducing unwanted intra-individual variability of PWV in an individual, as well as reducing the incidence of technical difficulties during measurement and identifying new confounding factors such as COVID-19, can facilitate the introduction of PWV measurement into clinical practice and interpreting longitudinal studies.

Keywords: arterial stiffness, pulse wave velocity, intra-individual variability, predictors, COVID19

3.8. Životopis

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The effects of experimental, meteorological, and physiological factors on short-term repeated pulse wave velocity measurements, and measurement difficulties: A randomized crossover study with two devices

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Background: Large longitudinal studies with repeated pulse wave velocity (PWV) measurements, a direct measure of arterial stiffness, are required to realize the full potential of arterial stiffness in clinical practice. To facilitate such studies it is important to increase the power of a study by reducing within-subject variability of PWV, and to ease the use of a PWV device in clinical settings by minimizing PWV measurement difficulties.

Methods: We systematically investigated experimental setting and meteorological conditions, as well as physiological factors and participant characteristics, to determine whether and to what extent they affected: between- and within-subjects variability of PWV recordings, and measurement difficulties of a particular device. We conducted a 2-week longitudinal block-randomized cross-over study with two blinded observers and two commonly used devices: applanation tonometry SphygmoCor CvMS and oscillometric Arteriograph to assess carotid-femoral (cfPWV) or aortic (PWVao) PWV, respectively. Our sample had uniform and wide-spread distribution of age, blood pressures, hypertensive status and BMI. Each participant (N = 35) was recorded 12 times over 3 visiting days, 7 days apart. On each day, recordings were made twice in the morning (7–10 a.m.) and afternoon (16–18 p.m.). Data were analyzed using multilevel mixed-effects models, separately for each device.

Results: In addition to age and mean arterial pressure (MAP) that strongly affected both cfPWV and PWVao, other significant factors appeared to indicate a measurement approach. cfPWV as a more direct measure of arterial stiffness was additionally affected by hypertension status, outdoor temperature, interaction of MAP with outdoor temperature and the order of visit, with MAP within-subject variability contributing on average 0.27 m/s to difference in repeated measurements at 5°C and 0.004 m/s at 25°C. PWVao measurements derived at a single brachial site were more dependent on age than cfPWV and also depended on personal characteristics such as height and sex, and heart rate; with within-subject MAP variability adding on average 0.23 m/s to the difference in repeated measures. We also found that female sex significantly increased, and recording in afternoon vs. morning significantly decreased measurement difficulties of both devices.

Conclusion: We identified factors affecting PWV recordings and measurement-difficulties and propose how to improve PWV measuring protocols.

KEYWORDS

carotid-femoral pulse wave velocity, pulse wave velocity, within subject variation, predictors, meteorological conditions, experimental conditions, measurement error, measurement difficulty

1. Introduction

Arterial stiffness is a phenomenon associated with vascular aging that refers to loss of arterial compliance or changes in vessel wall properties (1). Arterial stiffness increases with age and with prolonged exposure to risk factors that accelerate this process (2, 3). Numerous studies have found that increased arterial stiffness is associated with an increased risk of a first or recurrent major CVD event, independent of traditional risk factors, in both disease-specific and population-based samples (4–6). In addition, arterial stiffness has been shown to improve reclassification of patients at intermediate risk for cardiovascular disease by complementing the information provided by traditional risk factors (4, 5, 7–9). The potential clinical implication of arterial stiffness measurements in early detection of high-risk individuals (10, 11) and in driving hypertensive patient therapy (12) make arterial stiffness measurements a promising keystone in hypertension management and cardiovascular prevention (13, 14).

Pulse wave velocity (PWV) measurement is considered the simplest, non-invasive, robust, and reproducible method for assessing arterial stiffness, with the carotid-femoral vascular bed regarded as the most easily accessible pathway for aortic PWV measurements (2, 15). Because of the abundance of population data and reference values for the healthy population (16), carotid-femoral pulse wave velocity (cfPWV) recorded with the SphygmoCor CvMS applanation tonometer has been established in the literature as a reference for comparison and is recommended by the ARTERY Society guidelines as a standard against which new PWV devices can be validated (17). Devices that estimate PWV from other vascular beds and are technically less demanding, such as those that estimate aortic PWV (PWVao) from brachial cuff-based waveform analysis recorded at a single site—e.g., oscillometric Arteriograph device, are also commonly used in practice. While both the SphygmoCor CvMS and the Arteriograph device have been verified using invasively recorded aortic PWV (18, 19), their measurements are not interchangeable (20–22), likely because they employ different measuring techniques. For this reason, head-to-head comparison between the devices is hard to interpret.

Despite its considerable potential for cardiovascular disease prevention, measurements of PWV have limited use in clinical practice. The updated 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice argues against widespread use of PWV measurement in clinics because of difficulties in measurements and precision of measurements (23). While recommendations for minimizing confounding of arterial stiffness measurements in order to obtain reliable PWV values have been published and are included in study protocols (24), there are additional factors that have been proposed to alter PWV measurement (24–28). These factors, that are not controlled for in a protocol, may increase between- and

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within-subject variability of PWV measurements, lowering the device's resolution to detect a minimal clinically important change and decreasing precision of PWV measurements, which consequently reduce the power of a study. As demonstrated by the SPARTE study's low power (12), improving the power of longitudinal studies measuring PWV and reducing PWV-related measurement difficulties (e.g., the need to repeat the measurement) are critical to facilitating large longitudinal studies and improving PWV translation into clinical practice. Aside from clinically relevant PWV changes, factors such as mean blood pressure (MAP), heart rate (HR), different observers, time of day, or outdoor temperature have been implicated as additional sources of PWV variability (24, 25, 29–35). While individual factors have been studied in few studies, no study has systematically examined several factors together to assess the independent contribution of each factor while controlling for the others. To minimize the impact of additional factors that significantly affect PWV measurements in a clinical setting it is important to identify such factors and quantify their effects. Furthermore, it is essential to identify and control factors affecting measurement difficulties of a device, such as the need to repeat a PWV measurement, in order to ease the use of devices in clinical setting. Because different PWV devices, particularly those that use different measurement principles, do not necessarily have interchangeable PWV values (20–22), it is reasonable to assume that the aforementioned analyses will produce different results and should thus be performed separately for each device. The study had two goals: (a) to identify factors that affected PWV measurements that differed from those controlled in the standard PWV measurement protocol (e.g., meals, smoking, exercise), and to quantify their effects; and (b) to determine which of these factors affects a device's measurement difficulties (e.g., the need to repeat PWV measurement, or to manually select a signal for analysis), and to what extent. The experimental setting and meteorological conditions, as well as physiological factors and participant characteristics, were systematically assessed as factors. The analyses were performed separately for the two devices that use different measuring techniques—the applanation tonometer SphygmoCor CvMS and the oscillometric device Arteriograph.

To reach our goals we used the study design that yields strong evidence: a block-randomized cross-over longitudinal study with two observers that were blinded to each other's readings, the largest number of repeated measurements per participant ($N_{perparticipant}$ = 12 recordings; N_{total} = 420), uniform distribution of participants by age, sex, BMI and hypertensive status, and multilevel mixed-effects models used in data analysis. All the recordings were performed over 2 weeks. In such a short period of time, clinically relevant changes in vascular biology are not expected, so the variability of PWV during repeated recordings can primarily be attributed to chance and confounding factors.

2. Materials and methods

2.1. Participants

The study enrolled 36 participants aged 20–60 years. Participants were purposively sampled by age, sex, hypertension status (normotensive or hypertensive), and body mass index (BMI, ranging from normal weight to obese) to ensure even distribution across the inclusion criteria (Figure 1). Participants' hypertension status was established by self-report of physician diagnosis, with all such persons reporting receiving treatment, whereas BMI categories were determined according to the Centers for Disease Control and Prevention classification for adults (36). Exclusion criteria included self-reported arrhythmias, cerebrovascular disease, pregnancy, surgery amputation, oncology disease, psychiatric disease, and infections throughout the trial duration.

One person (female, 45 years old, hypertensive and obese) who was initially enrolled in a study was additionally later excluded because she contracted an infection over the course of the study, leaving a total of 35 participants.

The study was approved by the Ethics Committee at the University of Split School of Medicine, and all participants provided written informed consent.

2.2. Study design

This is a single-blind block-randomized cross-over longitudinal study.

The study took place between October 2019 and February 2020 at the University of Split School of Medicine in the Laboratory for vascular aging.

Each participant was recorded 12 times in total over the course of 2 weeks, four times during each of the 3 visit days that were separated by 1 week. On each visiting day, recordings were taken in the morning (7–10 h) and afternoon (16–18 h) by the two observers.

The order of devices—Sphygmocor CvMs and Arteriograph and of observers, was randomized using the block size of 4. The two observers were blinded to each other's readings.

2.3. PWV measurements

Pulse wave measurements were taken with the two devices that use different measuring techniques—the applanation tonometer SphygmoCor CvMS (Atcor Medical, Sydney Australia) with which we collected cfPWV data and the oscillometric device Arteriograph (TensioMed, Budapest, Hungary) used to collect PWVao data.

The measurements were taken in accordance with the American Heart Association's recommendations for improving

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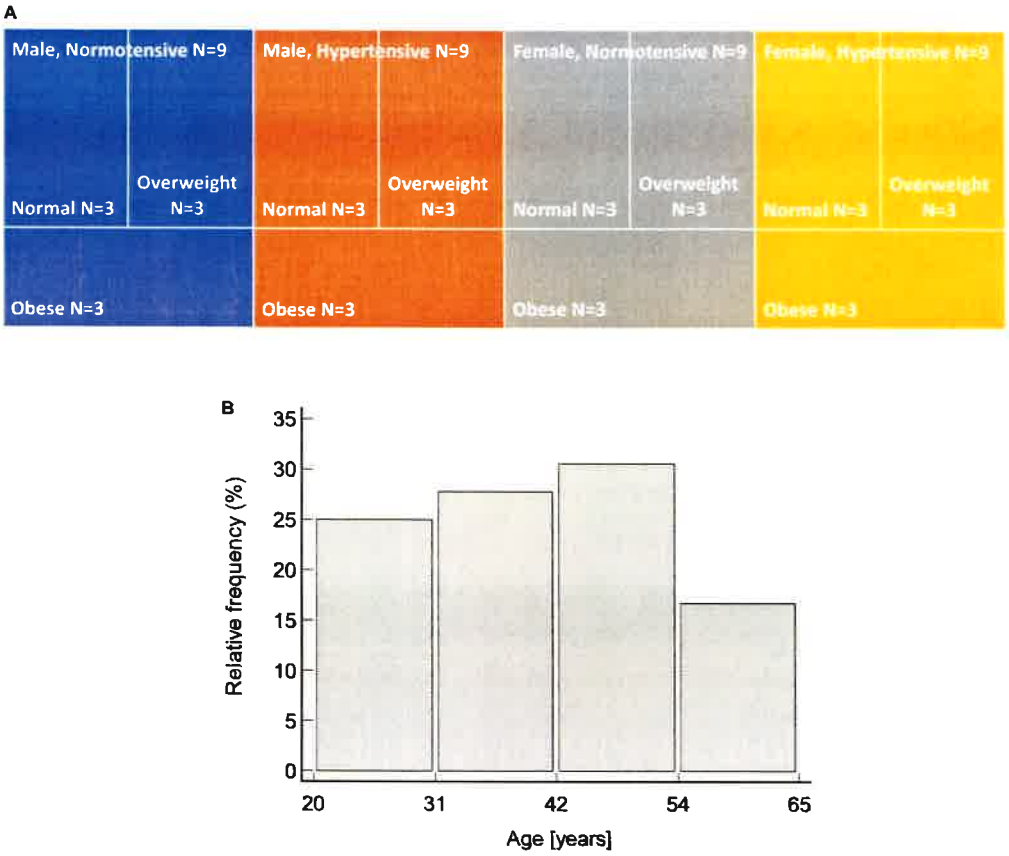

FIGURE 1
Uniform distribution of participants by **(A)** Sex, hypertensive status and BMI categories, and **(B)** age ($\chi^2 = 1.5$, $p = 0.692$).

and standardizing vascular research on arterial stiffness (24). The observers performed measurements in a quiet, temperature-controlled room at a comfortable temperature of 21–23°C. The participants rested in the supine position for 10 min before the first PWV measurement to ensure hemodynamic stability. After completion of the series of measurements with one device, participants were asked to stand up, walk around the room, and then rest supine for 10 min to prepare for measurements with the second device. This step was necessary to prevent participants from falling asleep while resting supine for an extended period, especially in the morning. During the measurements, participants were asked not to talk or sleep. All measurements were performed on the right hand (Arteriograph) and the right carotid and femoral artery (SphygmoCor).

Participants were requested to abstain from vigorous exercise and alcohol consumption for at least 24 h prior to a recording session (morning or afternoon). They were also instructed not to eat or drink anything except water or smoke for at least 3 h before any recording session. Those taking vasoactive medicines were advised to continue taking them as usual and not to change the dosage during the study.

Before the start of the study, both observers had received extensive 7-day training during which they performed approximately 40 high-quality measurements under supervision.

To calibrate the pulse wave signals acquired by the SphygmoCor, we obtained brachial blood pressure measurements with the validated oscillometric sphygmomanometer (Welch Allyn Connex ProBP 3400 digital blood pressure monitor with SureBP technology).

We used the subtracted distance method to calculate wave travel distance. The method was chosen over the direct method as per recommendation by the latest guideline (24). A large school divider (Figure 2) was used to measure the distance between the sternal notch and the femoral measurement site, as well as the distance between carotid measurement site and the sternal notch was than subtracted from this distance. The tool was selected because it measures straight-line distance independent of body shapes such as particularly large bellies and/or breasts in obese individuals. It is similar to a sliding

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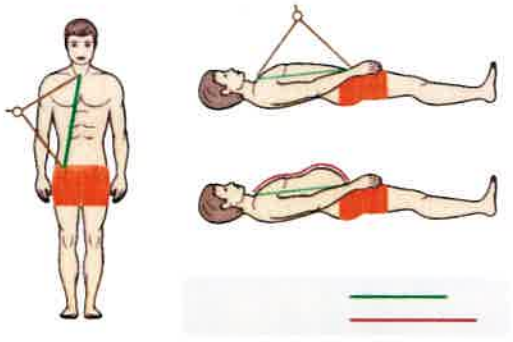

FIGURE 2
Measurement of a distance between the sternal notch and the femoral site with a tape (red line) and a school divider (green line).

caliper, which has been recommended for use when straight-line measurement with a tape measure is not possible (24, 37, 38). However, unlike slide caliper whose slide blades may still impede with the body shape in overweight and obese individuals to some extent, a divider is unaffected by it due to its long arms. We only measured the distance between the carotid and femoral sites during the first visit. Each observer obtained a single distance measurement during this visit. If the measured distances differed between observers, the process was repeated, and if the difference remained after the second round of measurements, the average of the four previous distance measurements was used as the true distance (N = 1 participant).

2.4. Collection of other data

All participants underwent a medical history.

The meteorological data: outdoor temperature, air pressure, and relative humidity, were provided by the Meteorological and Hydrological Service of Croatia's local office, and used to estimate weather conditions on each visit day and time. The contour plot is used to show the distributions of three meteorological parameters by a visit day (visit 1, 2, or 3) for all of the participants' visits, with relative humidity and air pressure as x and y dimensions, and temperature as a color coded z-dimension (Figure 3). The graph shows that outdoor conditions on the visit day 3 differed significantly from those on the visit days 1 and 2 (Figure 3).

2.5. Sample size consideration

The study employs a multilevel data structure, including 35 groups (participants, level-2) and 12 repeated measurements by a participant (level-1) totaling 420 observations. Because no random slopes are anticipated in any of the multilevel models that we developed, the sample sizes mentioned above (levels 2 and 1) are deemed adequate for estimating unbiased and accurate regression coefficients, variance components, and first level standard errors (39, 40), allowing for models with up to 12 independent variables (41).

2.6. Data analysis

We used descriptive statistics to describe distribution of quantitative (mean and standard deviation or median and IQR, depending the shape of distribution) and qualitative (absolute and relative frequencies) variables. To identify predictors of PWV single readings, or different types of measurement difficulties we employed several multilevel models with random intercept. Depending on the type of a dependent variable we used multilevel mixed-effects generalized linear models for continuous dependent variables like PWV readings, mixed-effects logistic regression models for dichotomous dependent variables like the occurrence of marginal-quality signals or manual signal selection, and mixed-effect Poisson regression for count data like the count of repeated attempts to record PWV values due to low-quality of a captured signal. All of the models were run with the robust estimator, which is resistant to certain types of misspecification in multilevel models such as heteroscedasticity or deviation from normality (42, 43), and sensitivity analysis was performed with the maximum likelihood (ML) method without robust estimator.

Each model was built in two steps. The experimental setting variables—order of visit, time of day, first device used in a session, and observer, meteorological variables—outdoor temperature (°C), air pressure (Pa), and relative humidity (%), and participants' characteristics—age, sex, BMI, hypertension status, MAP, SBP, DBP, HR—were all investigated for their relationship to a dependent variable by a simple multilevel regression analysis. Those independent variables that were associated with dependent variable at the $p < 0.2$ significance level, entered into multiple multilevel regression model. For independent variables that were non-significant in a multiple model, their contribution to the model (pseudo R^2) was investigated further to decide on their inclusion in the final model.

The metrics for assessment of individual variability of PWV readings was determined by considering the multilayered data structure. The average within-subject CV was calculated using the root mean square method (44). The intraclass correlation coefficient (ICC) was calculated by using random-effects model to estimate correlations between average measurements made on the same participant. As outliers may significantly affect this measure, we excluded the severe outliers from the calculation (45).

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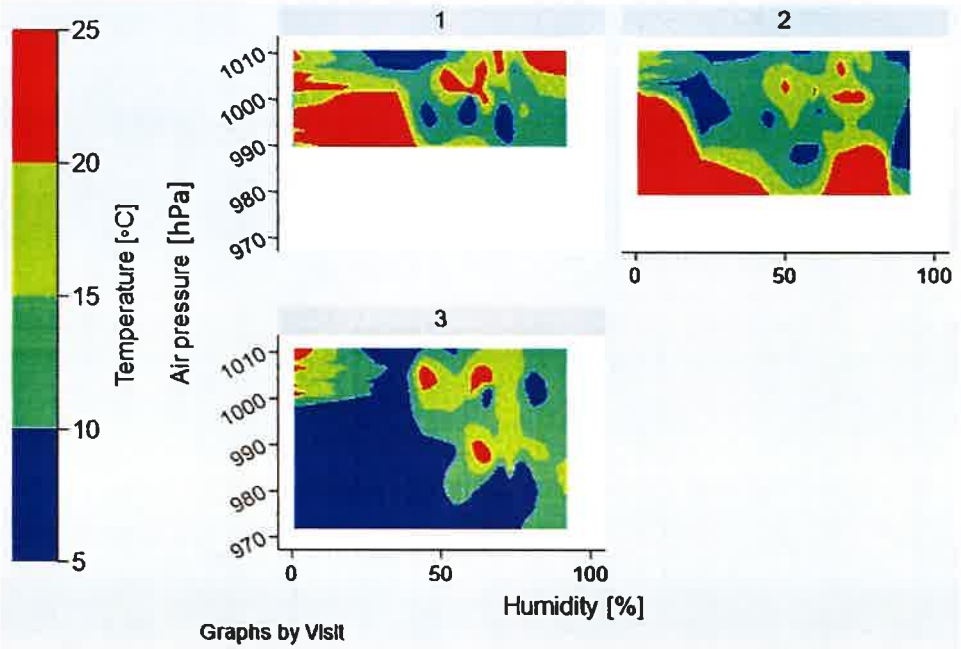

FIGURE 3
Meteorological factors during the 3 visit days—distributions of outdoor temperature, air pressure, and relative humidity are shown as contour plots for all the participants' visits assigned to a particular visit day—1, 2, or 3.

3. Results

The study included a total of 35 participants. Recorded PWV values ranged from 4.5 to 10.8 m/s for cfPWV and 5.5–15.8 m/s for PWVao. The validation sample also covered wide ranges of brachial blood pressures, age and BMI that were uniformly distributed (Figure 1). Participants' characteristics are shown in Table 1.

The difference in distance between the carotid and femoral sites measured with a tape vs. a school divider strongly correlated with the level of obesity as evaluated by BMI ($r = 0.79$, $P = 0.020$). Thus, the school divider was effective in removing this effect of BMI.

Overall, the coefficient of variation for within-subject variability was 9.9% (95% CI 9–11%) and 5.3% (95% CI 5–6%) for cfPWV and PWVao measurements, respectively; while ICC for within-subject average cfPWV measurements was 94% (95% CI 91–97%) and 96% (95% CI 94–98%) for PWVao measurements.

3.1. Factors significantly affecting PWV measurements

Table 2 lists the factors with a significant effect on PWV measurements, that were identified among experimental conditions, physiological and meteorological factors, as well as characteristics of the participants that we assessed in this study.

In both the cfPWV and PWVao models, the most prominent effects to which the largest difference in predicted PWV margins was attributed were age and MAP, in that order. The increase in PWV values with a 1-year increase in age was twice higher for PWVao than cfPWV measurements (Table 2, see 95% CI for B; Figures 4A, B). However, we could not directly compare the effects for MAP as the factor was involved in a significant interaction with outdoor temperature in the cfPWV model, which hampered interpretation of its main effect (Figure 4C).

As for the meteorological factors, only the outdoor temperature had a significant effect on cfPWV measurements. In cfPWV model, as mentioned earlier, outdoor temperature was involved in the interaction with MAP, suggesting that it modifies the relationship between cfPWV and MAP (Figure 4C). PWVao measurements were not influenced by any outdoor meteorological factor.

Except for the order of visit in the cfPWV model—notably the comparison of 2nd to 1st visit values, no other experimental factor was associated with differences in cfPWV or PWVao measurements.

Increased values of PWVao measurements were also associated with increased values of BMI and HR, and a female sex, whereas in the cfPWV model it was hypertensive status that was found to significantly increase cfPWV.

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TABLE 1 Characteristics of participants, $N = 35$.

Characteristics	Md (range) or N (%)
Age (years)	41 (20–60)
Sex	
Females	17 (49%)
Males	18 (51%)
BMI	27.3 (19.4–38.9)
Hypertension status	
Hypertensive	17 (49%)
Normotensive	18 (51%)
bSBP (mmHg)	126 (98–177)
bSDP (mmHg)	72 (53–98)
HR (beats per minute)	67 (48–94)

BMI, body mass index; bSBP, brachial systolic blood pressure; bDBP, brachial diastolic blood pressure; HR, heart rate.

During the development of multilevel mixed-effects models we also assessed if there was a significant interaction between sex and age. After including an interaction term between sex and age in the full multivariate models of both SphygmoCor and Arteriograph, and controlling for menopause status, we discovered that the interaction between age and sex is not significant ($p \geq 0.365$).

Finally, after developing multilevel mixed-effects models that aimed to identify factors influencing values of PWV measurements, we also evaluated which, if any, participants' characteristics are related to variability of repeated measurements in a person. We found for both cfPWV (Pearson's $r = 0.51$, $p = 0.002$) and PWVao ($r = 0.34$, $p = 0.047$) measurements that older age was associated with wider range of repeated PWV measurements observed in a person, whereas BMI, hypertension status or sex were not associated with this variability. However, we did find significantly larger variability of within-subject PWV measurements in menopausal than in women with menstrual cycle, for both devices ($P \leq 0.035$, Mann-Whitney test). Moreover, for women who menstruate, within-subject PWV variability was comparable to men ($P \geq 0.310$). In addition, we also assessed if the order of visit, the only experimental condition significantly affecting cfPWV values, is associated with decreasing variability of cfPWV measurement in a person and found this was not the case (repeated measurements ANOVA, $P = 0.781$).

Regarding the repeated measurements defined by the final models depicted in Table 2, within-subject cfPWV on average deviated by 0.73 m/s (95% CI 0.64–0.83), whereas PWVao deviated by 0.84 m/s (95% CI 0.58–1.20). These models described 59 and 67% of the repeated variability, respectively.

3.2. Factors significantly affecting difficulties in measuring PWV

Next, we wanted to know which factors influenced difficulties in measuring PWV with each device. We focused on the following difficulties: (a) the number of repeated measurements as a result of the SphygmoCor's pulse wave velocity signal failing the quality control, (b) the possibility of observing the marginal quality signal by SphygmoCor with the coefficient of variation for cfPWV estimation between 6 and 10%, or (c) the Arteriograph's oscillometric signal being manually selected for analysis (Table 3).

As opposed to the automatic selection of the oscillometric pulse wave signal by Arteriograph device, the manual signal selection is time-consuming and subject to uncertainty. In total, we had to manually select a signal for analysis in 78 (19%) of the Arteriograph recordings. We did not find, however, that manual selection affected PWVao values when this factor was added as independent variable to the simple ($p = 0.438$), or final ($p = 0.276$) multilevel model. The results demonstrate that it is very likely that the Arteriographs's oscillometric signal will be manually selected for analysis in a female participant. In fact, manual adjustment was required at least once for 11 (65%) of the women, with five women requiring it on ≥8 instances out of 12 measurements. In contrast, just five males (28%) required this modification on 1–3 occasions. Measuring in the afternoons was strongly to moderately associated with a lower chance of manual adjustment, reducing the odds ratio for it by 77% (95% CI from 46 to 90%). Finally, a chance for manual adjustment differed significantly between the measurers by increasing OR for the measurer 2 by 0.91 (95% CI 0.23–1.95).

In terms of the SphygmoCor, with which we were able to record all 12 measurements for each participant, we had to repeat a measurement 135 (32%) of the time. Female sex increased the incidence rate ratio (IRR) of a repeated measurement by 23% (95% CI 1–50%), whereas afternoon recording time and 3rd vs. 1st visit decreased IRR by 12% (95% CI 3–21%) and 14% (95% CI 4–27%), respectively.

We also discovered that the same two factors—afternoon recording time and 3rd vs. 1st visit—decreased the odds of a signal with marginal quality, which we observed in 95 (23%) of measurements, by: 40% (OR 95% CI −3 to 65%) and 50% (95% CI 1–74%), respectively. When the marginal quality of a signal was added as independent variable to the simple and final multilevel model we found that it increased cfPWV values by on average 0.37 m/s (95% CI 0.16–0.58, $p = 0.001$).

4. Discussion

We systematically examined a number of experimental and meteorological conditions as well as physiological factors and participants' characteristics to find if, and to what level,

TABLE 2 Factors that significantly affect PWV measurements, and expected between- and within-subject differences in PWV measurements that are due to a factor's observed range in a sample and its within-subject variability.

Device	Factor	B	95% CI for B		*P*-value	Between-subjects difference in PWV due to a factor, calculated from predicted margins† (m/s)	Within-subject average difference in repeated measurements due to within-subject variability of a factor (m/s)
SphygmoCor cfPWV (m/s)	Age (year)	0.04	0.02	0.06	<0.001*	1.63	No variability of the factor, but increase in variability of repeated cfPWV measurements with age
	MAP (mmHg)	0.05	0.02	0.08	<0.001*	1.14	In interaction
	Hypertension (yes vs. no)	0.44	−0.02	0.91	0.062**	0.44	No variability
	Outdoor temperature (°C)	0.18	0.06	0.31	0.005*	-0.25	In interaction
	Order of visit						
	2nd vs. 1st	−0.23	−0.43	−0.03	0.023*	−0.23	−0.03 (−0.25, 0.20) ††
	3rd vs. 1st	−0.05	−0.21	0.11	0.555	–	–
	Interaction Outdoor temperature × MAP	−0.002	−0.004	−0.001	0.003*	2.62 m/s at 5°C and 0.04 m/s at 25°C§	At 5°C average difference in repeated measurements due to variability in MAP is 0.27 m/s (0.24–0.30), and at 25°C 0.004 (0.004–0.005) m/s
	Snijders/Bosker R^2 Level 1: 39%, Level 2: 59%						
Arteriograph PWVao (m/s)	Age (year)	0.08	0.06	0.10	<0.001*	3.15	No variability of the factor, but increase in variability of repeated PWVao measurements with age
	MAP (mmHg)	0.04	0.02	0.05	<0.001*	2.30	0.23 (0.10–0.33)
	Height (cm)	0.06	0.02	0.09	0.001*	2.14	No variability
	Sex (female vs. male)	1.56	0.69	2.42	<0.001*	1.56	No variability
	HR (bpm)	0.02	0.002	0.04	0.031*	1.45	0.12 (0.01–0.27)
	Snijders/Bosker R^2 Level 1: 50%, Level 2: 67%						

B, regression coefficient. †Difference in predictive margins of PWV for an observed range of a factor, adjusted for other factors. ††Values in the brackets are calculated considering uncertainty of estimates for regression coefficient and factors' within-subject variability. §For the interaction term we calculated the maximal expected changes in cfPWV given the MAP range: at 5 and 25°C; Significant at the: *0.05, **0.1 level.

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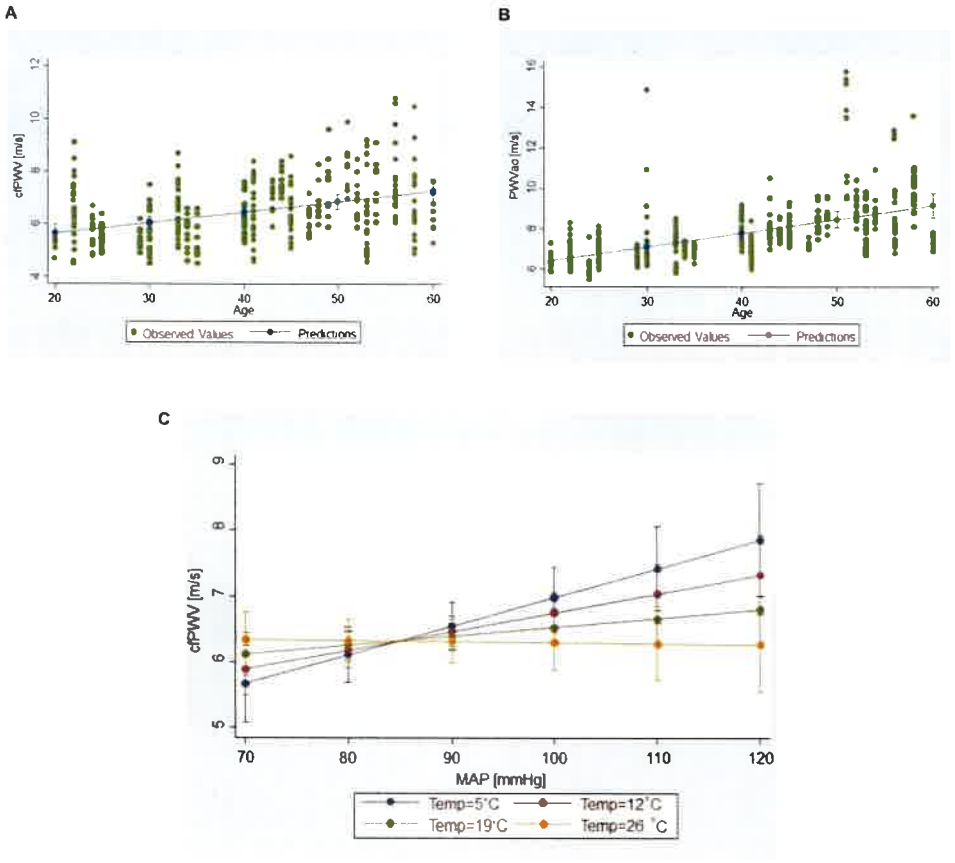

FIGURE 4
Scatter plots of (A) cfPWV measurements vs. age, and (B) PWVao measurements vs. age. (C) moderation interaction of the outdoor temperature on the relationship between cfPWV values and MAP. lines connect predictive margins of the multilevel regression model with 95% CI.

they affect (a) recorded PWV values, and (b) measurement difficulties associated with this recording. PWV values were repeatedly collected from the enrolled participants over the course of 2 weeks during which no clinically relevant change in PWV values is expected. The analyses were done separately for measurements acquired with two validated devices: cfPWV measurements acquired with applanation tonometry device SphygmoCor CvMS and PWVao values recorded with oscillometry Arteriograph device.

We utilized the study design that provides the strong evidence: block-randomized, cross-over, longitudinal study with as many as 12 repeated measurements per participant per device, recorded with observers blinded to each other's readings. Furthermore, our validation sample exhibited a reasonably wide range of PWV, age, BMI and brachial blood pressures values, and uniform distributions across age, sex, BMI, and hypertension status. Given that uniform distribution puts less emphasis on the center of the distribution and more on its extremes, it produces more precise validation estimates and is thus preferable as validation sample to a sample representative of an underlying population (46).

There haven't been many studies that look at the short-term repeatability or reproducibility of PWV measurements (47–51), with studies reporting from 2 to 6 repeated measurements per participant. Despite the fact that we recorded the most repeated PWV measurements per participant (N = 12), and that our sample had a reasonably wide range of PWV and PWV determinants, which tend to increase observed variability of PWV measurements, the agreement of repeated PWV measurements estimated in our study was comparable to what has been reported in the literature. Grillo et al. estimated within-subject CV, and ICC for cfPWV values recorded with SphygmoCor CvMS device in patients of predominantly normal weight who were hospitalized for suspected coronary artery disease. They used six repeated measurements per person and reported comparable metrics to our study with the CV of 9.5 (95% CI 7.7–11.0), and ICC of 0.85 (95% CI 0.78–0.90) (48). Our ICC value for the cfPWV measurements was also comparable to that reported by studies that were performed

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TABLE 3 Predictors of the measurement difficulties for Arteriograph and SphygmoCor devices.

Device	Predictors	OR	95% CI		*P*-value
Arteriograph—a need to manually select signal for analysis	Sex (female vs. male)	51.44	6.42	412.21	<0.001*
	Time of the day (afternoon vs. morning)	0.23	0.10	0.54	0.001*
	Measurer (no. 2 vs. no. 1)	1.91	1.23	2.95	0.004*
	Mixed-effects logistic regression model				
		IRR	95% CI		*P*-value
SphygmoCor—a need to repeat a measurement	Sex (female vs. male)	1.23	1.01	1.50	0.040*
	Time of the day (afternoon vs. morning)	0.88	0.79	0.97	0.014*
	Order of visit				
	2nd vs. 1st	0.95	0.86	1.06	0.361
	3rd vs. 1st	0.86	0.77	0.96	0.006*
	Mixed-effects Poisson regression model				
		OR	95% CI		*P*-value
SphygmoCor—occurrence of a marginal signal quality with cfPWV CV between 6 and 10%	Time of the day (afternoon vs. morning)	0.60	0.35	1.03	0.065**
	Order of visit				
	2nd vs. 1st	1.00	0.51	1.95	>0.999
	3rd vs. 1st	0.50	0.26	0.99	0.045*
	Mixed-effects logistic regression model				

CV, coefficient of variation; OR, odds ratio; IRR, incidence rate ratio.
Significant at the: *0.05, **0.1 level.

on patients with peripheral arterial disease (52, 53). As for the Arteriograph's PWVao measurements, Li et al. measured PWVao at 3 timepoints during a day in 70 participants including healthy young and elderly participants, and patients with cardiovascular disease treated in outpatient clinic; and reported CV of 6.1%, which is comparable to our study (54). Similarly, Ring et al. reported CV of 9.3 and 9.6% in 51 healthy non-smoking participants for Arteriograph's PWVao and SphygmoCor's cfPWV, respectively (55).

4.1. Factors affecting PWV readings

4.1.1. Age

Age was the factor with the largest effect on cfPWV and PWVao measurements, followed by MAP. This is consistent with the data from other studies that show a strong dependence of PWV on age and MAP (16, 24, 56, 57). On average, age accounted for approximately 2–3 m/s PWV difference between subjects, with apparently stronger effect of age on PWVao values: cfPWV increased by 0.4 m/s (95% CI 0.2–0.6), and PWVao by 0.8 m/s (95% CI by 0.6–1.0) every 10 years.

Other studies utilizing the SphygmoCor CvMS device reported comparable effects of age on cfPWV measurements ranging from: 0.2 m/s per 10 years (58), to 0.3–0.4 m/s (59), to somewhat larger effects of around 0.7–0.9 m/s reported by the same group of authors (16, 60). While age was identified as the strongest determinant of PWVao too (61), studies that investigated factors influencing Arteriograph's PWVao measurements did not reported comparable, unstandardized regression metrics. However, the study that investigated PWVao measurements recorded with oscillometric Vicoder device reported a comparable effect of 0.4–1.0 m/s per 10 years (62).

While we may assume that age remained constant during the 2 weeks of the study and thus did not contribute to an absolute change in a person's repeated measurements, we also discovered that older age increased the variability of repeated measurements for both cfPWV and PWVao, thereby increasing measurement error. Grillo et al. discovered that patients with increased arterial stiffness had greater variability in repeated PWV measurements acquired with various cfPWV-estimating devices (48). This relationship is thought to be due to the fact that PWV is defined as a ratio of traveled distance to pulse wave transit time (PWTT), in which case a small difference in PWTT can cause a relatively large difference in PWV in subjects with high arterial stiffness, whereas this difference is negligible in subjects with normal arterial stiffness. As age is the strongest determinant of PWV, the association reported by Grillo et al. corroborates the association between

age and within-subject short-term PWV variability that was reported in our study. Because both of the devices we tested estimate PWTT: SphygmoCor by determining pulse transit time from carotid to femoral location, and Arteriograph by determining the time difference between the first systolic wave and the second reflected wave; we found the said association for both devices. This finding suggests that when patients are monitored for longitudinal changes in PWV, it would be advantageous to increase the number of measurements in older persons during one visit (from 2 to 3–4) to improve precision of estimated PWV.

4.1.2. MAP and outdoor meteorological factors

The second strongest effect on PWV recording was due to MAP, which is considered the most significant physiological variable affecting arterial stiffness (30, 31, 34). With the increase of MAP, vessels stiffen, meaning that the effect of this variable on repeated PWV measurements should be considered whenever the measurements are taken under different BPs. MAP on average accounted for approximately 1–2 m/s PWV difference between subjects in our study, with PWVao increasing by 0.2 m/s (95% CI 0.10–0.25) for every 5 mmHg. This is comparable to the effect estimated with Vicorder, another oscillometric device: 0.05–0.20 m/s per 5 mmHg (62). Considering within-subject variability of MAP in our study, on average 0.23 m/s (from 0.10 to 0.33 when uncertainty in estimates is considered) discrepancy in repeated PWVao measurements may be assigned to MAP variations.

The expected changes in cfPWV due to MAP or outdoor temperature changes were less obvious due to the significant interaction between outdoor temperature and MAP, demonstrating that outdoor temperature moderates the relationship between cfPWV and MAP. The expected difference between predicted cfPWV assigned to lowest and highest observed MAP and adjusted for other factors at 5°C was 2.62 m/s, but at 25°C this difference was only 0.04 m/s. Individual differences in repeated measurements that are due to MAP variations are predicted to be 0.27 m/s (uncertainty 0.24–0.30) at 5°C and 0.004 m/s (0.004–0.005) m/s at 25°C. Interestingly, there was no significant main effect of outside temperature or its interaction with MAP for PWVao measurements.

While the negative relationship between outdoor temperature and BP readings has been observed in many studies (63–67), similar relationship was hypothesized for arterial stiffness, but studies reported inconsistent results. Di Pilla et al. reported that in an unadjusted regression analysis, cfPWV recorded by SphygmoCor was weakly and inversely associated with outdoor temperature, but not in a multiple regression analysis that controlled for age, BMI, SBP, DBP, daylight hours, O_3, CO, and N_2O (25). In repeated measures ANOVA analysis, Kita et al. demonstrated considerable sessional change of an arterial stiffness index—arterial velocity pulse index, with higher stiffness observed during a summer (26). The omission of a significant interaction term between temperature and MAP in these models may explain inconsistencies, as exclusion of the term from cfPWV model in our study also resulted in a non-significant main effect of temperature, while a simple correlation also showed that cfPWV is inversely associated with temperature. While the mechanism underlying this relationship is beyond the scope of our study, it has been proposed that cold-induced sympathetic activation may account for the dependence of cfPWV readings on outdoor temperature in conditions where the room temperature is constant (25, 68). Having said that, it appears that at lower outdoor temperatures, resting time in supine position in the temperature-controlled room might be extended beyond 10 min to allow for temperature accommodation, or alternatively, repeated measurements might be taken in a season with higher outdoor temperatures, such as those around 25°C. More research with different adaptation times is needed to determine whether temperature accommodation is indeed responsible for the observed effect.

4.1.3. HR

Aside from the effect of outdoor temperature on PWV measurements, there was also a disparity in the effect of HR between the cfPWV and PWVao models. This physiological factor had a moderate effect on PWVao readings explaining on average 1.45 m/s difference in PWVao between the subjects with PWVao increasing on average by 0.2 m/s (95% CI 0.02–0.40) per 10 bpm. The effect is similar to that reported by Tan et al., who used a hybrid applanation tonometry/oscillometric device SphygmoCor XCEL device and found an increase of cfPWV of 0.11–0.28 m/s per 10 bpm (35). In addition, individual variability in HR in our study accounted on average for 0.12 m/s (uncertainty, 0.01–0.27) discrepancy in repeated PWVao measurements. Considering these effects, PWVao is expected to have minimal physiologically relevant changes for small changes in HR, while larger changes in HR may be viewed as leading to considerable differences in PWVao. However, we did not observe a significant effect on cfPWV readings.

While current PWV estimation guidelines recommending that HR be considered as a confounding factor, there is still disagreement about the effect of HR on aortic PWV measurements, particularly considering that short-term studies investigating the relationship between heart rate and arterial stiffness reported varying results, including a positive, negative, and no association (24). O'Rurke et al. proposed that the apparent association between aortic PWV and HR might be due to systematic error introduced by certain devices when estimating such PWV (32), whereas Salvi et al. demonstrated, on data collected with applanation tonometry device PulsePen, that the said association was cofounded by the ventricular ejection time (69). Tan et al., however, used hybrid SphygmoCor XCEL and demonstrated BP-independent effects of HR on cfPWV

(35). In line with the O'Rurke's considerations, our results on two different devices suggest that the usage of devices with differing PWV measurement techniques might be the source of this disparity in findings. While many studies employing SphygmoCor CvMS or applanation tonometry technique in general reported no significant change in cfPWV with HR (60, 69, 70), studies that estimated aortic PWV using Doppler method (71), Arteriograph's oscillometric technique (61) or a hybrid applanation tonometry and oscillometric technique such as the one employed by SphygmoCor XCEL device (35) found independent HR effect on PWVao.

4.1.4. Experimental conditions

Out of all the experimental conditions tested only the visit order affected the PWV measurements. Specifically, whereas the order of visits had no effect on the PWVao measurements, the cfPWV measurements acquired during the second visit were on average 0.23 m/s (95% CI 0.03–0.43) lower than those of the first visit. However, no significant difference was found when the cfPWV data of the third visit were compared with those of the first visit. One possible explanation for the lack of a trend on the third visit is a confounding of this effect by outdoor temperature. Because outdoor temperature was identified as an important factor influencing cfPWV measurements, more pronounced meteorological changes on the third visit likely masked the effect of a visit order. In particular, for some subjects, the outdoor temperature varied by 8–10°C between the third and the other two visits.

The discovery of lower cfPWV values during the second visit may suggests that, despite receiving the amount of training recommended by current guidelines (24), both observers may have lacked some expertise in using SphygmoCor CvMS during the first visit. However, we did not observe a decrease in the within-subject variability of cfPWV measurements with increasing order of visits, as would be expected if the effect of poor training was present. Furthermore, Grillo et al. have shown that the 2-week training period is sufficient to achieve acceptable to excellent agreement of PWV recordings for various devices including SphygmoCor CvMS (48) and, as previously discussed, we have shown that the agreement of repeated cfPWV measurements estimated in our study was comparable to that reported by Grillo et al. Elliot et al. studied the influence of training on the repeatability of cfPWV values as well, but the authors assessed a hybrid SphygmoCor XCEL device with different mode of operation for which training differs (28).

Alternatively, the higher values of cfPWV during the first visit could point toward the white coat effect on arterial stiffness (72, 73). Indeed, the first measurement in our study was considerably higher than the second during the first visit cfPWV measurement series (median difference 0.35 m/s, 95% CI 0.05–0.65), and was also higher than the first measurement of the second visit (0.60 m/s, 95% CI 0.2–1.0). However, Barochiner et al. in an unadjusted analysis comparing isolated office uncontrolled hypertensive participants with sustained normotensive estimated much larger white coat effect on cfPWV with a median difference of 1.2 m/s (73). When we looked at SBP, we also found significant differences between the said measurements ($p \leq 0.015$; 1st measurement in first visit was on average higher for 3.3 mmHg, 95% CI 0.6–6.0 than 2nd, and for 3.8 95% CI 1.0–6.5 than 1st measurement in second visit), but these differences are not large enough to be classified as white coat effect. We also found no difference in DBP between these measurements ($p \geq 0.422$). A clinically significant white-coat effect is defined in terms of BPs as an office or clinic blood pressure exceeding the daytime ABPM by 20 mm Hg systolic or 10 mm Hg diastolic, either in the absence or presence of antihypertensive drug treatment (74). Thus, while the whitecoat effect, which describes a transient or persistent alerting reaction observed in the majority of patients, was likely present in our sample, the magnitude of it was insufficient to justify the first measurement discartion.

We did not find that time of day or different observers (conditional on observers having the same volume of training) affected cfPWV or PWVao measurements although the current guideline on PWV estimation recommends that repeated/follow-up measurements should be taken at the same time of day, preferably with the same observer (24). Several studies performed on different populations: young healthy volunteers (29), women with uncomplicated pregnancy (33), or healthy individuals of different ages and patients with heart disease (54) corroborate our result on lack of circadian variation in PWV measurements, whereas the study that reported increase in cfPWV with time of day revealed that diurnal PWV changes lost significance after adjustment for BPs suggesting that changes in arterial stiffening are mediated through changes in BP (75). As a result of the findings, future study protocols for follow-up PWV measurements could be simplified, as it is not necessary to measure cfPWV at the same time of day or with the same observer, provided that the quantity of training is adequate and comparable.

4.1.5. Participant characteristics: Sex, height, and hypertensive status

The differences in the PWV models of the two devices included participant characteristics such as sex and height which had a significant effect on PWVao, but not on cfPWV measurements; and hypertensive status, which on average increased cfPWV in hypertensive participants by 0.44 m/s but did fect PWVao measurements.

Female sex on average increased PWVao by 1.56 m/s, whereas height explained on average 2.14 m/s difference in PWVao measurements between the subjects, with one cm of height increasing PWVao by 0.06 m/s (95% CI 0.02–0.09).

Sex, with higher values of PWVao in women compared to men, was also identified as significant factor in another

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study investigating Arteriographs' PWVao measurements (61). However, while reports on the relationship between sex and central PWV measurements that were taken by devices other than Arteriograph were varied, all studies including those utilizing oscillometric devices reported either greater values of central PWV in men (16, 59, 60, 62, 76–78) or no association with sex (69, 79–82). Thus, it appears that higher values of PWVao measurements in women may be specific to Arteriograph device. Contrary to PWVao measurements, we did not find that cfPWV measurements were associated with sex. Vermeersch et al. estimated peripheral and central PWV with applanation tonometry in a large healthy, middle-aged population and concluded that while peripheral, carotid PWV measurements were associated with sex this was not the case with central arterial stiffness parameters such as cfPWV (80). Also, Reference Values for Arterial Stiffness' Collaboration in 2010 did not find significant differences between sexes in cfPWV while controlling for an age and MAP (57). Although cfPWV was markedly higher in males, the presence of male gender was also accompanied by marked differences in age and BP. After correction for age and MAP, the authors found negligible influence of gender on cfPWV (0.1 m/s difference, $P = 0.04$) and proceeded with the definition of the reference value population by including all untreated participants, regardless of sex. Similarly, sex was not identified as significant predictor of cfPWV in the multivariable linear regression models controlling for age, MAP, HR in the study by Mitchell et al. (83) which is based on apparently healthy Framingham Heart Study offspring cohort (83). Focusing solely on SphygmoCor's cfPWV measurements, the results are contradictory, with more studies reporting that in multivariate linear regression models, cfPWV increases with age similarly for both sexes (31, 80–82), others revealed that men have higher cfPWV values ranging from 0.27 to 0.72 m/s (16, 59, 60). We discovered no significant interaction between sex and age for either device.

As for the effect of height, similar to Jatoi et al. who also investigated Arteriograph's PWVao measurements, we found an inverse correlation between PWVao and height (data not shown). However, while in our final multiple model PWVao increased with the increase in height, in Jatoi's multiple model the association did not reach significance (61). Furthermore, our estimate of the regression coefficient of height was positive; similar to the estimates from the simple regression model developed by Mellin et al. on Vircorder measurements (62) and multiple regression model developed on PulsePen measurements in children (84).

4.1.6. Overall about PWV models

Overall, the findings suggest that SphygmoCor's cfPWV values are more sensitive to the state of the arterial tree because, in addition to age and MAP, they are also dependent on hypertensive status, the interaction between MAP and outdoor temperature, outdoor temperature, and visit order; whereas Arteriograph's PWVao values are more dependent on individual characteristics such as sex, height, and age as they are more heavily dependent on age than cfPWV.

It should also be noted that our findings are device-specific and cannot be generalized to other seemingly similar techniques, such as the oscillometric Mobil-O-Graph, which calculates PWV using a formula, because the factors affecting within-subject PWV variability would obviously differ.

4.2. Factors affecting measurement difficulties

The measurement difficulties appeared relatively frequently: from 19% of cases in which Arteriograph's oscillometric signal had to be manually selected for analysis, to over 24% of cases in which SphygmoCor's pulse wave signal was of marginal but acceptable quality (with a coefficient of variation for cfPWV estimates ranging from 6 to 10%), to 32% of cases in which SphygmoCor's recordings had to be repeated. While all of these difficulties are time-consuming, we also found that one of them—a marginal quality of SphygmoCor's signal, affected accuracy of estimated cfPWV values by raising cfPWV values on average by 0.37 m/s (95% CI 0.16–0.58 m/s). Concerning the difficulty in manually selecting the Arteriograph's signal, which increases the uncertainty of PWVao estimation and could be expected to affect the accuracy of PWVao readings, we did not find that it was associated to a change in PWVao levels.

All of aforementioned measurement difficulties were reduced when PWV measurements were performed in the afternoon (16–18 h) compared to the morning (7–10 h) sessions. The effect of time of day on need to manually select Arteriograph's signal was strong with afternoon session reducing the OR for automatic selection by on average 75%, and reducing the probability of the event from 23% during morning sessions to 6% in the afternoon. The time of day also had moderate effect on a need to repeat a SphygmoCor measurement by reducing its IRR in the afternoon measurements on average by 12%. Finally, the OR for the marginal quality of SphygmoCor's signal reduced by 40% in the afternoon, with a probability of such a signal decreasing from 26% during mornings to 19% in the afternoon. As time of day did not show significant effect on absolute PWV values of either cfPWV or PWVao, just by performing measurement in the afternoon measurement difficulties could be significantly reduced.

Sex was another factor that affected measurement difficulties for both devices. It strongly increased the OR of manual selection of a Arteriograph's signal by 51 times, and also increased IRR of repeated measurements by SphygmoCor by 1.23 times. The difficulties we observed in obtaining PWV estimation for female participants with both devices might be attributed to men being more likely prototype examinees due to the menstrual cycle's effect on PWV recordings in

women, which, as a result, would make signal acquisition and processing suboptimal for women. However, because the literature describes a validation sample rather than a developmental sample for prototypes, and the manufacturers do not provide a description of developmental sample, there is a lack of evidence to support or refute this assumption.

We also found that the order of visits, which affected repeated cfPWV measurements, affected the occurrence of measurement difficulties with the SphygmoCor too, in such a way that both the need for repeated measurement and the occurrence of a signal with marginal quality were significantly reduced during the third visit compared with the first visit, whereas the frequency during the second visit was comparable to that during the first visit. So it seems that in terms of measurement difficulties with SphygmoCor, there is still a learning curve to be observed in the first 2 weeks.

Finally, while inter-observer differences in PWV readings are not expected for oscillometric devices nor were observed in our study, we show that the need for manual selection of the Arteriograph's signal is observer-dependent with one observer increasing the odds of it by 91%. Whether this difference between observer is due to positioning or adjusting the cuff should be explored in further studies.

Considerations about the supine and sitting positions with Arteriograph.

While we did not examine the effect of supine and sitting positions on Arteriograph's PWVao measurements, Arteriograph is frequently used in clinic in a sitting position. Nurnberger et al. estimated the mean difference in PWVao between supine and sitting positions to be −0.18, with the limits of agreement ranging from −1.55 to 1.21 (85). Such wide limits include differences of more than 1 m/s thus demonstrating that PWVao values recorded in two different positions are not the same and only one position, preferably supine, should be used when assessing PWVao.

4.3. Limitations

Potential limitation of our study is referred to the pre-training of observers. Despite providing observers with the necessary pre-training, we discovered a learning curve with the SphygmoCor device, as measurement difficulties were significantly reduced during the third visit compared to the first. Furthermore, we also discovered that repeated cfPWV measurements recorded during second visit were on average 0.23 m/s lower than those recorded during the first visit, indicating that there may be a learning curve affecting cfPWV accuracy too, but we were unable to show this tendency for measurements collected during the third visit compared to first. Nonetheless, even if this learning curve affected repeated measurement accuracy, we do not expect it to have a significant effect on our results: the estimated difference in cfPWV between second and first visit is not large, we did not find that variability of repeated measurements taken during one visit was related to visit order, and we reported comparable agreement between repeated measurements to a study that reported this agreement using observers for which it proved they received a sufficient training period (48). In addition, estimated decrease in within-subject cfPWV measurements during 2nd vs. 1st visit was just −0.03 m/s (uncertainty −0.25, 0.20).

To capture short-term variability of PWV, we monitored within-subject PWV changes for 2 weeks only. Consequently, we were not able to control an effect of menstrual cycle on arterial stiffness by studying all women who still have menstrual periods at a similar phase in the menstrual cycle as this would imply that repeated measurements are also taken at similar phase of the cycle, which would prolong the monitoring period. However, we do not expect that effect of menstrual cycle substantially affected PWV variability in young women as we found significantly larger variability of within-subject PWV measurements in menopausal women than in women with menstrual cycle, for both devices ($P \leq 0.035$, Mann-Whitney test). Moreover, for women who menstruate within-subject PWV variability was comparable to men ($P \geq 0.310$).

While the study included 35 participants, we developed multilevel models with 12 repeated measurements per participant for a total of 420 measurements, which considerably increased the study's power (86). Furthermore, as indicated in the methodology section, the sample size consideration for our model choice allowed for up to 12 independent variables, and we also demonstrated that our estimates of the effects of factors influencing PWV readings were realistic, whenever we could compare our estimates to those estimated in other studies.

One potential limitation of our study was mitigated with the choice of cross-over randomized design. Although we did not directly compare the devices for reasons explained before, it was important that devices be used in a comparable setting as there was a possibility that measurements taken with the first device in a series may affected measurements taken with the second device and that an identified significant factor for a device may be due to the fact that that device is always applied second in the measurement series. To account for this effect, we randomized the order of devices in a measurement series and developed multilevel regression models that showed that the identity of the first device used in a measurement series for a person had no effect on PWV measurements, supporting the assumption of comparable settings.

Finally, because the PWV measurements were taken within 2 weeks, we were unable to investigate any seasonal effects on the PWV measurements. Given that blood pressure can be significantly lower in the summer compared to the winter, it remains to be seen whether the short-term estimates made in this study are sensitive to the season in which the recordings were taken.

5. Conclusion

In conclusions, we systematically assessed a large number of experimental, meteorological, physiological factors and personal characteristics to identify those that affect PWV measurements and contribute to differences in short term repeated measurements. We quantified these effects separately for the two devices that use different measurement techniques, using study design that provides strong evidence.

We discovered that increasing age increases not only the values of cfPWV and PWVao but also the variability of their repeated measurements, suggesting that in older people precision of measurement should be increased, possibly with the use of 3–4 measurements in a series instead of only 2.

For SphygmoCor's cfPWV measurements, we also found a significant interaction between MAP and outdoor temperature, as well as significant mean effect of temperature, which could lead to significant fluctuations in short-term repeated measurements. Recording cfPWV during a season with a higher outdoor temperature (e.g., 25°C) when differences in MAP result in smaller differences in cfPWV may reduce some of the short-term fluctuations. Also, it should be further investigated if prolonging resting time during periods with low outdoor temperature might help with reduction of short-term fluctuations.

Time of day or different observers who received the same amount of pre-training did not affect cfPWV or PWVao measurements. However, measurement difficulties occurred significantly less frequently in the afternoon for both instruments. To facilitate future longitudinal studies, it would be advisable that PWV is measured in the afternoon whenever possible, not necessarily with the same observer.

For both devices, we found a relationship between a measurement difficulty and female sex. Whereas, the number of retaken measurements with SphygmoCor was moderately increased in women, the association with sex was very strong for manual signal selection with the Arteriograph device which was much more common in women than in men. In addition, the PWVao measurements by Arteriograph revealed that PWVao values were on average significantly higher in women than in men. This is in contrast to other devices that found higher PWV values for men, or no association with sex. Overall, the findings indicate that devices' design may be suboptimal for women, with a possible systematic bias in Arteriograph's measurements.

The differences in factors affecting the PWV measurement in the two models most likely reflect differences in the measurement techniques of the two devices. In addition to age and MAP, cfPWV measurements which are considered a more direct measure of arterial stiffness were also influenced by hypertension status, the interaction MAP-outside temperature, temperature and the order of visits. PWVao values which were estimated from a single site were additionally influenced by patient characteristics such as sex, height, and HR, with higher PWVao values in women being specific to the Arteriograph device.

Data availability statement

The original contributions presented in this 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 Ethical Committee of University of Split School of Medicine. The patients/participants provided their written informed consent to participate in this study.

Author contributions

MP and AJ conceived and designed the study, analyzed the data, and wrote the draft of the manuscript. MP, BŠ, AB, PK, VĐ, and IM participated in data collection. MB, IM, and AJ supervised the data collection and the findings of the study. All the authors interpreted the data and approved the final version of the manuscript.

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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.

Publisher's note

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What Is the Smallest Change in Pulse Wave Velocity Measurements That Can Be Attributed to Clinical Changes in Arterial Stiffness with Certainty: A Randomized Cross-Over Study

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Abstract: Pulse wave velocity (PWV), a direct measure of arterial stiffness, is a promising biomarker of cardiovascular risk and a cardiovascular surrogate outcome. The resolution for detecting its smallest clinically significant change is dependent on the expected reproducibility, but there is currently no consensus on this. We estimated the PWV reproducibility in a range of intra-subject values that were observed over a 2 week period in a broad range of participants and under clinically relevant experimental conditions (two observers, morning/afternoon sessions, and number of visits) using SphygmoCor and Arteriograph devices. Each participant was recorded 12 times with each device over three visits, one week apart, and two morning and two afternoon recordings were taken per visit. The factors affecting reproducibility and the discrepancies between the consecutive PWV measurements for each device were also examined using multilevel mixed-effect models. We show that current PWV estimation guidance recommending 2 + 1 measurements is suboptimal because the PWV range was outside of the 1 m/s threshold for most of the participants, which is proposed as a minimal clinically important difference. The best reproducibility was yielded with median of four measurements and a 1.1 m/s threshold. Although PWV reproducibility and repeatability are frequently used interchangeably in studies, we demonstrated that despite their relative measures of variability (e.g., coefficient of variation) being comparable, their ranges revealed a clinically significant difference between them. We also found that different physiological variables were predictors of the discrepancy between the consecutive measurements made by the two devices, which is likely due to their distinct modes of operation. The evidence base for PWV reproducibility is limited, and more research is needed to deepen our understanding of the variation in arterial stiffness over time, as well as fluctuations within a population group and in an intervention setting.

Keywords: arterial stiffness; pulse wave velocity; reproducibility; repeatability; minimal clinically important difference; MCID; intra-subject variability; measurement errors

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1. Introduction

Arterial stiffening is the most characteristic clinical feature of the aging process in the arterial system, which is characterized by a decrease in the arterial compliance and/or changes in the arterial wall characteristics [1,2]. Numerous studies performed on both patient- and population-based samples have found that more arterial stiffness is independently associated with an increased risk of having a first or recurrent major cardiovascular disease event [3–5]. Because the measurement of aortic stiffness is

viewed as an integrator of all of the damage that has been incurred in previous years to the arterial wall in response to both traditional and poorly identified/unidentified cardiovascular risk factors, arterial stiffness is considered to be a good biomarker for the detection of early vascular aging [2,6–8], as well as a surrogate endpoint for cardiovascular disease [9,10]. Measurements of arterial stiffness have been shown to improve the reclassification of patients who are at intermediate risk for cardiovascular disease by supplementing the information provided by the traditional risk factors [3,11–13]. Additionally, it has been demonstrated that arterial stiffness is associated with target organ damage [14–17]. Recently, clinical trials evaluating arterial stiffness as a surrogate endpoint for cardiovascular disease in hypertensive patients have begun [18,19].

In a 2006 consensus document, the measurement of carotid–femoral pulse wave velocity (PWV) was defined as the gold standard for measuring arterial stiffness [20].

Despite their significant potential for cardiovascular disease prevention and hypertensive treatment management, the use of PWV measurements in clinical practice is limited. One of the barriers impeding PWV translation to clinical practice is a lack of methodological consensus, which hampers accurate comparisons of PWV within and between studies. The number of consecutive measurements used in the estimation of the true PWV value is an example of such an inconsistency [21]. To estimate a true PWV value, the American Heart Association (AHA) recommends averaging at least two PWV measurements, and if their difference exceeds 0.5 m/s, a third measurement should be taken, and the median value should be reported [22]. Nonetheless, some researchers employ a measurement protocol that entails repeating the measurements until two values are within 0.5 m/s of each other [23–26]. Furthermore, duplicate measurements are averaged in many studies regardless of their difference [27–30], while some studies use a single PWV measurement [31,32]. Despite its significance, the AHA recommendation is based on a single study [33], and it is classified as having a weak evidence [22]. No study, so far, has evaluated the effect of the number of measurements used in PWV estimation on the reproducibility of the PWV.

Another issue with translating PWV measurements to clinical practice is a lack of consensus on the expected reproducibility of PWV, which is defined as the precision of the measurements obtained under different conditions over a short period, typically days or weeks. Such a quantification is required to correctly interpret the results of longitudinal studies monitoring PWV changes in an individual over time and, consequently, detect the minimal clinically important change. However, currently, the reproducibility of PWV appears to have mainly been investigated in validation studies comparing new, to the reference device [34]. Only a few studies that investigated the PWV reproducibility of a single device typically used small sample sizes ($N \leq 21$) and reported reproducibility as the coefficient of variation (CV), which is a relative measure expressed that is in units of standard deviation and is difficult to interpret when one is looking for clinically relevant changes or the correlation coefficient, e.g., interclass correlation coefficient [35,36]. Some of these studies additionally reported repeatability, which they expressed as the mean difference between two PWV measurements and corresponding limits of agreements [36], whereas others reported repeatability as a precision of measurements obtained under the same conditions within 24 h, rather than reproducibility [33,37–40]. To assess PWV reproducibility, the variability that occurs due to different experimental settings and random factors other than clinically relevant change, powered studies on a specific device are required, with PWV reproducibility being expressed in a measurement unit that is easily interpretable in the context of determining the minimal clinically relevant change, and with PWV measurements spanning more than two measurements that are separated by a longer period than 1–2 days.

The goal of this study was to determine the amount of intra-subject PWV variability that could be attributed to conditions/factors other than the clinically relevant change by monitoring the PWV over 2 weeks in a broad range of participants and under different experimental conditions resembling those in clinical practice (different observers, time of day, and number of visits). The resolution for detecting the smallest clinically significant

change is determined by this value. We also wanted to see how the number of repeated measurements used in PWV estimation affected the PWV reproducibility and what factors contributed to discrepancies between the consecutive PWV measurements. The analyses were performed separately for the two devices that use different PWV measurement techniques: the applanation tonometry device SphygmoCor CVMs, a gold standard device for PWV measurement, and the Arteriograph, an oscillometric device.

2. Methods

The methods for this study are detailed in another study in which we investigated the factors influencing PWV measurements and measurement difficulties [41], and they are summarized herein.

2.1. Participants

This 2 week long longitudinal study enrolled 36 participants between 20 and 60 years of age. The participants were evenly distributed by age (in decades), sex, hypertension status (normotensive or hypertensive), and body mass index (BMI) (normal weight, overweight, or obese). All of the invited participants provided their medical history, and those with arrhythmias, cerebrovascular sickness, pregnancy, surgery amputation, oncology disease, psychiatric disease, infections throughout the trial duration, and medical nonadherence were excluded from the study.

The Ethics Committee at the University of Split School of Medicine approved the study, and all of the participants provided written informed consent.

2.2. Study Design

This is a single-blind randomized cross-over longitudinal study that was conducted at the University of Split School of Medicine's Laboratory for Vascular Aging.

Over two weeks, each participant was recorded 12 times in total, four times on each of the three visit days, which were separated by one week. The two observers recorded a participant in the morning (7–10 h) and afternoon on each visit day (16–18 h) (Figure 1).

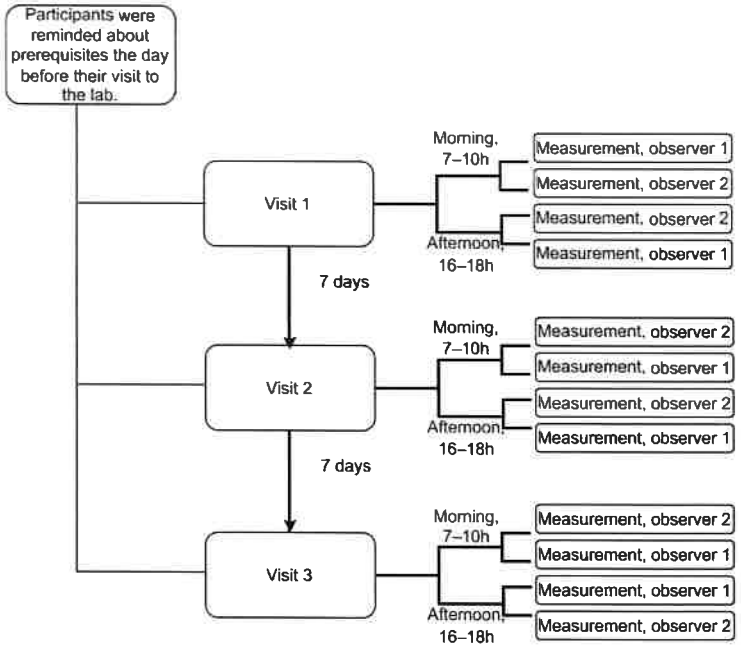


Figure 1. Overall study design showing recordings with a single device. The order of the observers was randomized, and their ID numbers are provided here just for illustration purposes. Prerequisites: participants were reminded to refrain from strenuous exercise and alcohol for 24 h, as well as from eating, drinking (except water), or smoking for at least 3 h before each recording session.

To ensure that the measurements were taken under comparable conditions by two observers, we randomized the order of the observers who were also blinded to each other's readings. Similarly, we randomized the order of devices to ensure that the Sphygmocor CvMs and Arteriograph devices were used under the same conditions. To do so, we used randomization with a permutated block size of four and a random number generator algorithm to generate a random sequence of blocks.

2.3. PWV Measurements

The pulse wave measurements were taken separately using two different measuring devices: the applanation tonometer SphygmoCor CvMS (AtCor, Australia) and the oscillometric device Arteriograph (Tesniomed, Hungary). The SphygmoCor device measured the PWV between the carotid and femoral sites, and the measurements are thus referred to as carotid–femoral PWV or cfPWV in the following text. The Arteriograph, on the other hand, estimated the aortic PWV from a single site at the brachial vascular bed, which is thought to accurately approximate the cfPWV. We refer to the Arteriograph measurements in the following text as PWVao.

For both of the devices, the measurements were taken following the American Heart Association's (AHA) recommendations for improving and standardizing the vascular research on arterial stiffness [22].

The observers performed the measurements in a quiet, temperature-controlled room at a comfortable temperature of 21–23 °C. The participants were asked to refrain from strenuous exercise and alcohol for 24 h before the recording was taken. They were also told not to eat or drink anything other than water for at least 3 h before the recording and not to smoke. Those taking vasoactive medicines were advised to continue taking them as usual and not to change the dosage during the study. To ensure hemodynamic stability, the participants rested in the supine position for 10 min before the first PWV measurement. Following the completion of the series of measurements using one device, the participants were asked to stand up, walk around the room, and then lie down for another 10 min to prepare for measurements using the second device. This step was required to keep the participants from falling asleep while lying supine for an extended period, especially in the morning. The participants were not allowed to talk or sleep during the measurements. All of the measurements were taken on the right hand (Arteriograph), and the right carotid and femoral arteries (SphygmoCor).

Before the start of the study, both of the observers had received extensive training for 7 days, during which they performed approximately 50 high-quality measurements under supervision.

To calibrate the pulse wave signals acquired using the SphygmoCor, we obtained brachial blood pressure measurements using a validated oscillometric sphygmomanometer (Welch Allyn Connex ProBP 3400 digital blood pressure monitor with SureBP technology).

To calculate the wave travel distance, we used the subtracted distance method. The method was chosen over the direct method as per the AHA guideline, the most recent guideline on arterial stiffness measurements [22]. Additionally, as per the AHA guideline, instead of using a tape measure, we used a large school divider to measure the distance between the sternal notch and the femoral measurement site, and then, we subtracted the distance between the carotid measurement site and the sternal notch. The distance between the carotid and femoral sites was only measured during the first recording session.

2.4. Meteorological Conditions

To describe the meteorological (outdoor) conditions under which the measurements were taken, we obtained data of the outdoor temperature, air pressure, and humidity from the Meteorological and Hydrological Service of Croatia's local office, and we used them to estimate the weather conditions during each recording session. Throughout the study, the temperature ranged from 4.5 to 23.3 °C, the air pressure ranged from 972 to 1011 hPa, and the humidity ranged from 32 to 92%.

2.5. Sample Size Consideration

Assuming that we obtained a level of confidence of 95%, a population standard deviation of the intra-subject PWV changes measured using the Arteriograph of 0.57 m/s, and a margin of error of 0.2 m/s, the study would require a minimum sample size of 35 to achieve the envisaged level of precision [42]. This sample size is also sufficient to produce a level of confidence of 95% for the SphygmoCor measurements too.

2.6. Definitions of PWV Repeatability and Reproducibility

The AHA guideline defines the variability between the intra-subject PWV measurements separated by at least 24 h as reproducibility, which is a precision of measurements obtained under different conditions over a short period, usually days or weeks [22]. Consequently, the variability of measurements taken within 24 h and recorded under same the conditions is defined as repeatability, which isa precision of measurements obtained under the same conditions within 24 h. Usually, the repeatability and reproducibility of PWV measurements are expressed as relative measures: coefficient of variation (CV), which is reported in units of standard deviation; or correlation coefficients, such as the intraclass correlation coefficient (ICC). In this study, we express the variability as a range in m/s, which is easily interpretable in a clinical setting.

2.7. Data Analysis

We used descriptive statistics to describe the distribution of quantitative (mean and standard deviation or median and interquartile range (IQR), depending on the shape of distribution) and qualitative (absolute and relative frequencies) variables.

Multilevel regression models were used to account for repeated PWV measurements while identifying the factors associated with the per person count of discordant pairs of consecutive PWV measurements, the size of a discordance, or an occurrence of a discordant pair of measurements. As per the AHA guidelines, a discordant pair of PWV measurements is one where the values are more than 0.5 m/s apart [22]. Depending on the type of a dependent variable, we used multilevel mixed-effects generalized linear models for models where the size of the PWV discordance was the dependent variable, multilevel mixed-effects logistic regression models for a dichotomous dependent variable such as an occurrence of a discordant pair of consecutive PWV measurements, and multilevel mixed-effect Poisson regression for count data such as the per person count of discordant pairs of consecutive PWV measurements. All of the models used the robust estimator, which is robust to certain types of misspecification in multilevel models [43,44]. The sensitivity analysis was performed with the maximum likelihood (ML) method, without the robust estimator.

Each multiple regression model (the model including several independent variables (IVs)) was built in two steps. The experimental conditions' variables: the order of the visit and the time of day; the meteorological (outdoor) conditions' variables: the temperature (°C), air pressure (Pa), and humidity (%); physiological variables: the blood pressure or heart rate; participants' characteristics: age, sex, BMI, and hypertension status, were all investigated for their relationship to the dependent variables by a simple regression analysis. Those IVs that were associated with the dependent variables at the $p < 0.2$ significance level were entered into a multiple regression model. For the IVs that were nonsignificant in a multiple model, the contribution of an IV to the model (pseudo R^2, log pseudolikelihood, and random variance) was investigated further to decide on the final model.

3. Results

The study enrolled a total of 36 participants, and one participant was later removed from the study due to an ongoing infection, leaving a total of 35 participants.

We observed a wide range of PWV values (median 6.3, range 4.5–10.8 m/s, as measured using the SphygmoCor), brachial blood pressures (systolic: 126, 98–177 mmHg; diastolic: 72, 53–98 mmHg), age (41, 20–60 years), and BMI (27.3, 19.4–38.9) in our sample. In addition, the participants were distributed evenly across age (in decades), sex (17 or

49% females), hypertensive status (17 or 49% hypertensives), and BMI categories (12 or 34% normal, 11 or 32% overweight, 12 or 34% obese patients), ($p \geq 0.692$ for all of them).

Overall, the CVs for the within-subject variation were 9.9% (95% confidence interval (95% CI) 1–19%) for the SphygmoCor and 5.3% (95% CI 0.4–10%) for the Arteriograph.

3.1. An Examination of Large Differences in Consecutive PWV Measurements (>0.5 m/s), for Which the AHA Recommends the Inclusion of a Third Measurement in PWV Estimation

3.1.1. The Requirement for a Third Measurement

The prevalence of two consecutive PWV measurements taken within 1 h, which were more than 0.5 m/s apart, was high for the Sphygmocor device (51%, 95% CI 45–58%), and it was lower, but not insignificant, for the Arteriograph (27%, 95% CI 21–33%). In fact, the odds ratio (OR) of observing a pair of measurements with discrepancies of greater than 0.5 m/s, was 3 times higher (95% CI 2.0 to 4.6; mixed-effects logistic regression, $p < 0.001$) for SphygmoCor than for Arteriograph.

3.1.2. Do Pairs of PWV Measurements with Unacceptable Large Differences Cluster within Specific Individuals?

Out of six pairs of consecutive PWV measurements that were recorded per person, the median number of discordant pairs with a difference of greater than 0.5 m/s was three (range, 1–6) for SphygmoCor and one (0–5) for Arteriograph. However, the counts of the discordant pairs of PWV measurements per person did not deviate significantly from chance (Figure 2), implying that large differences emerge at random and independently of each other. Indeed, for the Arteriograph PWVao measurements, we found no relationship between the counts of the discordant pairs of measurements per participant and the participant's characteristics such as age, gender, BMI, hypertension status, or the median values of the participants' HR or BPs (simple Poisson regressions, $p \geq 0.074$ for all of them). For the cfPWV discrepancies, these counts were weakly positively correlated to a person's median mean arterial pressure (MAP), (simple Poisson regression, incidence rate ratio 1.017, 95% CI 1.003–1.031, $p = 0.014$) and median diastolic blood pressure (DBP) (incidence rate ratio 1.018, 95% CI 1.002–1.033, $p = 0.027$), with the models describing only up to 2% of the variation, and no other factors having been identified as predictors ($p \geq 0.299$).

3.1.3. Factors Affecting an Occurrence of a Pair of PWV Measurements with Unacceptable Large Differences

Next, we examined if any of the experimental conditions (order of visit: one, two, or three; or time of day: morning or afternoon), outdoor conditions (outdoor temperature, air pressure, or humidity) or characteristics of participants (age, gender, BMI, hypertension status, heart rate (HR) or blood pressures (BPs): MAP, systolic blood pressure (SBP) and DBP) could predict an occurrence of a discordant pair of PWV measurements with a difference of greater than >0.5 m/s. We found that increasing the median MAP and DBP values increased the odds of a discordant pair of SphygmoCor readings (simple mixed-effects logistic regressions, $p \leq 0.025$ for both of them), while the other factors were not identified as predictors ($p \geq 0.263$ for all of them). A one mmHg increase in the MAP and DBP raised the odds by 4% (95% CI 1–7%) and 4% (0.04–7%), respectively. In terms of the Arteriograph measurements, a one bpm increase in the median HR increased the odds of failed measurements by 7% (95% CI 1.03–1.12, $p = 0.002$), but the other factors were not significant predictors ($p \geq 0.252$ for all of them).

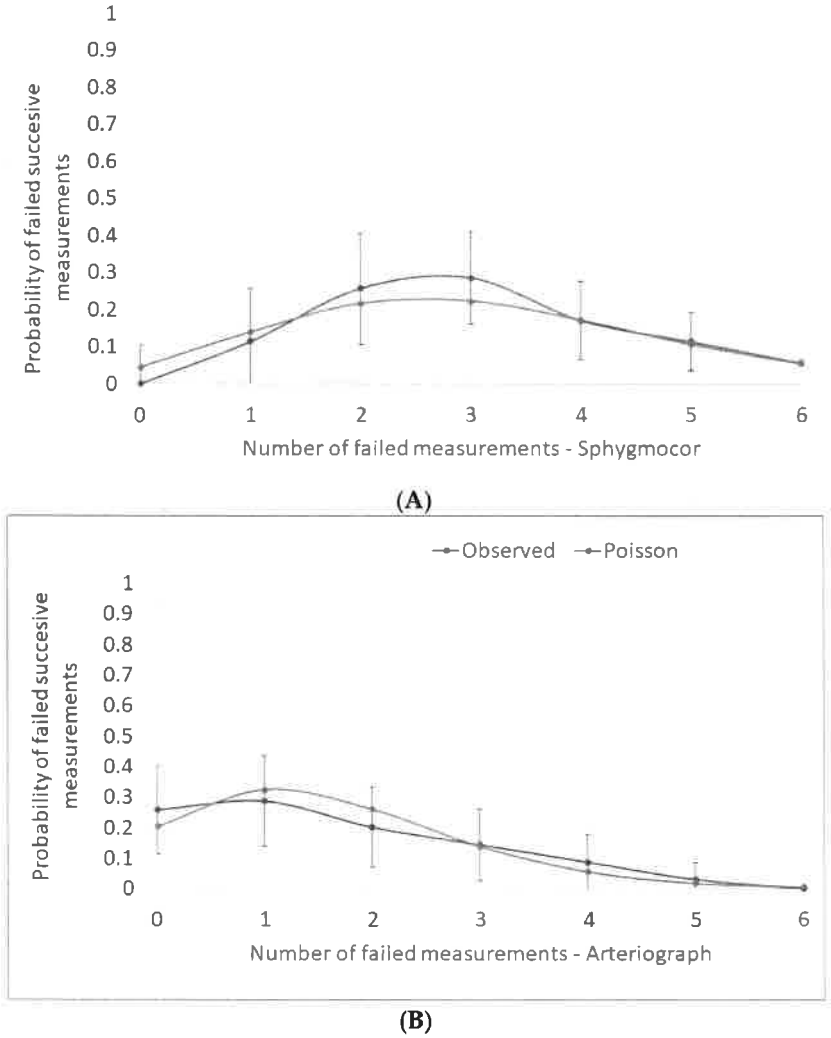


Figure 2. The distribution of counts per person of consecutive PWV measurements with discrepancies >0.5 m/s is shown here with the associated 95% confidence intervals (blue line). The orange line represents the theoretical Poisson distribution, which depicts discordant pairs of measurements occurring at random and independently: (**A**) SphygmoCor and (**B**) Arteriograph devices.

3.2. Factors Affecting the Size of Differences between Consecutive PWV Measurements

To find out if the characteristics of the participants, experimental conditions, or outdoor conditions affected the size of the differences between the pairs of measurements, we built mixed-effects ML regression models.

We discovered that age, median MAP, but also outdoor temperature, and the interaction between the outdoor temperature and the MAP predicted the size of the discrepancies between two consecutive cfPWV measurements (Table 1). As for the PWVao discrepancies, while experimental and outdoor conditions did not affect these discrepancies ($p \geq 0.229$ for all of them), the patients' characteristics such as BMI, sex, or hypertension status did. The ICC for the SphygmoCor and Arteriograph revealed that the discrepancies in the consecutive PWV measurements were not well correlated within a person, for both the Sphygmocor (no correlation) and the Arteriograph (poorly correlated).

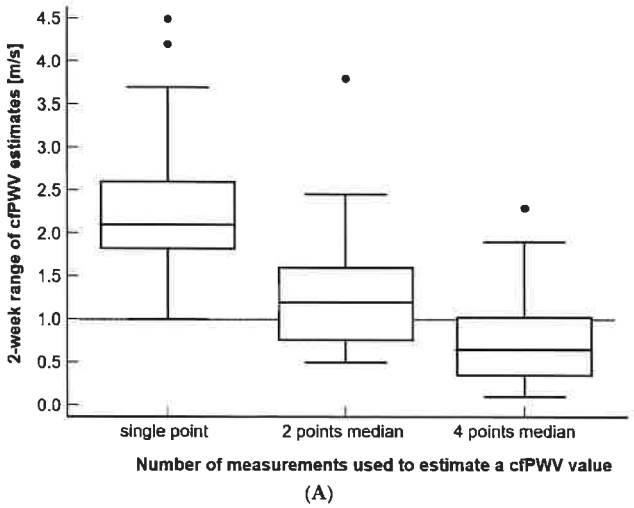
Table 1. Predictors of the size of discrepancies between the two consecutive quality-passed PWV measurements.

Discrepancies		B	95% CI		*p*-Value
SphygmoCor (cfPWV)	**Age (years)**	0.01	0.004	0.02	0.003 *
	Outdoor temperature (°C)	0.25	0.05	0.44	0.015 *
	MAP (mmHg)	0.05	0.02	0.08	0.002 *
	Interaction Outdoor temperature × MAP	−0.003	−0.005	−0.0008	0.007 *
	ICC at a participant level 3.17 $\times 10^{-14}$ (95% CI 3.17 $\times 10^{-14}$–3.17 $\times 10^{-14}$), Snijders/Bosker R^2 Level 1: 12%, Level 2: 21%				
Arteriograph (PWVao)	**BMI**	0.03	0.003	0.05	0.025 *
	Sex (Female vs. Male)	0.29	0.08	0.50	0.007 *
	Hypertension (Yes vs. No)	−0.23	−0.44	−0.01	0.037 *
	ICC at a participant level 0.11 (95% CI 0.05–0.21), Snijders/Bosker R^2 Level 1: 10%, Level 2: 30%				

The models used are the mixed effects ML regression models with a robust estimator. * Significant at the 0.05 level.

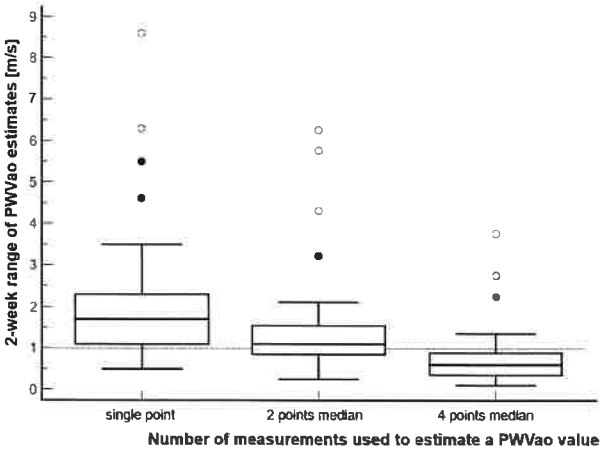
3.3. The Effect of the Number of Consecutive Measurements Used in PWV Estimation on 2 Weeks Reproducibility of PWV Measurements

To investigate the effect of the number of consecutive measurements utilized to estimate the daily PWV value on the variability of PWV values observed in an individual over 2 weeks, we compared the 2 week ranges of the intra-subject PWV values when these values were estimated from a single, two (recorded within 1 h), or four consecutive measurements (recorded within 24 h) (Figure 3). We found that the reproducibility of the PWV estimates significantly decreased with the increasing number of measurements used in the estimation for both the cfPWV and PWVao values (Figure 3A and 3B, Friedman test, $p < 0.001$ for both of the measures and all of the comparisons), with the median of four consecutive daily measurements yielding the best results. Even after fully implementing the AHA recommendation for two points median/mean (two measurements plus a third measurement included in the calculation if two measurements were more than 0.5 m/s apart), the results still showed that the range for two point median PWV estimates for both the cfPWV and PWVao was greater than 1 m/s in 19 or 54% of participants. For the three points median, which is not presented in Figure 3, the respective percentages were fourteen or 40% of the participants for cfPWV and six or 17% with PWVao. On the contrary, by implementing the four points median approach, 26 participants (74%) for cfPWV and 30 of them (86%) for PWVao had their 2 week PWV values within ≤1 m/s range, whereas 30 participants (86%) for cfPWV and 31 (89%) for PWVao had 2 weeks PWV values that were within the ≤1.1 m/s range.

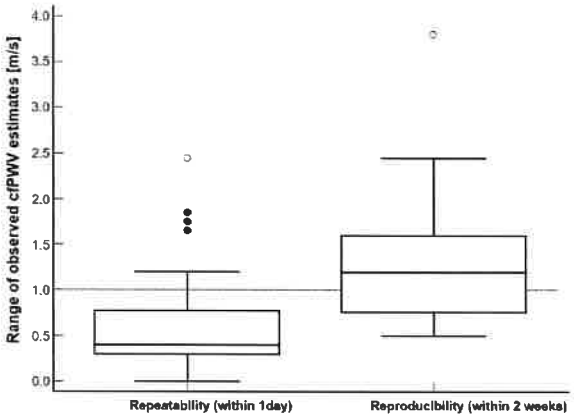


(A)

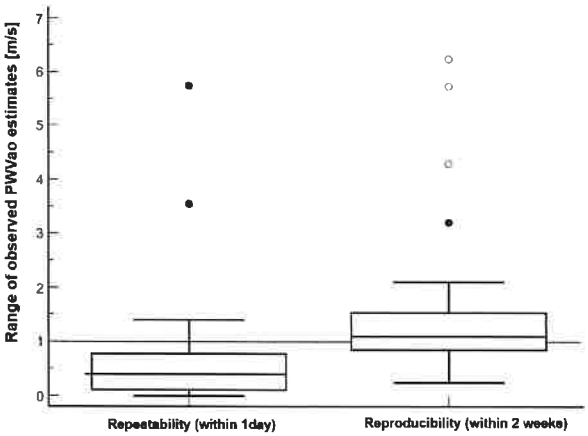
Figure 3. *Cont.*



(B)



(C)



(D)

Figure 3. (**A**–**B**) The effect of estimating PWV from the different number of measurements that were recorded within a day. (**C**–**D**) Comparison of repeatability and reproducibility range estimated using the currently recommended strategy. The reference line at 1 m/s is noted. Additionally, please note that the value marked as 2 points median is equivalent to 2 points mean.

3.4. Comparison of the Reproducibility and Repeatability of PWV Measurements, When Expressed as a Range of Values

Using the currently accepted strategy of PWV estimation (mean or median of the two consecutive measurements), we then compared the repeatability of the PWV values observed in the same participant within a day to their reproducibility that was observed

over two weeks (Figure 3C,D). We found that the reproducibility, when it was expressed as a range of observed values, was significantly higher for both of the PWV measures than the repeatability range was (Wilcoxon test for paired samples, $p < 0.001$ for both of the PWV measures). Specifically, the reproducibility range of cfPWV was higher by a median of 0.70 m/s (95% CI 0.55–0.87) and that of the PWVao was higher by a median of 0.68 m/s (95% CI 0.50–0.88) than their respective repeatability ranges. The repeatability, which is expressed as a range of 1 day PWV estimates, fell out of the ≤1 m/s range in eleven (10%, cfPWV) and nine (9%, PWVao) cases out of one hundred and five 1 day recording sessions, whereas the reproducibility fell out of this range in twenty-one (60%, cfPWV or PWVao) out of thirty-five participants.

3.5. Is There a Difference in the Size of Discrepancies between PWV Measurements Taken on the Same Day up to One Hour Apart and Those Taken on the Same Day, but Over a Longer Period?

To see if the PWV repeatability definition of the measurements needing to be taken within 24 h is supported by the data, we compared the discrepancies between the pairs of measurements taken within the same day up to 1 h apart and those taken over longer periods (8 and more hours apart), and we found no difference: mean discrepancy: 0.04 m/s, 95% CI—from 0.21 to 0.14, $p = 0.663$.

4. Discussion

With as many as 12 measurements per participant having been recorded over two weeks in a wide variety of experimental settings and weather conditions and over a wide range of participants, this study is the first one to estimate the reproducibility of PWV measurements that may be applicable in clinical practice, with the results being expressed using a simple measure of variability: a range of values.

We showed that depending on the number of repeated measurements used in the PWV estimation, the PWV repeatability ranged from unacceptable (a single measurement, two measurements, or two measurements with the inclusion of a third measurement as per the AHA guidance) to acceptable (four measurements). With there being only a slight increase in the proposed threshold for the minimal clinically important difference from 1 m/s (O'Connor, Koufaki et al., 2017) to 1.1 m/s, the four measurements represent an acceptable 11–14% of the participants with an intra-subject PWV range that is wider than the 1.1 m/s limit.

Our evaluation of the two precision components that are frequently confused in PWV research, the reproducibility and repeatability of PWV, showed that the former one is significantly larger than the latter one is, on average, by 0.7 m/s. It was also demonstrated that both of the devices exhibit an adequate repeatability PWV range according to the 1 m/s criteria.

We also investigated large discrepancies between the consecutive PWV measurements, which are defined by the AHA as discrepancies that are larger than 0.5 m/s, and we found they are prevalent with both of the devices. This fact, along with the seemingly random occurrence of large discrepancies per individual, demonstrates that identifying individuals with a significantly higher probability of large discrepancies is unlikely, and this supports the need for a new PWV estimation protocol. Instead of the sporadic inclusion of a third measurement when a discrepancy is large, the number of measurements should be increased for all of the participants from the current two measurements.

Finally, we identified several factors that influence the amount of variation between consecutive PWV measurements, with a clear difference between the devices in terms of the significant predictors detected. Age, MAP, the outdoor temperature, and the interaction between the outdoor temperature and MAP were all predictors for SphygmoCor. This implies that in the case of a low or high outdoor temperatures, the body's adaptation to the room temperature in the lab may need to be extended beyond the standard 10 min resting time to minimize the differences between the consecutive measurements. For the Arteriograph, however, the predictors included personal characteristics such as BMI, sex,

and hypertension status, which are less directly associated with arterial stiffness than SphygmoCor's predictors are.

4.1. Number of Measurements Used in PWV Estimation

When one is translating the cfPWV research into clinical practice, the issue of the number of measurements used in the PWV evaluation is crucial, not only in the context of the desired precision of the PWV estimation, but also regarding time spent recording, as shorter procedure times would be required to ensure the proper workflow at the physician's office. The evidence supporting the choice of the number of measurements used in PWV estimation, regardless of its importance, is scarce, and it currently relies on a single study [33]. Despite claiming that the reproducibility was assessed, the authors of this study, which included 80% men, took three PWV measurements that were taken using the Compilor device in a single recording session approximately 1 min apart and essentially assessed the effect of the number of measurements on PWV repeatability. Additionally, a poster by Souza et al. evaluated the impact of the number of measurements on the repeatability of PWV measurements taken in the elderly using the SphygmoCor device [45]. To our knowledge, this is the first study to investigate this effect in terms of PWV reproducibility, which were assessed here under a broad range of conditions and in a broad range of participants. Our results show that based on the effect on repeatability, we do not recommend using single or two measurements, including the procedure proposed by the current PWV guidance [22,46] to estimate the PWV. Currently, the optimal precision is observed when four measurements were taken, and the threshold of 1.1 m/s represents the minimal clinically important difference, but these results should be supported with future studies to increase the strength of the evidence and precisely balance the findings with a need to simplify the measurement techniques in clinics and utilize as few measures as possible to decrease the time spent testing.

4.2. PWV Repeatability and Reproducibility: Is There a Difference?

Except for the clinical change in PWV measurements due to underlying pathophysiological mechanism(s), the estimate of PWV reproducibility should account for as many sources of PWV variability as one might reasonably expect to encounter in clinical settings. Having said that, different observers or times of day may also be sources of variation in the data. However, in the context of estimating PWV repeatability, which is defined as the precision of PWV measurements obtained under the same conditions within 24 h, the intra-subject PWV variability that we recorded within 1 day might reflect a less conservative assessment of repeatability. Namely, this estimate might be inflated by additional variability due to different conditions such as different observers or different times of the day. Still, as was shown in our previous study, these sources (which involved the same amount of training for the observers) did not affect the PWV measurements, and they are unlikely to inflate the repeatability [41].

When PWV repeatability from our study was expressed as CV, it was consistent with the previously reported repeatability estimates for both the SphygmoCor [39] and the Arteriograph (Li, Cordes et al. 2014; Ring, Eriksson et al. 2014), including the Li Y et al. study, in which the measurements were taken at different times of the day. Furthermore, our finding, as well as the findings of other studies reporting PWV repeatability using CV, were consistent with the estimates of PWV reproducibility from the three randomized controlled studies that monitored the PWV changes over time [40]. The consistency of all of these CVs demonstrates the difficulty in estimating precision when a relative measure of variability is used for the estimation (CV, but also ICC, Pearson's correlation coefficient, and others), and it may be one of the reasons why repeatability and reproducibility are often confused in studies [33,37–40]. Although some of these studies employed different observers, which may justify their use of the term reproducibility, their definition does not correspond to the AHA's definition of PWV reproducibility for measurements that are taken more than 24 h apart.

Contrary to CV comparisons, when we compared the PWV repeatability and reproducibility using ranges of values estimated from two consecutive measurements, as is currently recommended by AHA, our results demonstrated a clinically important difference of 0.7 m/s between the PWV repeatability and reproducibility. Moreover, the PWV repeatability was good for both of the devices, as just 14% (SphygmoCor) and 13% (Arteriograph) of the recording days presented with a range of wider than $\leq$1 m/s for and just 10% of them had a range of wider than $\leq$1.1 m/s.

4.3. Discrepancies between Consecutive PWV Measurements

To the best of our knowledge, this is the first study in which discrepancies between the consecutive PWV measurements including the occurrence of large discrepancies (>0.5 m/s) and the size of the discrepancies were investigated in more detail. The results altogether point towards important differences between the devices.

We showed that the odds of observing one pair of measurements with a large discrepancy increases with the median value of a person's MAP or DBP for SphygmoCor or HR for Arteriograph, but they are not affected by the experimental or outdoor meteorological conditions we tested or personal characteristics such as age, sex, BMI, or hypertensive status. At least part of the effect of these physiological variables is due to their natural variation within a person. The variability of the BPs increases with the average value of the BP, and it is positively associated with the severity of organ damage and cardiovascular morbidity and mortality in hypertensive patients and the general population [47]. The variability of the HR, on the other hand, results from complex, nonlinear interactions in a number of different physiological systems [48]. On average, both the MAP and DBP deviated within a person by 6 mmHg, whereas the HR deviated by 6 bpm with an intra-subject ranges from 7 to 32 mmHg (for MAP) and from 6 to 30 bpm (for HR). The difference between the devices, in terms of the physiological variables identified, which are significant predictors of a pair of measurements with a large discrepancy, is most likely due to their distinct modes of operation, with the applanation tonometry's cfPWV seemingly providing a more direct estimate of the arterial stiffness due to its association with the BPs [1,49,50].

In line with the abovementioned results, we also identified factors that influence the size of the discrepancy between the consecutive PWV measurements, and we again found a distinct difference in terms of significant predictors detected between the devices. The findings of the SphygmoCor show that this variation is greater in older adults with stiffer arteries. Furthermore, we discovered a significant interaction between a person's MAP and outdoor temperature, implying that the outdoor temperature has a moderating effect on the relationship between MAP and the amount of variation. This finding suggests that in the case of an externally low or high temperature, the body's adaption to the room temperature in the lab may need to be extended beyond the typical 10 min resting time to minimize the differences between the consecutive measurements. Personal characteristics such as BMI, sex, and hypertension status affect the size of differences between the consecutive PWV measurements taken using the Arteriograph. However, these characteristics do not directly link to arterial stiffness, as is the case with the SphygmoCor, whose predictors included age, MAP, and temperature, nor can they be targeted to reduce the amount of variation.

4.4. On Variability in PWV in General

While the variability in the PWV can be an annoyance when one is attempting to determine the true PWV value, and while it affects the resolution of minimal clinically important differences in the PWV, the sensitivity of PWV measurements to the current status of the arterial tree, including the pulse pressure distension due to changes in the BPs, is critical if one wishes to monitor clinical PWV changes over time. In that context, while some devices may appear to be superior in terms of PWV repeatability and reproducibility to others [39], it is questionable how sensitive their mode of operation is to pathological changes. Only the clinical evaluation of these devices in terms of comparisons of the efficacy

of therapies driven by PWV or with PWV as a therapeutic target can determine which device is best one to be used in the clinical context.

4.5. Limitations

The fact that individuals with hypertension were told to keep taking their vasoactive medication as directed throughout the study is one of the potential limitations of the study. The reason for such guidance was that we were interested in the intra-subject variability in the PWV measurements, rather than the absolute PWV levels. That being said, we anticipated that irregular compliance with vasoactive medication would have had a significantly greater impact on our results than taking vasoactive medication throughout the study would have had, as in the latter case the same effect of the medicine is expected on all of the measurements. In addition, in future clinical trials involving PWV, we expect that PWV levels will be evaluated in patients undergoing treatments. The same approach as ours was used in a recent study by Keehn L et al. [40] focusing on longitudinal changes.

The study did not assess the effect of changing therapies on the PWV reproducibility in patients, which could have occurred over a longer follow-up period. Drugs being introduced or changes in dosage after the baseline PWV measurement has been taken (e.g., sartans, which affect the arteries differently than calcium antagonists, statins, or diabetic therapies do) may affect this reproducibility [51,52]. Future studies should investigate these pharmacological effects.

We included measurements that were taken within 1 day but under different conditions—by different observers and at different times of day—when we were estimating the PWV repeatability, which may have inflated the repeatability estimate. However, as previously stated, these conditions did not affect the PWV measurements in our previous study (the observers received an equal amount of training), and we do not expect them to significantly affect the repeatability estimate [41].

5. Conclusions

We showed that the current AHA PWV estimation guidance is suboptimal as the PWV range was outside the 1 m/s threshold, which is a proposed minimal clinically important difference, for most of the participants. We yielded the best reproducibility with a median of for measurements and a 1.1 m/s threshold.

Regarding the PWV reproducibility and repeatability, which are frequently used interchangeably in studies, while the range showed the clinically relevant difference between them, the relative measures of variability were comparable.

We also found that different physiological variables were predictors of the discrepancy between the consecutive measurements, which points toward their distinct modes of operation. Age, MAP, the outdoor temperature, and the interaction between the outdoor temperature and MAP were all predictors for SphygmoCor. This implies that in the case of a low or high outdoor temperature, the body's adaptation to the room temperature in the lab may need to be extended beyond the standard 10 min resting time to minimize the differences between the consecutive measurements. For the Arteriograph, however, the predictors included personal characteristics such as BMI, sex, and hypertension status, which are less directly associated with arterial stiffness than the SphygmoCor's predictors are.

Author Contributions: M.P. and A.J. conceived and designed the study; M.P., B.Š., P.K., V.Đ. and I.M. participated in data collection; M.P. and A.J. analyzed the data; M.B., I.M. and A.J. supervised the data collection and the findings of the study; M.P. and A.J. wrote the draft of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of the University of Split School of Medicine (No. 2181-198-03-04-20-0015, of 10.02.2020.).

Informed Consent Statement: The study was approved by the Ethics Committee at the University of Split School of Medicine, and all participants provided written informed consent.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: Authors declare no conflict of interest.

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Long-Term Adverse Effects of Mild COVID-19 Disease on Arterial Stiffness, and Systemic and Central Hemodynamics: A Pre-Post Study

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Abstract: COVID-19-associated vascular disease complications are primarily associated with endothelial dysfunction; however, the consequences of disease on vascular structure and function, particularly in the long term (>7 weeks post-infection), remain unexplored. Individual pre- and post-infection changes in arterial stiffness as well as central and systemic hemodynamic parameters were measured in patients diagnosed with mild COVID-19. As part of in-laboratory observational studies, baseline measurements were taken up to two years before, whereas the post-infection measurements were made 2–3 months after the onset of COVID-19. We used the same measurement protocol throughout the study as well as linear and mixed-effects regression models to analyze the data. Patients (N = 32) were predominantly healthy and young (mean age ± SD: 36.6 ± 12.6). We found that various parameters of arterial stiffness and central hemodynamics—cfPWV, AIx@HR75, and cDBP as well as DBP and MAP—responded to a mild COVID-19 disease. The magnitude of these responses was dependent on the time since the onset of COVID-19 as well as age ($p_{regression\_models} \leq 0.013$). In fact, mixed-effects models predicted a clinically significant progression of vascular impairment within the period of 2–3 months following infection (change in cfPWV by +1.4 m/s, +15% in AIx@HR75, approximately +8 mmHg in DBP, cDBP, and MAP). The results point toward the existence of a widespread and long-lasting pathological process in the vasculature following mild COVID-19 disease, with heterogeneous individual responses, some of which may be triggered by an autoimmune response to COVID-19.

Keywords: arterial stiffness; central hemodynamics; COVID-19; vascular remodeling; long COVID-19 syndrome; autoimmune response

1. Introduction

In December 2019, the first official case of coronavirus disease (COVID-19) was detected in the Chinese city of Wuhan. Since its first breakout, the virus has swiftly spread over the world, and the World Health Organization (WHO) declared a pandemic in March 2020. At the time of writing, there had been 660,378,145 confirmed cases of COVID-19, with 6,691,495 fatalities globally (1). While COVID-19 was initially thought of as an acute respiratory illness, it is now recognized as a complex multisystemic disease with extensive and deleterious cardiovascular involvement [1,2]. In addition to direct consequences and complications due to acute COVID-19 infection, a recent study showed that 12 months after the onset of COVID-19 infection, up to 25% of patients who were otherwise healthy and free of underlying diseases exhibited the long COVID-19 syndrome [3].

Subclinical myocardial and vascular dysfunction have been linked to worse outcomes and an increased risk of death in patients with COVID-19 disease [4]. Even in patients with mild COVID-19 disease severity, the infection has been linked to impaired subclinical markers of cardiovascular and endothelial function [5]. It is presumed that COVID-19-associated vascular disease complications may be precipitated by direct endothelium damage [6] or immune-mediated vascular damage [7,8]. However, it is unknown to what extent structural alterations of the vascular wall occur in addition to endothelial damage. Even fewer data exist regarding the long-term effects of COVID-19 infection on vascular structure and function. Current fragmented evidence suggests that COVID-19 disease reduces systemic vascular function and increases arterial stiffness [9,10].

Arterial stiffness is a vascular aging phenomenon that refers to a loss of arterial compliance or changes in artery wall characteristics [11]. Arterial stiffness worsens with age and exposure to risk factors that hasten the stiffening process [12,13]. Various measures of arterial stiffness and central hemodynamics can reveal a decline in arterial elasticity brought on by structural wall changes in the arterial system. The most validated and direct measure of arterial stiffness is the carotid–femoral pulse wave velocity (cfPWV) (Townsend, Wilkinson et al., 2015). In addition, augmentation indices are indirect measures of arterial stiffness which are believed to capture the negative impact of systolic wave reflection on cardiac workload [14]. Finally, the central blood pressures refer to the pressure in the ascending aorta. These are the pressures that the target organs are subjected to, and they are lower than brachial cuff pressures due to arterial pressure amplification [15].

We hypothesized that even mild cases of COVID-19 disease could have long-term detrimental effects on arterial structure and function. To investigate this, we examined individual pre- and post-infection changes in arterial stiffness as well as systemic and central hemodynamic parameters in patients diagnosed with mild COVID-19. Baseline measurements were taken up to two years before a participant became infected, and post-infection measurements were taken two to three months after the onset of the disease.

2. Materials and Methods

2.1. Study Design

This is a pre–post study design in which measures of arterial stiffness and central hemodynamic were recorded in a group of participants before and after the COVID-19 infection.

All the recordings were made between October 2019 and April 2022 in the Laboratory for Vascular Aging at the University of Split School of Medicine.

To assess arterial stiffness and central hemodynamic parameters prior to COVID-19 infection, we utilized the stored recordings of enrolled participants from in-laboratory observational studies that applied the same measurement protocol as was used for the post-COVID-19 measurements. The post-COVID-19 measurement was performed between 8 and 12 weeks after the COVID-19 infection had ended, as evidenced by the absence of symptoms. This timeframe corresponded to 50 ± 2 to 90 ± 2 days after the onset of the first

symptoms. The maximum amount of time between pre- and post-COVID measurements was set at 24 months.

2.2. Participants

The participants who had their arterial stiffness and central hemodynamic outcomes measured in our laboratory prior to infection with COVID-19 and who were afterwards infected with the virus were considered eligible for inclusion in the study. For all of those invited to the study, COVID-19 diagnosis was made by real-time Polymerase Chain Reaction test. During their first visit to the laboratory (pre-COVID measurements), all the participants underwent a medical history, and those with arrhythmias, cerebrovascular sickness, pregnancy, surgery amputation, oncology disease, psychiatric disease, infections throughout the trial duration, medical nonadherence, those that were unable to provide fully informed written consent or had any other serious medical condition that may affect data interpretation were excluded from the study.

While this was not originally our inclusion criteria, all of the participants reported mild severity of COVID-19.

In total, we invited 36 adults to participate in our study, with 32 (89%) agreeing to take part. All participants provided written informed consent to participate in the study, which conformed to the Declaration of Helsinki.

2.3. Study Procedures

Before undergoing testing, participants filled out a health history questionnaire, which inquired about personal and family medical history as well as medication use. They arrived for testing in a fasted state having abstained from food, caffeine, or smoking for at least 3 h and from exercise, alcohol and smoking for 24 h before testing. Those taking vasoactive medications (3 or 9%) maintained the same dosage throughout the duration of the study.

All study procedures were carried out in a quiet, temperature-neutral environment with the temperature range of 21–23 °C after participants had lain supine for 10 min.

To avoid possible confounding, each participant was recorded at the same time of day and with the same device during both visits.

2.4. Study Measurements

The arterial stiffness and central hemodynamics measurements were taken in accordance with the American Heart Association's recommendations for improving and standardizing vascular research on arterial stiffness (Townsend, Wilkinson et al., 2015).

Office blood pressure (BP) was measured during each visit using the validated oscillometric sphygmomanometer (Welch Allyn Connex ProBP 3400 digital blood pressure monitor with SureBP technology). The BP measurements were taken in a supine position after 5 min of resting and prior to PWV measurements. The participants did not change body posture between the two measurements.

Carotid–femoral pulse wave velocity (cfPWV); central blood pressures including: central systolic (cSBP) and diastolic (cDBP) blood pressures and pulse pressure (cPP); pulse pressure amplification; augmentation pressure (AP); augmentation indices: AIx calculated as AP/PP, AIx©75—AIx calculated as AP/PP and normalized to the heart rate of 75 beats per minute (bpm), and AIx index calculated as the ratio of late to early systolic pressure P2/P1; and heart rate (HR) were measured by either applanation tonometry using the Sphygmocor CvMS device (Atcor Medical, Sydney, Australia) or by the hybrid applanation tonometer—oscillometric device SphygmoCor Xcel (Atcor Medical, Sydney, Australia), as described previously [16,17]. While the validation studies comparing two devices indicated that they were comparable in terms of assessment of carotid–femoral pulse wave velocity (cfPWV) and augmentation index (AIx) [18,19], each participant was

recorded using only one device to ensure that intra-individual changes are not affected by the type of device used.

A single operator (M. P.) carried out all of the measurements. For cfPWV measurements, recordings were performed on the right carotid and the right femoral artery. Central BPs and other parameters derived from the pulse wave analysis (PWA) were estimated after calibration of the pulse waveform recorded at the radial artery to mean and diastolic brachial pressures. We used the subtracted distance method to calculate the wave travel distance. The method was chosen over the direct method as per recommendation by the latest guideline [20].

2.5. Sample Size Considerations

Thirty-two participants are sufficient to detect moderate to strong effects on parameter changes using a simple linear regression model and strong effects when two-predictor linear regression model is used. Namely, using a two-predictor multiple linear regression model with $\alpha = 0.05$, $f^2 = 0.35$, and power of 80%, a sample size of 31 is required to detect a strong association between pre–post changes in vascular function/structure and potential predictors. For estimations based on a simple linear regression model, the sample size of 32 was sufficient to detect moderate to strong associations under assumptions of $\alpha = 0.05$, $f^2 = 0.27$, and power of 80%.

2.6. Data Analysis

To describe the distribution of a quantitative variable, we used mean and standard deviation (SD) or median and interquartile range (IQR), depending on the shape of the distribution. To decide if a distribution is asymmetrical, we used skewness and kurtosis tests for normality. The distribution of a qualitative variable was described with absolute and relative frequencies.

We utilized one-sample tests to determine whether a pre–post change in a parameter was statistically significant: either the parametric one-sample t-test or its non-parametric counterpart, the sign rank test, depending on the symmetry of the variable's distribution.

There were two sets of the regression models developed. We employed simple or multiple linear regression (MLR) models to identify predictors of the change from baseline (pre-COVID) for different arterial stiffness and hemodynamic parameters. These models were preferred as they use individual pre–post changes as the dependent variable. In addition, we built mixed-effects regression models to identify predictors affecting the values of a modeled parameter. Due to the fact that mixed-effects regression models employ repeated measurements of a parameter, these models had greater analytical power than simple or MLR models.

The model building was performed in two steps. In the first step, potential predictors—including age, sex, the amount of time that passed since the start of COVID infection, the amount of time that passed between the pre- and post-COVID-19 measurements, pre-COVID baseline values of a modeled parameter, and the type of device used to estimate its values—were used as single predictors in a simple linear regression or a mixed-effects model. For the final model, only those predictors that were significant at the 0.1 level or higher were considered ($p \leq 0.01$). Requirements for inclusion in the final model were significance at the 0.05 level or an increase in adjusted R^2 of at least 2% and a p-value of less than 0.2.

The above-mentioned potential predictors that were initially evaluated were selected to estimate the dependence of parameter values on the time that passed since the COVID infection and account for potential confounding variables. As an example, even though we did not anticipate any significant changes in vascular function over a 24-month period in the predominantly young participants (Table 1), we included the time between the first and second measurements as a potential predictor to control for its confounding effect.

We interpreted the strength of association between a predictor and a modeled parameter by applying the Cohen's effect size magnitudes for R^2 (small from 0.02 to <0.13,

medium from 0.13 to <0.26, large from ≥0.26) to the adjusted R^2 of a single predictor model. [21]

As this is an exploratory study, no control for multiple testing was performed. The analysis was performed in STATA (version 17.0, Stata Corp LP, College Station, TX, USA). We applied the significance level of $p = 0.05$.

Table 1. Demographic and clinical characteristics of participants, N = 32.

Characteristic	Statistics
Sex, N (%)	
Male	18 (56%)
Female	14 (44%)
Age (years), mean ± SD	36.6 ± 12.6
BMI, median (IQR)	28 (24.5 to 31.4)
Hypertension, N (%)	3 (9%)
Diabetes, N (%)	2 (6%)
Dyslipidemia, N(%)	0 (0%)
Familial history of CV disease, N (%)	7 (22%)
Smoking, N (%)	
No	17 (53%)
Yes	7 (22%)
Ex-smoker	8 (25%)
Smoking [cigarettes per day], median (range) *	5–10 cigarettes (1–10)

BMI—body mass index; * Calculated on N = 7 smokers.

3. Results

In this study, 32 participants were recruited; each participant visited the laboratory twice; and all of their data were collected. Table 1 shows their demographic and clinical characteristics at baseline prior to the COVID-19 infection. The participants were predominantly young (≤40 years) and healthy with only 9% (n = 3) of the cohort being hypertensive. None of the participants had dyslipidemia, and only two had diabetes (6%, one person was also hypertensive). The majority of the cohort was overweight or obese (69%) and did not smoke (78%).

The average time since the onset of COVID-19 infection in our sample was mean ± SD: 73 ± 10 days, with this time ranging from 51 to 92 days. The median time that elapsed between two measurements was 327.5 days (IQR, 129 to 458), with the range between 74 and 730 days. The majority of participants, 23 or 72%, were recorded with the SphygmoCor XCEL device.

In terms of the severity of COVID-19 infections, none of our participants have developed any of the cardiovascular, pulmonary, thromboembolic, or other COVID-19-associated complications, and there were no hospitalizations. Participants were evenly distributed according to the year they became infected (chi-square test, $p = 0.084$). There was no significant pre–post change in weight: median change 0 kg, 95% CI from −0.3 to 0.5.

When we looked to see if the mean individual pre–post changes were significantly different from 0, we found no significant pre–post change in any of the arterial stiffness or hemodynamic parameters tested ($p \geq 0.122$). We did, however, see an average increase of 0.19 m/s in carotid–femoral pulse wave velocity (cfPWV) from pre-infection values but at the significance level of 0.1 ($p = 0.052$). Table 2 shows the distribution of vascular parameters at baseline and after the infection.

Table 2. Distribution of arterial stiffness, and central and systemic hemodynamic parameters at baseline (pre-infection) and 2-3 months after the onset of COVID-19 disease (post-infection), N = 32.

Parameter	Pre-Infection	Post-Infection
Systemic Hemodynamics		
SBP (mmHg), mean ± SD	120 ± 9	119 ± 9
DBP (mmHg), mean ± SD	70 ± 8	71 ± 9
MAP (mmHg), mean ± SD	86 ± 8	85 ± 10
PP (mmHg), median (IQR)	47 (43, 54)	47 (43, 51)
HR (bpm), mean ± SD	65 ± 10	64 ± 7
Central Hemodynamics		
cSBP (mmHg), mean ± SD	107 ± 7	107 ± 9
cDBP (mmHg), mean ± SD	71 ± 8	72 ± 9
cPP (mmHg), mean ± SD	36 ± 6	35 ± 6
Carotid–Femoral Pulse Wave Velocity		
cfPWV (m/s), mean ± SD	6.3 ± 0.7	6.5 ± 1.0
Pulse Wave Analysis		
Aortic Augmentation (mmHg), mean ± SD	7 ± 5	7 ± 6
Aortic AIx, P2/P1 (%), mean ± SD	19% ± 13%	20% ± 16%
Aortic AIx, AP/PP (%), mean ± SD	123% ± 13%	126% ± 19%
Aortic AIx@HR75, P2/P1 (%), mean ± SD	15% ± 14%	15% ± 17%

AIx, augmentation index; AIx@HR75, augmentation index corrected for HR; cDBP, central diastolic blood pressure; cPP—central pulse pressure; cSBP, central systolic blood pressure; BP, blood pressure; cfPWV, carotid–femoral pulse wave velocity; DBP, diastolic blood pressure; HR, heart rate; IQR—interquartile range; MAP—mean arterial pressure; P1, first systolic peak; P2, second systolic peak; PP—pulse pressure; SBP, diastolic blood pressure; SD—standard deviation.

Further analysis, however, revealed a widespread and complex pattern of confounders that affect the pre–post infection changes, and parameter values, in the majority of the assessed arterial stiffness and hemodynamic parameters (Tables 3 and 4). The size of these changes, as well as the direction in which they went, were dependent not only on the length of recovery time that had passed since the onset of the COVID-19 infection, confirming the existence of a response to the COVID-19 infection, but also, and more commonly, on cardiovascular health status at baseline (ascertained by age of an individual at baseline or the value of a parameter at baseline), as well as the amount of time that had passed between the two measurements. In accordance with the Cohen's interpretation of R^2, the majority of identified associations, regardless or the type of model, were moderate to strong [21].

Table 3. The predictors of the pre–post COVID-19 changes in systemic and central hemodynamic parameters and arterial stiffness parameters, as estimated by the linear regression models.

Pre–Post Change in:	Predictor	B (95% CI)	*p*-Value	Adjusted Simple Model R^2	Adjusted Final Model R^2
Systemic and Central Hemodynamics					
SBP (mmHg)	Baseline value *	−0.46 (−0.68 to −0.24)	<0.001	21 §	21 §
DBP (mmHg)	no significant model				
PP (mmHg)	Baseline value	−0.35 (−0.68 to −0.02)	0.041	26 §§	30 §§
	Device, XCEL vs. CvMs ¶	4.16 (−0.02 to 8.34)	0.051 †	12	
MAP (mmHg)	no significant model				
cSBP (mmHg)	Baseline value	−0.24 (−0.44 to −0.03)	0.026	3	3
cDBP (mmHg)	no significant model				
cPP (mmHg)	Baseline value	−0.36 (−0.71 to −0.18)	0.040	12	12
Carotid–Femoral Pulse Wave Velocity					
cfPWV (m/s)	no significant model				
Pulse Wave Analysis					
Aortic AP (mmHg)	Age	0.11 (0.02–0.21)	0.023	7	7
Aortic AIx, AP/PP (%)	Age	0.003 (0.0008–0.006)	0.013	10	10
Aortic AIx, P2/P1 (%)	Age	0.005 (0.002–0.008)	0.001	18 §	33 §§
	Time between measurements	0.0003 (−0.00003, 0.0005)	0.076 †	17 §	
Aortic AIx@HR75 (%)	Time from COVID	0.004 (0.001–0.006)	0.003	20 §	26 §§
	Age	0.002 (−0.0001, 0.004)	0.061 †	10	

AIx, augmentation index; AP, aortic augmentation pressure; B, unstandardized regression coefficient; cDBP, central diastolic blood pressure; cfPWV, carotid–femoral pulse wave velocity; cPP, central pulse pressure; cSBP, central systolic blood pressure; P1, first systolic peak; P2, second systolic peak; PP, pulse pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; SBP, diastolic blood pressure; * Refers to the pre-COVID value of a predictor; † The predictor is not significant, or is significant at 0.1 level, but its inclusion in the multiple linear model increased adjusted R^2 from 2 to 15%; § moderate and §§ strong association of pre–post changes with time from COVID or confounders, in accordance with the Cohen's effect size magnitudes for R^2 [21]; ¶ Variable is strongly correlated to the amount of time that passed between two measurements.

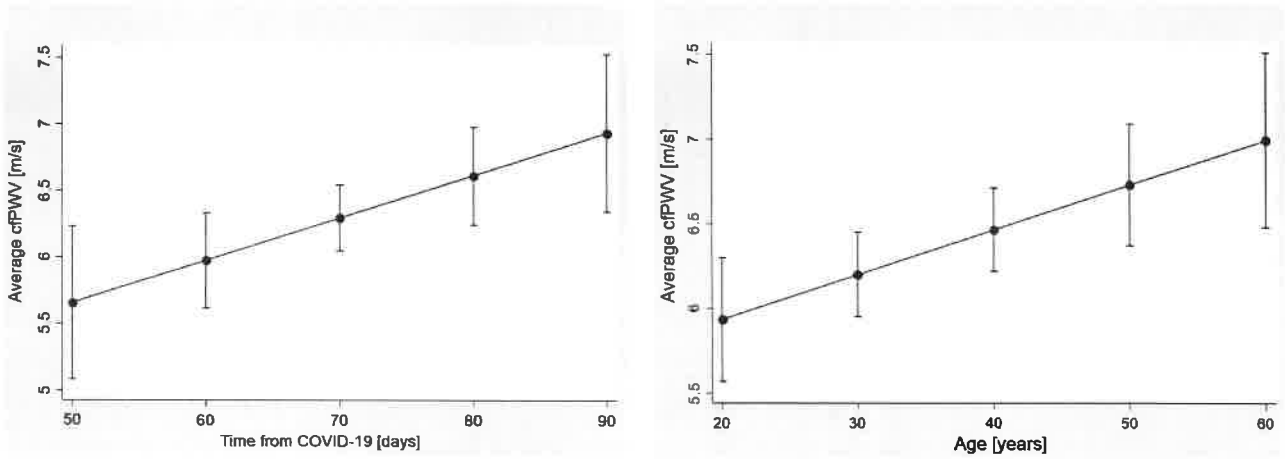
Table 4. Predictors of values of systemic and central hemodynamic parameters, as well as arterial stiffness parameters, estimated by the mixed methods regression models.

Measure:	Predictor	B (95% CI)	*p*-Value	The One-Predictor Model	The Final Model
				Snijders/Bosker's R^2 Level 1, Level 2 ¶	
Systemic and Central Hemodynamics					
DBP (mmHg)	Time from COVID	0.20 (−0.01, 0.41)	0.063 †	8%, 9%	29% §§, 32% §§
	Age	0.32 (0.13, 0.51)	0.001	24% §, 27% §§	
cDBP (mmHg)	Time from COVID	0.19 (−0.02, 0.39)	0.082 †	7%, 8%	28% §§, 31% §§
	Age	0.33 (0.13, 0.52)	0.001	24% §, 27% §§	
MAP (mmHg)	Time from COVID	0.19 (−0.04, 0.42)	0.113 †	7%, 8%	31% §§, 34% §§
	Age	0.35 (0.17–0.53)	<0.001	27% §§, 30% §§	
Carotid–Femoral Pulse Wave Velocity					
cfPWV (m/s)	Time from COVID	0.03 (0.003, 0.05)	0.030	13% §, 15% §	28% §§, 32% §§
	Age	0.03 (0.008, 0.05)	0.005	18% §, 21% §	
	Pre–post change in cfPWV	0.19 (−0.03, 0.40)	0.094 †	1%, 0%	

B, unstandardized regression coefficient; cDBP, central diastolic blood pressure; cfPWV, carotid–femoral pulse wave velocity; DBP, diastolic blood pressure; MAP, mean arterial pressure; † The predictor is not significant or is significant at 0.1 level, but its inclusion in the mixed model increased R^2; § moderate and §§ strong association of parameter values with time from COVID or confounders, in accordance with [21]; ¶ Level 1 defines how well the model describes changes at the level of the entire sample, whereas Level 2 depicts how well the model describes individual changes.

3.1. Arterial Stiffness—cfPWV

Regarding the cfPWV response to COVID-19 infection, defined as the pre–post change in this parameter, we found that post-infection values increased by 0.19 m/s (95% CI −0.04 to 0.41) but only at the significance level of 0.1 ($p = 0.052$). We also found no evidence that age, time since the onset of COVID 19, time between measurements, or cfPWV baseline values influence individual cfPWV responses. Individual pre–post changes were also significant at the 0.1 level according to the mixed-effects model (Table 4). However, age and time were moderately and positively associated with the cfPWV change since the onset of COVID infection at a group level. This model, which explains 32% of the intra-individual variability and 28% of variation at the group level, predicts an increase of 1.14 m/s in the average cfPWV value as a result of variation in the time since the onset of COVID-19 infection (51–92 days). The relationship between cfPWV and two predictors is shown in Figure 1. Although the age dependence is to be expected, we included it for comparison purposes. The change in cfPWV was not determined by the pre–post change in HR or the baseline HR value, nor was it affected by the change in BMI or the baseline BMI value ($p \geq 0.308$).



(a) (b)

Figure 1. The relationship between the average cfPWV values in the sample and: (**a**) the time since COVID infection; (**b**) age, as estimated with the mixed-effects model (R^2=27% at the level of sample, R^2=31% for individual changes). Shown are predictive margins of cfPWV values with 95% CIs.

3.2. Arterial Stiffness—Augmentation Indices

As previously stated, no significant pre–post changes in augmentation indices were observed following the COVID-19 infection ($p \geq 0.244$). However, we discovered that the pre–post changes increased with age in AP and all of the AIx indices: Aix AP/PP, Aix P2/P1, and AIx@HR75 (Table 3). Except for the pre–post change in Aix P2/P1, which was moderately associated with age, this dependency was generally weak (Table 3).

Aside from age, which was found to be a common predictor of AIx pre–post changes, we discovered additional time-related predictors of these changes in Aix P2/P1 and AIx@HR75 indices.

Time since the onset of COVID-19 infection was a moderate and positive predictor of pre–post changes in the AIx@HR75, accounting for 20% of their variance (Table 3, Figure 2). Within a range of 51 to 92 days after the onset of COVID infection, the AIx@HR75 pre–post change was predicted to move from −5% to +10%.

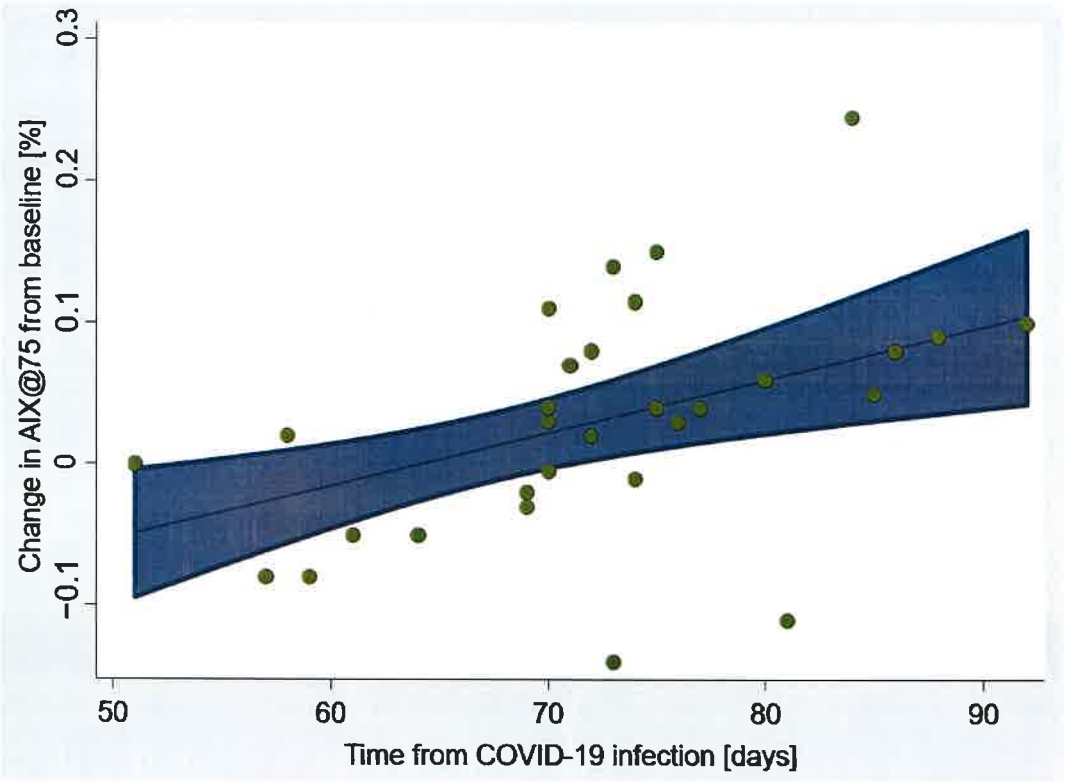


Figure 2. The change in AIx normalized to the heart rate of 75 bpm (AIx@HR75) from the baseline depends on the time that passed from acquiring COVID-19 infection. Shown are predictive margins with 95% CI estimated by the LR model (R^2=26%) and the scatter plot of observation.

3.3. *Peripheral and Central Hemodynamics*

We found no significant changes from baseline in any peripheral or central hemodynamics parameters ($p \geq 0.122$).

Pre–post changes from baseline in SBP, cSBP, PP, and cPP were negatively dependent on their baseline values (weak—cSBP and cPP, moderate—SBP, strong—PP; Table 3). For example, given the range of baseline values for the PP parameter, the predicted post-COVID increase in PP ranges from +4 to -11 mmHg. It should also be noted that we also found a significant association of pre–post changes in PP with the time from onset of COVID-19, but at a 0.1 level of significance (p = 0.098). The variable was not included in the final model of pre–post PP changes because it did not meet our protocol's inclusion requirements.

Using the mixed-effects models, we were able to identify that age, and to a lesser extent the time since acquiring COVID-19, were positively associated with parameter values for DBP, cDBP, MAP, and cfPWV (Table 4). According to these models, the estimated change in average pressure values caused by variations in the amount of time that has passed since the beginning of the COVID-19 infection is as follows: DBP is envisaged to increase by 8.1 mmHg, cDBP by 7.6 mmHg, and MAP by 7.6 mmHg. As for individual pre–post changes, we were unable to identify significant mean changes nor the associations of these changes with any of the predictors listed above, nor were we able to find significant individual pre–post changes within mixed-effects models. Such a finding suggests that individual changes are likely heterogeneous and that that the effect that we identified at the group level is probably an average effect.

3.4. The Age Dependence of Pre-Post Changes in Investigated Parameters

We examined the pattern of pre–post changes in age dependence to see if there is a possibility that age modifies responses to COVID-19 infection. The scatter plots in Figure 3a–c depict a distinct pattern for those aged under and over 40. We demonstrated that the change in AIx P2/P1 from baseline for those over 40 years old is significantly greater than 0 (median 6%, 95% CI 0.7–24%, $p = 0.005$), whereas no significant change was observed for those under 40 years old ($p = 0.976$) (Figure 3a).

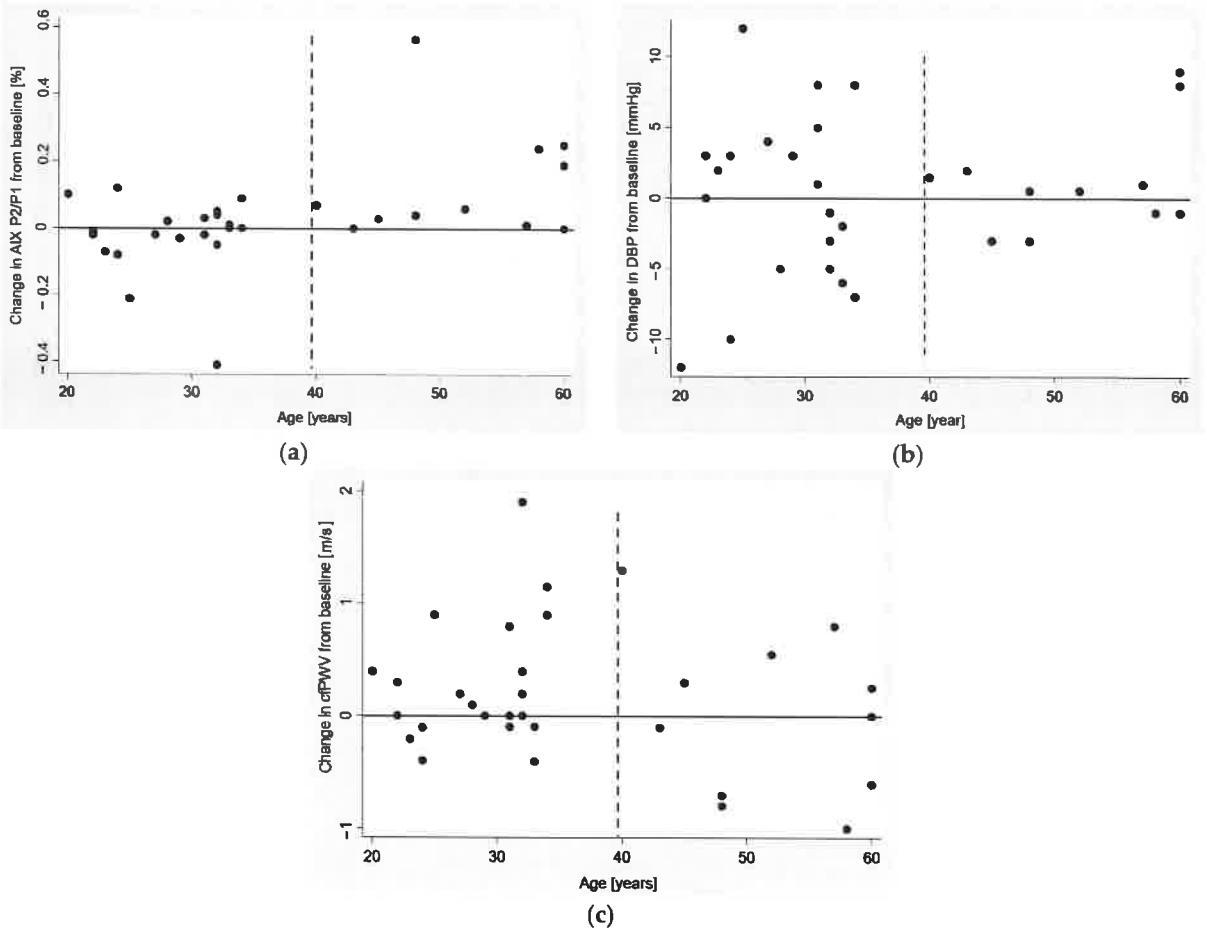


Figure 3. Scatter plots showing the relationship between age and pre–post change in: (**a**) augmentation index AIx P2/P1, (**b**) diastolic blood pressure, and (**c**) cfPWV. The horizontal reference line set at zero pre–post change, and the vertical dashed line set at 40 years that separates parts of a plot with apparently different dispersion patterns.

4. Discussion

This is the first study to compare pre- and post-COVID-19 infection levels across a wide range of arterial stiffness and hemodynamics parameters in the same group of participants. We discovered that the responses of the vascular system to a mild COVID-19 disease, defined here as systematic, individual pre–post differences in investigated parameters, are not simple in the sense that COVID-19 on average either increases or decreases a parameter in infected patients by a comparable amount of measurement units. In fact, except for a non-significant trend for cfPWV, we were unable to detect any parameter with a mean pre–post COVID-19 change that differed from 0.

Instead, responses to COVID-19 infection are dynamic and depend on the time since the onset of COVID-19 infection. We identified such time-dependent responses in the arterial stiffness parameters—cfPWV and AIx@HR75, the central hemodynamic

parameter—cDBP, and the systemic hemodynamics parameters—DBP and MAP; and we showed that their values increased with the length of time that passed from the onset of COVID-19 infection, independent of age or other confounders. In addition, the vascular impairment predicted by our models for observation period of two to three months post-infection is clinically significant as shown by an increase in cfPWV of +1.4 m/s, +15% in AIx@HR75, +8 mmHg in DBP, and +7.6 mmHg in cDBP and MAP.

The finding that the longer the period from COVID-19 infection the worse the vascular impairment was surprising, as we expected inflammation burden associated with COVID-19 to decrease with time. While we can only speculate on what causes this phenomenon, emerging evidence suggests that it stems from a failure to resolve autoantibodies observed during the acute phase of disease [22–24], or alternatively, that generating de novo pathogenic autoimmune responses post-recovery contributes to long COVID with evidence of residual inflammatory cytokines [25–27]. Hence, what we observed at the group level, 2–3 months after infection, may be related to inflammation-induced arterial stiffening in some individuals [28], which is caused by inflammation from an autoimmune response or chronic inflammation that precedes one [29]. Furthermore, the heterogeneous responses observed in our study could be explained by the fact that inflammation in post-recovery was not triggered in all patients. Indeed, a recent study found that the circulating levels of anti-/extractable nuclear autoantibodies (ANA/ENAs) were higher at 3 months post-recovery in patients who had COVID-19 and were free from autoimmune diseases at the time of infection compared to healthy and non-COVID infection groups. High circulating ANA/ENA titers, which correlated with long COVID symptoms, were maintained up to 6 months after recovery but significantly reduced by 12 months. Even after 12 months, several pathogenic ANA/ENAs were still detectable in up to 30% of COVID survivors. Furthermore, a retrospective study of 4 million participants found an increased risk of autoimmune diseases in patients with COVID-19 with an adjusted hazard ratio for different autoimmune diseases ranging from 1.78 to 3.21 [30].

All the time-dependent responses to COVID-19 disease were also affected by age in a way that each additional year at baseline added to vascular impairment post-infection. The effect of age was not the result of the time gap between pre- and post-COVID-19 measurements, as this confounding variable was controlled for in our analyses. In addition, we could not assign the effect of age only to the increased variability of investigated parameter with age [16], because in that case, pre–post differences would go in both directions—positive and negative, and we would not be able to find an increasingly positive relationship with age. Age, however, may modulate the response to a mild COVID-19 disease in arterial stiffness and central hemodynamics parameters in different age groups. Previous studies have suggested an age modulation of vascular responses to various triggers, including an infection [31,32], and the association of age with autoimmune inflammation [33]. While our results suggest the role of age as a modifiable factor in the response to mild COVID-19 disease, such a role should be further examined in studies with a larger sample size.

We detected the responses to COVID-19 disease in a variety of arterial stiffness measures and measures of its hemodynamic consequences, including: the direct (cfPWV) and indirect (augmentation index) measures of arterial stiffness as well as central (cDBP) hemodynamic parameter. Each of these three parameters represents a distinct aspect of the atherosclerotic process, which involves morphological and/or functional alterations to the vessel wall [34]. Therefore, the simultaneous detection of responses to COVID-19 disease in various vascular structure and function parameters supports the existence of a widespread and long-term pathological process in the vasculature following infection [35].

So far, only a handful of studies investigated the effect of COVID-19 infection on arterial stiffness and central hemodynamics. Most of them were case control studies with small sample sizes (10–22 per arm) comparing patients recovering from COVID-19 with

controls [4,9,10]. Despite the possibly limited power of these studies, their results support our conclusions regarding the existence of vascular impairment after COVID-19.

The fact that cfPWV is increased in participants after the COVID-19 infection when compared to controls was found in several studies performed on: young healthy patients and their controls 3–4 weeks after the onset of COVID-19 (increase of 0.7 m/s) [9], acutely ill elderly patients (increase of 3.3 m/s) [4], as well as middle-aged patients that were compared to controls at 4 months (increase of 2.05 m/s) [36] and 12 months (increase of 1.15 m/s) after the COVID-19 onset [37].

Aix, like cfPWV, has been found to be higher in COVID-19-infected participants compared to controls. A 10% increase in the augmentation indices AIX AP/PP and AIx@HR75 has been reported in those infected with COVID19 when comparing 15 young adults 3–4 weeks after a positive COVID-19 test to healthy young controls. [10].

Finally, in terms of cSBP, at the 4- and 12-month follow-ups, COVID-19 patients have had a persistent increase of 10 mmHg in cSBP compared to controls [36,37]. In addition, Akpek et al. [38] reported an increase in systemic hemodynamics parameters during short-term follow-up in patients diagnosed with COVID-19.

Only one case control study did not find significant differences in arterial stiffness parameters—PWV and AIx75—at 4 weeks post-infection when young adults who were infected with COVID-19 were compared with their controls [39]. In addition, two small longitudinal studies reported results contradicting our findings. In the first study that followed 14 young participants from the first to sixth month post-infection, the authors reported a decrease in cfPWV (decrease by 0.82 m/s), SBP (by 11 mmHg), MAP (by 11 mmHg) with time; and no change in time was found for AIx@HR75 [40]. The second study followed 10 young adults for 6 months after the COVID-19 infection and found that SBP and DBP decreased throughout the study: with SBP decreasing by 15 mmHg and DBP decreasing by 10 mmHg [41]. Given that both of these longitudinal studies reported participant attrition on very small sample sizes, used inappropriate statistics (mean and standard deviation) to describe the distribution of limited data, and removed outliers from a small sample size [40], the reported results could be the result of methodological issues. On the other side, the lack of a uniform individual response to COVID-19 in the investigated parameters of vascular structure and function, which may be the consequence of age modulation (and possibly modulation by other factors), may have caused such results.

Our study had some limitations, the most significant of which was that the sample size only allowed us to detect moderate to strong associations. This means that even though we may have confidence in the significant associations observed in our study, we may have overlooked a relationship between the time since the onset of COVID-19 and several other parameters. For example, pre–post changes in PP were significantly associated with the time from COVID at the lower significance level of 0.1. As our sample size was limited by the number of recent pre-COVID recordings in our laboratory, we were not able to expand the sample size further.

Another potential drawback is the possibility that age and perhaps other factors relate to moderate responses to COVID-19 and that certain patient subgroups respond differently to COVID-19 than it was predicted in the overall model for that parameter. The fact that our models did not detect that individual pre–post changes in the tested parameters are significantly different from 0 but were able to detect in the mixed-effects models that parameter values depend on time from COVID-19, at the level of the entire sample, suggests that heterogeneous responses are possible. If this was the case, given that our results suggested an average response to COVID-19 disease, further analyses should be performed in studies with larger sample sizes.

Finally, because MAP in our study was estimated rather than directly measured, its estimation may be unreliable for some individuals [42], which could affect the accuracy of parameters derived from pulse wave analysis. However, since we were able to identify

general patterns of change and interdependence, we do not expect this had a significant impact on the results.

The findings of this study demonstrated that there is a widespread and long-lasting pathological process in the vasculature following the mild COVID-19 infection which keeps deteriorating during 2–3 months post-infection. In light of the recent finding that up to 25% of otherwise healthy and disease-free patients exhibited the long COVID-19 syndrome 12 months after the onset of COVID-19 infection [3], and in light of the fact that vascular impairment increases the risk of future cardiovascular events, it is crucial that future studies explore these changes with larger sample sizes and with more synchronous population regarding the onset of COVID-19.

5. Conclusions

We found that various parameters of arterial stiffness and central hemodynamics respond simultaneously to the mild COVID-19 disease in predominantly healthy individuals. While we were unable to demonstrate this effect on all of the parameters tested, the worsening of values of those found to be responsive (cfPWV, AIx@HR75, cDBP, DBP, and MAP) points toward the existence of a widespread and long-lasting pathological process in the vasculature following the infection.

The detected responses to COVID-19 disease are not straightforward but rather deteriorate with the time since the onset of COVID-19 infection and age.

Within the period of 2–3 months following infection, our models demonstrated a clinically significant progression of vascular impairment.

Finally, we discovered that individual responses to COVID-19 are likely heterogeneous and possibly moderated by age.

Emerging evidence suggests that post-recovery autoimmune response to COVID-19 may be the cause of this phenomenon, although we can only speculate on its origin.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author A.J.

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