Pulse wave imaging as an indicator of arterial stiffness in patients affected with periodontitis

Imagen de onda de pulso como indicador de la rigidez arterial en el paciente periodontal

Imatge de l'ona del pols com indicador de rigidesa arterial en el pacient periodontal

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Patologia i Terapèutica Odontològica i Maxil·lofacial

PULSE WAVE IMAGING AS AN INDICATOR OF ARTERIAL STIFFNESS IN PATIENTS AFFECTED WITH PERIODONTITIS

IMAGEN DE ONDA DE PULSO COMO INDICADOR DE LA RIGIDEZ ARTERIAL EN EL PACIENTE PERIODONTAL

IMATGE DE L'ONA DEL POLS COM INDICADOR DE RIGIDESA ARTERIAL EN EL PACIENT PERIODONTAL

Memòria presentada per *Elena C. Sanz Miralles* per optar al grau de doctor/a per la Universitat de Barcelona

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PWV	Pulse Wave Velocity
aPWV	Aortic PWV
baPWV	Brachial PWV
PWI	Pulse Wave Imaging
IMT	Intima and media thickness
cIMT	Carotid IMT
FMD	Flow-mediated dilation
NMD	Nitroglycerin-mediated dilation
PP	Pulse pressure
PPA	Pulse pressure amplification
PWUM	Pulse Wave Ultrasound Manometry
Aix	Augmentation index
MAP	Mean arterial pressure
CAV	Cardio-ankle vascular index
BP	Blood pressure
CBP	Central blood pressure
LVMI	Left ventricular mass index
ASI	Arterial stiffness index
ABI	Ankle brachial index
CT	Computed tomography

MRI	Magnetic resonance imaging
CV	Cardiovascular
ASVD	Atherosclerotic vascular disease
CVD	Cardiovascular disease
CHD	Coronary heart disease
CAD	Coronary artery disease
ACS	Acute coronary syndrome
CVA	Stroke and cerebral vascular accident
AAA	Abdominal aortic aneurysm
MI	Myocardial infarction
DM	Diabetes mellitus
HBP	High blood pressure
Pg, P. gingivalis	Porphyromonas gingivalis
LPS	Lipopolysaccharides
NO	Nitrous oxide
CPR	C reactive protein
TNF	Tumor necrosis factor
IL-	Interleukin
INF	Interferon
MMP	Matrix metalloproteinase
MCP	Monocyte chemotactic protein
TLR	Toll-like receptors
HSP	Heat shock proteins

Gro-El	Chaperonin 60
NF-κB	Nuclear factor- κB
Th	T helper
SMC	Smooth muscle cells
TGF	Transforming growth factor
LDL	Low-density lipoproteins
hFH	Heterozygous familial hypercholesterolemia
PD	Periodontal Disease
LOA	Loss of attachment
CAL	Clinical attachment level
BoP	Bleeding on probing
NHANES	National Health and Nutrition Examination Survey
UEIL	Ultrasound and Elasticity Imaging Laboratory
RCT	Randomized clinical trial
cm/sec	Centimeters/second
r	Linear regression
SD	Standard deviation
n	Sample size
OR	Odds ratio

ABSTRACT - SÍNTESIS (CASTELLANO)

En esta tesis doctoral hemos investigado la relación entre el estatus periodontal y la rigidez arterial, determinada mediante la utilización de una nueva técnica llamada Imagen de Onda de Pulso (PWI). PWI es una técnica basada en ultrasonidos que ha sido desarrollada por integrantes de nuestro equipo y permite determinar la Velocidad de Onda del Pulso (PWV) y valorar la homogeneidad de la transmisión de dicha onda (R2). A su vez, se realizó un estudio de factibilidad en la determinación de la Presión de Pulso (PP) central en una arteria central de gran calibre de manera no invasiva mediante el uso de la misma tecnología.

Se llevó a cabo una investigación transversal en una muestra de 80 voluntarios sin antecedentes de enfermedad cardiovascular exceptuando hipertensión, de los cuales 40 presentaba enfermedad periodontal moderada-severa, definida por la presencia de un mínimo de 2 dientes/cuadrante con profundidades de bolsa (PD)>5mm, pérdida de inserción (CAL) concomitante >3mm y sangrado al sondaje (BoP) en >30% de las localizaciones. El grupo control fue constituido por una muestra pareada con los casos en cuanto al género y la edad (± 5 años) y periodontalmente se definió por la ausencia de PD >4mm y CAL interproximal >2mm. Los pacientes se sometieron a un examen periodontal completo y el grado de rigidez arterial de la carótida derecha e izquierda

fueron evaluadas para determinar las variables resultado PWV y R2. Debido al hecho de que PWI es una técnica nueva, se utilizaron dos umbrales para analizar los resultados obtenidos. El primero (umbral A) fue diseñado como un umbral genérico mientras que el segundo (umbral B) fue individualizado para cada paciente, ya que se calculó a partir de los resultados obtenidos de la medición de todos los ciclos cardiacos recogidos para cada individuo. En lo que respecta al estudio de factibilidad en el cálculo de la PP, se realizó en una submuestra de pacientes clasificados conforme sus niveles de presión arterial braquial en normotensos, pre-hipertensos e hipertensos; calculándose la PP en tres localizaciones diferentes del árbol arterial (arteria braquial, radial y aorta) mediante la utilización de tres técnicas diferentes (esfigmomanómetro, tonometría de aplanación y PWI respectivamente). Los resultados de PWV y R2 obtenidos en las muestras pareadas de pacientes periodontales y controles fueron comparados mediante el test de Wilcocon (Wilcoxon Signed Rank Test) con la finalidad de explorar diferencias entre los grupos. También se realizaron análisis univariables y multivariables para analizar la asociación entre PWV y R2 y otras variables potencialmente explicativas. Por otra parte, los resultados de PP fueron comparados mediante el test de ANOVA bilateral y la corrección de Bonferroni.

La aplicación del umbral A permitió la formación de 30 pares de casos-controles, basados en género y edad (± 5 años), mientras que la aplicación del segundo umbral

(umbral B) resultó en 33 pares. Los valores resultantes tras la aplicación del umbral A no revelaron diferencias estadísticamente significativas en cuanto a PWV (mediana de PWV de 2.37m/seg vs. 2.64m/seg en periodontitis y controles respectivamente, p=0.74) ni en cuanto a la variable resultado R2 (0.74 vs. 0.71, respectivamente, p=0.81). El análisis univariante reveló asociaciones negativas no significativas entre R2 y las variables: presencia de periodontitis (p=0.60), edad (p=0.87), presión arterial sistólica (p=0.62), presión arterial diastólica (p=0.42) y las variables continuas relacionadas con la presencia de periodontitis. En cuanto al análisis de regresión multivariable, solo se detectaron diferencias significativas respecto a la variable resultado R2 y género (p=0.04). La aplicación del umbral individualizado (umbral B) muestra algunas diferencias respecto a los resultados tras aplicar el umbral genérico (umbral A). En este caso, los pacientes con periodontitis mostraron valores menos harmónicos de transmisión de la onda del puso (R2) respecto a los pacientes control (p=0.01), mientras que no se hallaron diferencias significativas en cuanto a los resultados obtenidos para PWV entre los dos grupos. El análisis univariable mostró una asociación significativa negativa entre R2 y las variables periodontitis, uso de tabaco y las variables incluidas relacionadas con la condición periodontal. En cuanto al análisis multivariable, la única asociación estadísticamente significativa fue la establecida entre R2 y la variable periodontitis. Por otra parte, en los resultados derivados de la porción del estudio dedicada a estudiar las diferencias de la PP, se observaron diferencias estadísticamente significativas en los valores obtenidos al evaluar la PP en la aorta en el grupo de pacientes hipertensos (siendo éstos más

elevados), mientras que las diferencias en los resultados obtenidos en la arteria radial y braquial no fueron significativas entre los diferentes grupos de pacientes.

Podemos concluir que se observó un menor grado de uniformidad en la transmisión de la onda del puso en la arteria carótida en una muestra de pacientes periodontales sin antecedentes de enfermedad cardiovascular. Esto sugiere una asociación entre periodontitis y una alteración funcional resultando en una disminución de la elasticidad arterial. Para el estudio de la onda de pulso se utilizó una técnica novedosa, recientemente desarrollada por miembros de nuestro equipo que pretende superar algunas de las limitaciones de las técnicas clásicamente utilizadas. Respecto al análisis de datos, cabe destacar el impacto en los resultados al considerar diferentes umbrales y las diferencias observadas al utilizar umbrales genéricos y personalizados. En cuanto al análisis de la PP, concluimos que es factible la utilización de métodos no invasivos para su determinación en arterias centrales de gran calibre como la aorta.

SUMMARY – RESUMEN (CASTELLANO)

INTRODUCCIÓN

En 1989 Mattila et al. describieron la asociación entre la salud dental y el infarto agudo de miocardio en un estudio epidemiológico (Mattila et al., 1989). Desde ese momento, se ha publicado una copiosa cantidad de literatura, en forma de estudios mecanísticos y epidemiológicos, en los cuales se han utilizado variables finales o subrogadas relacionadas con la enfermedad cardiovascular y la arteriosclerosis. Los resultados de la evidencia son controvertidos, especialmente después de controlar los posibles factores de confusión (Kebschull et al., 2010).

En 2012, la "American Heart Association (AHA)" publicó una declaración científica, basada en estudios observacionales, en la que se ratifica la asociación entre la enfermedad periodontal y la enfermedad vascular arterioesclerótica (Lockhart et al., 2012).

RELACION ENTRE PERIODONTITIS Y RIGIDEZ ARTERIAL

La enfermedad periodontal constituye un proceso inflamatorio crónico de origen bacteriológico que tiene como consecuencia la destrucción de la inserción de tejido

conectivo y el hueso alveolar que soportan al diente. La respuesta proporcionada por el huésped determinará la susceptibilidad individual de cada paciente a la colonización bacteriana.

El punto en común entre periodontitis y determinadas enfermedades sistémicas, lo constituye la inflamación local y sistémica, resultado de la invasión local de los tejidos y la bacteriemia y endotoxemia. Dichos micro-organismos, sus productos y la respuesta inflamatoria e inmunológica proporcionada por el huésped susceptible desencadenarán una serie de procesos con consecuencias tanto locales como sistémicas. La plausibilidad de la relación entre periodontitis y la arteriosclerosis está basada, en primer lugar en la afectación de las células endoteliales por parte de *P. gingivalis* y otras bacterias que presentan fimbria. Posteriormente, una serie de receptores y procesos se activarán como respuesta, que tendrán como consecuencia el deterioro del endotelio y el reclutamiento de monocitos en el espacio de las capas intima y media. Entre estos procesos, se encuentran la activación de los TLR-2 y receptores a diversas moléculas de adhesión (VCAM-1), procesos que posibilitan el mimetismo molecular (HSP60-related GroEL), el aumento en la producción de citoquinas y otros mediadores de la inflamación y la apoptosis que directamente puede causar *P. gingivalis* y otras microorganismos sobre le epitelio. Estos mecanismos, como se ha mencionado anteriormente contribuyen al reclutamiento en el espacio sub-endotelial de monocitos activados, la invasión, activación y apoptosis de las células endoteliales por parte de los microorganismos y procesos desencadenados y la disminución en la producción de NO, tan importante para los

procesos de dilatación y contracción de los vasos sanguíneos. Posteriormente, la formación de células espumosas, consecuencia de la fagocitación de LDL-oxidado por parte de los macrófagos (provenientes de los monocitos), junto con proliferación de células del músculo liso y matriz extracelular entre otros procesos, contribuirán a la formación de la placa de ateroma. Las plaquetas, reclutadas a consecuencia de la perdida de continuidad del epitelio también contribuirán en el proceso. En último lugar, la placa de ateroma se desprenderá (provocando y continuando el proceso pro-coagulante y de expansión) a consecuencia de la necrosis de las células endoteliales y la degradación de la matriz extracelular al tiempo que el proceso de proliferación continua (Faxon et al., 2004, Kebschull et al., 2010, Schenkein and Loos, 2013).

RELACION ENTRE LA ENFERMEDAD CARDIOVASCULAR Y RIGIDEZ ARTERIAL

Una de las variables subrogadas más destacadas en el estudio de las enfermedades cardiovasculares es la cuantificación de la rigidez de las paredes arteriales. El término rigidez arterial se refiere a la disminución de la capacidad arterial de expandirse y contraerse, adaptándose a los requerimientos circulatorios y a los cambios de presión.

La existencia de rigidez arterial, especialmente en las arterias centrales, constituye un predictor fuerte e independiente de mortalidad cardiovascular producida por diversas causas, eventos coronarios y accidente cardiovascular fatal en pacientes con hipertensión (Laurent et al., 2001), pacientes con enfermedad renal avanzada, diabetes tipo dos y también en la población general (Luo et al., 2012). En la literatura cardiovascular se han utilizado distintas variables para definir la elasticidad de las

arterias (Laurent et al., 2006) o lo que es igual, la rigidez arterial. Entre los métodos usados, contamos con métodos directos, como la velocidad de onda de pulso –PWV- y otros parámetros asociados, como la presión de pulso –PP- y métodos indirectos, como el grosor de la intima y media –IMT- y la dilatación mediada por el flujo –FMD-. En general, los resultados obtenidos son altamente sensibles a la técnica utilizada, conllevan dificultades en su interpretación (ya que para facilitar su ejecución utilizan arterias periféricas) y, además, su precio suele ser elevado, ya que utilizan aparatología adicional.

La presión de pulso (PP) se corresponde a la diferencia entre la presión sistólica y diastólica y se considera un predictor de mortalidad y morbilidad cardiovascular. El cálculo de la PP contempla la interacción entre el volumen sistólico y las propiedades de la circulación arterial, por lo que un aumento en la rigidez arterial llevará a un aumento en la PP debido a la reducción de la distensibilidad arterial y un aumento en la velocidad en las ondas reflejadas originadas con el paso del pulso. La PP se calcula normalmente en las arterias periféricas se ha calculado principalmente mediante esfigmomanometría braquial y tonometría de aplanamiento (Li, 2017). La PP se ha propuesto como un marcador de riesgo cardiovascular y en estudios epidemiológicos se ha demostrado que la mortalidad cardiovascular se relaciona positivamente con valores altos de presión arterial sistólica y bajos de presión arterial diastólica, es decir, con valores altos de PP (Asmar et al., 2001). Por otro lado, no es un índice ideal, ya que la PP especialmente si es calculada en arterias periféricas, no es una medida directa de elasticidad arterial y que no siempre se corresponde a la presión observada

en arterias de mayor calibre y más cercanas al corazón, sobre las cuales se reconoce un papel crucial en la etiopatogenia de las enfermedades cardiovasculares (Pini et al., 2008). Además, la PP no es una medición directa de elasticidad vascular, y sus resultados se encuentran influidos por la cantidad de sangre presente en el ventrículo izquierdo al final de la diástole y justo antes de la sístole, el nivel de hipertrofia ventricular y la capacidad de las arterias coronarias para irrigar el corazón (Laurent et al., 2001). Por otro lado, el hecho de realizar mediciones en arterias periféricas y extrapolar los datos a las arterias centrales tendría en este caso las limitaciones de que se ha observado un efecto diferente de algunos antihipertensivos sobre las arterias periféricas y centrales (Williams et al., 2006) y que la repercusión de factores fisiológicos, como la edad y factores patológicos, como la hipertensión en arterias funcional y ultraestructuralmente diferentes puede a su vez, ser diferente. Cabe destacar que es posible calcular la PP en arterias de mayor diámetro, aunque los métodos comúnmente utilizados son invasivos (Li, 2017).

Mediante tonometría de aplanamiento es posible calcular la velocidad de onda de pulso (PWV) en dos puntos distantes del sistema vascular. PWV se considera el “gold” estándar en la medición de la rigidez arterial (Vappou et al., 2011a). Basada en esta técnica, surge la técnica “Imagen de la Onda de Pulso”, del inglés “Pulse Wave Imaging” (PWI), técnica basada en la visualización de un determinado vaso sanguíneo mediante ecografía o ultrasonidos, y por lo tanto, no invasiva. Este método aporta una serie de ventajas con respecto al calculo del PWV mediante tonometría de aplanación, que serán expuestos más adelante. Se trata de un método de fácil acceso,

que utiliza medios tecnológicos y técnicas comunes y extendidas. PWI es una novedosa técnica, desarrollada en el laboratorio "Ultrasonidos, Elasticidad e Imagen" de Columbia University. En esencia, esta técnica proporciona información cualitativa y cuantitativa sobre la transmisión de la onda de pulso. Mediante ultrasonidos, permite la visualización de la propagación de la onda generada cuando el pulso se trasmitirse a lo largo de las paredes de una determinada arteria, aportando información sobre la homogeneidad de transmisión de la onda. A su vez, es capaz de estimar la velocidad de transmisión del pulso (en m/s) ("Pulse Wave Velocity" (PWV)) siendo este índice el criterio de referencia de rigidez arterial establecido entre la comunidad científica (Luo and Konofagou, 2011).

ESTUDIO DE LA RIGIDEZ ARTERIAL EN LA LITERATURA PERIODONTAL

Diversos métodos se han utilizado para su estudio, siendo los más extendidos la Dilatación Mediada por Flujo (FMD), la cuantificación del Grosor de la Intima-Media (IMT) que estudia la anatomía del vaso, y la cuantificación de la Velocidad de la Onda del Pulso (PWV), siendo solamente la última una medida directa de la rigidez arterial.

En la literatura periodontal IMT se ha utilizado demostrando la asociación de su incremento con la enfermedad periodontal en estudios epidemiológicos de gran volumen (Beck et al., 2001, Desvarieux et al., 2005). Generalmente IMT se determina con ultrasonidos y al compararla con PWV se considera una técnica inferior en algunos aspectos, sobre todo por el hecho de que su asociación con la rigidez arterial aún no está clara y por su aumento fisiológico con la edad, pudiendo presentarse

como un factor de confusión en el campo periodontal.

FMD estudia la funcionalidad de la pared arterial. Normalmente FMD se compara con la dilatación ocasionada tras la administración de nitroglicerina con la finalidad de desestimar la dilatación independiente del endotelio. La dilatación obtenida con y sin nitroglicerina se calcula de manera bidimensional mediante ultrasonidos tras la provocación de hiperemia mediante oclusión.

PWV ha sido tradicionalmente evaluada en dos arterias distantes y su cálculo es el resultado de la determinación de la velocidad de propagación de la onda, determinada mediante tonometría de aplanación, entre los dos puntos dividido por la distancia entre ellos. Metodológicamente, la mayoría de las investigaciones son transversales y algunos trabajos limitan su muestra a pacientes que presentan factores de riesgo de arteriosclerosis (pacientes con hipertensión primaria, hipercolesterolemia familiar, diabetes mellitus, enfermedad cardíaca isquémica, etc.), difieren en la definición de periodontitis utilizada y en la técnica y localizaciones usadas para determinar PWV. Los resultados reflejados en la literatura periodontal son heterogéneos y, aunque en la mayoría encuentran una asociación significativa entre periodontitis y valores más elevados de PWV (Miyaki et al., 2006, Vieira et al., 2011, Hayashida et al., 2013, Shanker et al., 2013, Vidal et al., 2013, Jockel-Schneider et al., 2014, Kapellas et al., 2014a, Houcken et al., 2016), dicha asociación se atenúa o no se detecta después de ajustar para variables como edad, presión sistólica, tabaco y otros factores de riesgo comunes entre la enfermedad cardiovascular y periodontitis

(Miyaki et al., 2006, Vieira et al., 2011). Por el contrario, en nuestra revisión de la literatura observamos que en uno de los estudios las diferencias permanecieron después de ajustar para factores de riesgo cardiovasculares (Houcken et al., 2016) mientras que en la mayoría de los estudios no se detectaron diferencias en la PWV obtenidas en pacientes periodontales y controles (Franek et al., 2009, Franek et al., 2012, Hanaoka et al., 2013) mientras que en otros trabajos, las diferencias solo existían al considerar los casos más severos de periodontitis (Vieira et al., 2011, Kapellas et al., 2014a). Nuestra búsqueda bibliográfica detectó tres estudios intervencionales, de los cuales únicamente uno es un RCT (Kapellas et al., 2014b). Respecto a los posibles cambios en PWV tras el tratamiento periodontal se observaron diferencias significativas en tan solo una investigación, cuantificándose la mejora en una reducción en PWV de 0.9 m/s (Vidal et al., 2013). Por otra parte, no se hallaron diferencias en las dos publicaciones restantes (Kapellas et al., 2014b, Houcken et al., 2016). Por último, en una revisión sistemática y mata-análisis reciente se obtuvo una diferencia de 0.85 m/s; 95% CI: 0.53-1.16; $p<0.00001$ en PWV en pacientes periodontales vs. controles. Por otro lado, los resultados del tratamiento periodontal fueron inconsistentes (Schmitt et al., 2015).

OBJETIVOS DE LA INVESTIGACION

Dada la notable evidencia que asocia la periodontitis crónica con la enfermedad cardiovascular, y la reducida y contradictoria literatura que ahonda en el estudio de la rigidez arterial en el paciente periodontal, se ha utilizado la técnica conocida como "Pulse Wave Imaging (PWI)" para investigar su asociación.

Asimismo se comparó el parámetro "presión del pulso (PP)" obtenido en tres localizaciones diferentes del árbol arterial (arteria braquial, radial y aorta) mediante la utilización de tres técnicas diferentes respectivamente (esfigmomanómetro, tonometría de aplanación y PWI) en una submuestra de pacientes clasificados conforme a sus niveles de presión arterial braquial en normotensos, pre-hipertensos e hipertensos.

MATERIAL Y METODOS

DISEÑO DEL ESTUDIO

Se ha llevado a cabo un estudio transversal en el que se han comparado la velocidad de transmisión de la onda del pulso (PWV) obtenida mediante PWI en una muestra de pacientes diagnosticados con enfermedad periodontal y una muestra de pacientes periodontalmente sanos, pareada respecto a edad (±5 años) y género con los casos.

Los pacientes fueron reclutados en las clínicas odontológicas de la Facultad de Odontología de la Universidad de Columbia. Aquellos pacientes que potencialmente cumplían con los criterios de inclusión del estudio fueron contactados e invitados a participar en el estudio. Los objetivos y procedimientos del estudio fueron aprobados por el Comité Ético competente (Institutional Review Board of the Columbia University Medical Center (IRB# AAAL1851)) y todos los participantes firmaron el pertinente consentimiento informado previamente a su inclusión en el estudio. La confidencialidad de los pacientes se aseguró gracias a la asignación de un identificador a cada participante.

CRITERIOS DE INCLUSION

Los pacientes seleccionables debían presentar enfermedad periodontal moderada-severa con un mínimo de 2 dientes/cuadrante con profundidades de bolsa (PD)>5mm, pérdida de inserción (CAL) concomitante >3mm y sangrado al sondaje (BoP) en >30% de las localizaciones. El grupo control (pacientes periodontalmente sanos) fue constituido por una muestra pareada con los casos en cuanto al género y la edad (± 5 años) definida por la ausencia de PD >4mm y CAL interproximal >2mm.

Además, todos los individuos incluidos debían cumplir con los siguientes requisitos: tener una edad comprendida entre 25 y 65 años, más de 20 dientes presentes, no haber recibido tratamiento antibiótico durante los 3 meses anteriores y no presentar ninguna patología sistémica o desorden genético compatible con el diagnóstico

periodontal de “periodontitis asociada a una enfermedad sistémica (1999)” (Lindhe et al., 1999), diabetes mellitus, embarazo, cáncer o enfermedades reumáticas.

VARIABLES

La raza, etnia y datos sobre la historia médica (incluyendo fármacos y existencia de hábito tabáquico) y dental fueron recogidos durante la anamnesis. El peso y la altura fueron medidos para calcular el Índice de Masa Corporal (BMI). La presión arterial sistólica y diastólica fue el resultado de la media de las mediciones obtenidas por triplicado en cada brazo mediante un monitor automático.

PARÁMETROS PERIODONTALES

Todos los pacientes fueron examinados por la autora (ESM), previamente calibrada mediante la sonda manual UNC-15. El examen periodontal incluyó seis localizaciones por diente (vestibular, lingual/palatino, mesio-vestibular, mesio-lingual/palatino, disto-vestibular y disto-lingual/palatino), incluyendo todos los dientes presentes. Los siguientes parámetros fueron recogidos: profundidad de sondaje clínica (PD), posición del margen gingival (GM), presencia de sangrado al sondaje (BoP) y presencia de placa (PI). A partir del PD y GM se calculó el nivel de inserción clínica de cada diente (CAL). La unidad empleada para medir PD, GM y CAL fue el milímetro (valor redondeado al milímetro más cercano) y constituyeron variables continuas. Tanto BoP como PI fueron tratados como variables dicotómicas (ausencia/presencia).

OBTENCION DE PWV Y R^2 MEDIANTE ULTRASONIDOS Y PROCESADO DE DATOS

La técnica de cuantificación de la onda del pulso mediante la imagen (PWI) ha sido desarrollada recientemente en el Laboratorio de Ultrasonidos, Elasticidad e Imagen de Columbia University. Permite el cálculo de la velocidad de la onda del pulso (PWV), la presión del pulso (PP) y a su vez, el estudio de las imágenes doppler facilita una evaluación cualitativa de la moción de la arteria al transmitirse la onda de pulso.

La pared de la arteria fue delineada gracias al uso de una frecuencia baja de ultrasonidos mientras que la velocidad de la onda del pulso (PWV) se cuantificó mediante la utilización de una frecuencia alta de ultrasonidos, ya que ésta permite obtener información del rápido movimiento provocado por el pulso en la pared arterial, analizado mediante el rastreo de ultrasonidos. Todas las mediciones se realizaron con el uso de un ultrasonidos comercialmente disponible en el laboratorio de Elasticidad e Imagen de Columbia University (SonixTouch, RP or MDP System, Ultrasonix Medical Corporation).

La profundidad de imagen fue ajustada hasta permitir la visualización de las paredes arteriales. Las imágenes se tomaron en la carótida común derecha e izquierda, a 1mm de la bifurcación. Cada captura tuvo una duración de 2.5 segundos, conteniendo aproximadamente dos ciclos cardíacos. El número de capturas realizadas a cada paciente varió entre 5 y 12, dependiendo de la calidad de la visualización de la arteria y la homogeneidad de transmisión de la onda de pulso. Todos los ciclos cardiacos fueron analizados por la autora (ESM), estando previamente calibrada (valor kappa de

0.88 para PWV y 0.85 para R^2).

El tiempo de llegada del pie de la onda para cada ciclo, expresado en milisegundos fue representado gráficamente junto con la distancia viajada, medida en milímetros (diagrama x-y). Posteriormente, se calculó la regresión lineal de la distribución de los datos. PWV se calculó como la inversa de la pendiente de la regresión lineal, mientras que R^2 constituyó el coeficiente de determinación de la regresión lineal.

La presión de pulso (PP) central se obtuvo mediante PWI en la aorta abdominal de los pacientes en los que su medición se podía hacer de manera clara y sin obstrucciones. Las PP periféricas se obtuvieron mediante tonometría de aplanación en la arteria radial y esfigmomanometría en la arteria braquial. La primera se obtuvo mediante el tonómetro de pulso SPT-301 y la unidad de control de presión PCU-200. La PP braquial se calculó mediante un monitor de presión arterial automático (HEM-705CP) en el brazo izquierdo a partir de tres mediciones (la primera de las cuales fue desechada) realizadas durante un tiempo máximo de 15 minutos. Los pacientes fueron divididos en tres grupos: normotensos, pre-hipertensos e hipertensos basado en la medición de la presión arterial braquial mediante esfigmomanómetro. Los resultados fueron analizados mediante el test ANOVA bidireccional y la corrección de Bonferroni fue utilizada para analizar diferencias entre los tres grupos de pacientes.

DETERMINACIÓN DE LOS UMBRALES EN PWV

PWI ofrece la ventaja de aportar datos cualitativos (R2) y cuantitativos (PWV) sobre la

transmisión del pulso. Dos umbrales fueron establecidos para el análisis de los datos, basados en los valores de $R^{2.}$

Esta decisión se tomó basada en la alta variabilidad en los resultados observada en la literatura (en contraposición a la literatura de IMT y FMD), la escasez de estudios sobre PWI en pacientes relativamente sanos y el interés en analizar los resultados dependiendo del tipo de definición utilizada. Los umbrales se establecieron basados en el análisis de la homogeneidad de transmisión del pulso (R^2), ya que existen investigaciones previas donde se muestra que los resultados de R^2 representan mejor la salud de los vasos en comparación a PWV en pacientes que no han sufrido eventos cardiovasculares graves.

El primer umbral aplicado fue establecido en $R^2 \geq 0.6$ y se basó en la idea de que cuando una captura es menor a dicho resultado, es probable que éste se deba a una mala técnica de adquisición, por lo tanto, todos los resultados de PWV acompañados por un resultado menor a 0.6 fueron descartados. El segundo umbral se creó con la finalidad de individualizar los resultados y fue calculado a partir de restar una desviación estándar (SD) de la media de los resultados del paciente para la carótida izquierda y derecha a la media (media – SD).

Todos los ciclos cardiacos disponibles fueron medidos y los diferentes umbrales fueron aplicados por separado para las mediciones provenientes de la carótida común derecha y la izquierda. Con los resultados disponibles, se obtuvo la media para PWV y R^2 para la carótida común derecha e izquierda. Con la finalidad de crear un modelo

que representase la máxima patología del paciente (máxima PWV, indicando mayor rigidez de las arterias, y menor R^2 indicando menor homogeneidad en la transmisión del pulso), de los dos resultados de PWV y R^2, el mayor PWV y menor R^2 fueron seleccionados para representar a cada paciente.

Los resultados tanto para PWV como R^2 fueron analizados por parejas, obteniendo el delta (Δ) entre test y control para cada pareja y el test estadístico correspondiente se aplicó dependiendo de la distribución de los datos. Análisis uni y multivariables se realizaron posteriormente con la finalidad de explorar posibles asociaciones.

RESULTADOS

DEMOGRAFÍA

La muestra estaba compuesta por un total de 80 voluntarios (39% hombres, con una media de edad de 47.5 años, SD 11.6, rango 24-78), incluyendo 40 pacientes con periodontitis crónica y 40 controles periodontalmente sanos. El 64% de los individuos se definió como Hispánico y la distribución de Hispánicos en el grupo de periodontitis fue del 70% y del 57.5% en el grupo control. Los datos demográficos de los pacientes se encuentran en la Tabla 8. Tal y como fue establecido en los criterios de exclusión, no se incluyeron pacientes afectados por diabetes mellitus. Los dos grupos fueron comparables, excepto en edad y hábito tabáquico. También se observó una mayor tendencia de pacientes que usaban anti-hipertensivos en el grupo de pacientes

afectados por periodontitis. No se observaron diferencias en lo que respecta a “índice de masa corporal (BMI)”, presión arterial sistólica y diastólica.

PARÁMETROS PERIODONTALES

La información referente al estado periodontal de los pacientes se encuentra recogida en la Tabla 9. Como se puede observar en ésta, en cuanto a los parámetros que indican presencia de inflamación, la media de profundidad de sondaje en los pacientes fue de 4.0 ± 0.72 mientras que en los controles fue de 2.2 ± 0.29 y que el porcentaje de sitios con BoP fue de 63 ± 0.04 vs. 9.0 ± 0.04.

En cuanto a CAL, los valores obtenidos fueron de 4.7 ± 1.22 vs. 1.3 ± 0.52 para pacientes con periodontitis vs. controles.

VELOCIDAD DE TRANSMISION DEL PULSO (PWV) Y HOMOGENEIDAD EN LA TRANSMISION DE LA ONDA DE PULSO (R^2)

De los 80 pacientes que se sometieron a ecografía, se obtuvieron resultados pertenecientes a 78 pacientes. Dos de los escáneres, uno perteneciente a un paciente periodontal y el otro a un control periodontalmente sano, no pudieron ser leídos debido a fallos técnicos durante la adquisición de la imágenes.

UMBRAL A: SELECCIÓN DE RESULTADOS DE PWV Y R^2 CUANDO LOS VALORES OBTENIDOS PRESENTABAN UN $R^2 \geq 0.6$

Después de la aplicación del "umbral A" a todos los resultados disponibles (78 resultados: 39 pertenecientes a casos y 39 a controles), se contó con datos de los ultrasonidos provenientes de 73 individuos (5 individuos presentaban todos sus resultados de R^2 menores a 0.6). De estos 73 individuos, se pudieron formar 30 pares caso-control basados en género y edad (± 5 años).

Normalidad: la distribución de ambas variables (PWV y R^2) siguieron una distribución no normal, como se demuestra en los histogramas 1-4.

Análisis estadístico comparando las parejas caso-control: dado que la distribución de los resultados no seguía los parámetros de normalidad, se calcularon las medianas y el rango intercuartil en las 30 pares caso-control incluidas. Posteriormente, se calculó el test de Wilcoxon (Wilcoxon Signed Rank Test) con la finalidad de explorar diferencias entre los grupos. En los resultados se observó que no existían diferencias significativas entre los grupos en cuanto a PWV (mediana PWV 2.37m/seg vs. 2.64m/seg en periodontitis y controles periodontalmente sanos respectivamente, Wilcoxon test p=0.74) ni en cuanto a la variable resultado R^2 (0.74 vs. 0.71, respectivamente, p=0.81). Los resultados se exponen en los diagramas de caja 1 y 2 (Boxplot 1,2). Cabe destacar la mayor dispersión de datos (rango) tanto para la variable resultado PWV como R^2 observada en pacientes periodontales.

El análisis estadístico univariante, en el que se utilizaron PWV y R^2 como variables resultado y el resto de variables (presencia de periodontitis, género, edad, etnia, BMI, hábito tabáquico, medicación para el control de la hipertensión arterial, presión arterial sistólica y diastólica, número de dientes, porcentaje de localizaciones que presentan PD ≥ 4 y 6mm, CAL ≥ 4 y 6 mm, placa y BoP) como variables explicativas no reveló ninguna asociación estadísticamente significativa. Se observaron asociaciones negativas entre R^2 y las variables: presencia de periodontitis (*p*=0.60), edad (*p*=0.87), presión arterial sistólica (*p*=0.62), presión arterial diastólica (*p*= 0.42) y todas las variables continuas relacionadas con la presencia de periodontitis (porcentaje de sitios que presentan BoP (*p*=0.54), porcentaje de sitios que presentan placa (*p*=0.56), porcentaje de sitios con PD ≥4mm (*p*=0.32), PD ≥6mm (*p*=0.34), CAL ≥4mm (*p*=0.47) y CAL ≥6mm (*p*=0.35). Por otro lado, la asociación entre PWV fue negativa respecto a las variables periodontitis (0=0.35), género (*p*=0.78), medicación para presión arterial (p=0.47) y todas las variables continuas relacionadas con la presencia de periodontitis (Tabla 10).

Tras el ajuste para las variables género, edad, etnia, BMI, hábito tabáquico, medicación para el control de la hipertensión arterial, presión arterial sistólica y diastólica, número de dientes, la regresión lineal multivariable solo reveló diferencias estadísticamente significativas respecto a la variable resultado R^2 y género (*p*=0.04) (Tabla 11).

Se realizó, así mismo, un análisis multivariable con el fin de analizar la asociación de las tres variables más importantes relacionadas con la presencia de periodontitis y las variables resultado PWV y R^2, tras realizar el ajuste de las variables género, edad, etnia, BMI, hábito tabáquico, medicación para el control de la hipertensión arterial, presión arterial sistólica y diastólica, número de dientes. Los parámetros analizaron fueron porcentaje de sitios con CAL≥4mm (Tabla suplementaria 1), CAL≥6mm (Tabla suplementaria 2) y BOP (Tabla suplementaria 3). La asociación fue estadísticamente significativa para las variables % sitios con CAL≥4mm y PWV (p=0.03) y género y R^2 (p=0.04) (Tabla suplementaria 1), % sitios CAL≥6mm y PWV (p=0.008), y género y R^2 (p=0.05) (Tabla suplementaria 2) y % sitios BOP y PWV (p=0.03), y género y R^2 (p=0.04) (Tabla suplementaria 3).

- UMBRAL B: SELECCIÓN DE RESULTADOS DE PWR Y R^2 CUANDO LOS VALORES OBTENIDOS PRESENTABAN UN $R^2 \geq$ AL RESULTADO DE CALCULAR LA MEDIA DE R^2 OBTENIDOS -EN CADA LOCALIZACION (CAROTIDA IZQUIERDA Y DERECHA) RESPECTIVAMENTE Y PACIENTE- Y RESTAR UNA UNIDAD DE DESVIACION STANDARD (SD)

Tras aplicar el “umbral B” a todos los resultados disponibles (78 resultados: 39 pertenecientes a casos y 39 a controles), se obtuvieron resultados de los 78 pacientes que presentaban con escáneres que permitieron su lectura. Los 78 pacientes se

pudieron dividir en dos grupos y se formaron 33 pares caso-control basados en género y edad (± 5 años).

Normalidad: la distribución de ambas variables (PWV y R^2) para los 33 pares de pacientes siguieron una distribución no normal, como se demuestra en los histogramas 5-8.

Análisis estadístico comparando las parejas caso-control: dado que la distribución de los resultados no seguía los parámetros de normalidad, se calcularon las medianas y el rango intercuartil en los 33 pares caso-control incluidos. Posteriormente, se calculó el test de Wilcoxon (Wilcoxon Signed Rank Test) con la finalidad de explorar diferencias entre los grupos. En los resultados se observó que no existían diferencias significativas entre los grupos en cuanto a PWV (mediana PWV 2.81m/seg vs. 3.35m/seg en periodontitis y controles periodontalmente sanos respectivamente, Wilcoxon test p=0.31) mientras que se observó una mediana inferior en cuanto a la variable resultado R^2 en el grupo de pacientes periodontales vs. controles periodontalmente sanos (0.43 vs. 0.52, respectivamente, p=0.01). Los resultados para ambas variables se exponen en los diagramas de caja 3 y 4 (Boxplot 3,4). Cabe destacar la mayor dispersión de datos (rango) para la variable resultado R^2 observada en pacientes periodontales (Boxplot 3).

El análisis estadístico univariante basado en los valores de los 78 pacientes para los que se contaba con resultados (Tabla 12) mostró una asociación significativa y negativa entre la uniformidad de la propagación de la onda de pulso (R^2) y la

presencia de periodontitis (p=0.004), hábito tabáquico (p=0.02) así como de las variables continuas relacionadas con la enfermedad periodontal: porcentaje de sitios presentando BoP (p=0.02), porcentaje de sitios presentado placa dental (p=0.03), porcentaje de sitios con PD ≥4mm (p=0.008), PD ≥6mm (p=0.02), CAL ≥4mm (p=0.003) and CAL ≥6mm (p=0.002). Por otro lado, no se encontraron asociaciones significativas entre la variable resultado PWV y ninguna de las variables explicativas consideradas, a excepción de porcentaje de sitios presentando BoP (p=0.04).

El análisis de regresión multivariante, tras ajustar para las variables presencia de periodontitis, género, edad, etnia, BMI, hábito tabáquico, medicación para el control de la hipertensión arterial, presión arterial sistólica y diastólica y número de dientes, reveló solamente asociaciones estadísticamente significativas entre R^2 y la presencia de periodontitis (p=0.01) (Tabla 13) (Sanz-Miralles et al., 2017)

Se realizó, asimismo, un análisis multivariable con el fin de analizar la asociación de las tres variables más importantes relacionadas con la presencia de periodontitis (porcentaje de sitios con CAL≥4mm -Tabla suplementaria 4-, CAL≥6mm -Tabla suplementaria 5- y porcentaje de sitios que muestran BoP -Tabla suplementaria 6-) y las variables resultado PWV y R^2. Tras realizar el ajuste de las variables periodontales, género, edad, etnia, BMI, hábito tabáquico, medicación para el control de la hipertensión arterial, presión arterial sistólica y diastólica, número de dientes, los resultados mostraron una asociación negativa y estadísticamente significativa para las variables % sitios con CAL≥4mm y las variables resultado PWV (p=0.04) y R^2 (p=0.03)

(Tabla suplementaria 4), % sitios CAL≥6mm and y la variable resultado R^2 (p=0.04) (Tabla suplementaria 5) y % sitios mostrando BoP y la variable resultado PWV (p=0.02) (Tabla suplementaria 6).

PRESION DEL PULSO (PP)

Se incluyeron 19 pacientes en total (9 pre-hipertensos, 5 hipertensos y 5 normotensos). Se buscaron asociaciones entre los tres grupos de pacientes (pre-hipertensos, hipertensos y normotensos) y las 3 variables resultado (PP braquial, PP radial y PP en la aorta) mediante la aplicación del test de ANOVA bilateral y la corrección de Bonferroni. Se encontraron diferencias estadísticamente significativas para el parámetro PP medido en la aorta entre el grupo normotenso e hipertenso ($0.001 < p < 0.01$). Además, se determinó una relativa elevada correlación entre PP medido en la aorta y en las arterias braquiales y radiales pero solo en pacientes hipertensos (Li, 2017).

DISCUSIÓN

Se detectaron diferencias en las propiedades elásticas de la arteria carótida común entre un grupo de pacientes periodontales y un grupo de pacientes periodontalmente sanos, emparejados en cuanto a género y edad. Ninguno de los pacientes presentaba antecedentes de episodios cardiovasculares graves ni diabetes mellitus. El análisis multivariante reveló una menor uniformidad en la propagación de la onda de pulso en un punto reproducible de las arterias carótidas derecha e izquierda en los pacientes periodontales, tras controlar las variables demográficas, BMI, hábito tabáquico y presión arterial. Nuestros resultados se suman a la amplia cantidad de literatura que respalda la asociación entre periodontitis e inflamación sistémica, disfunción endotelial y episodios cardiovasculares adversos (Sanz-Miralles et al., 2017).

La rigidez arterial está íntimamente relacionada con la enfermedad cardiovascular y es una característica del proceso de arteriosclerosis. La base biológica que explica la relación entre la periodontitis y el incremento de la rigidez arterial reside en el rol de los procesos inflamatorios, característicos en ambas patologías. La enfermedad periodontal es una enfermedad inflamatoria crónica que provoca efectos locales y sistémicos. Sus efectos sistémicos se deben a la inflamación *per se*, a procesos de mimetismo molecular, bacteriemia e infección vía patógenos periodontales, es decir, se deben a las bacterias o a alguno de los mecanismos que desatan, junto con la respuesta del paciente frente a estos.

En el estudio de la relación entre rigidez arterial y periodontitis se han utilizado distintas metodologías, como las que evalúan la anatomía de los vasos (grosor de la capa íntima y media -IMT-) y las que evalúan su función (dilatación mediada por el flujo -FMD-, la presión de pulso –PP- y la velocidad de transmisión de la onda de pulso -PWV-). Tanto las investigaciones que utilizan IMT como en los que se utiliza FDM generalmente llegan a la conclusión de que los pacientes periodontales presentan un mayor grado de patología cardiovascular. Cabe destacar un estudio intervencional en el que se observó que inmediatamente después del tratamiento periodontal los niveles de FMD empeoraban, posiblemente a consecuencia de la bacteriemia. A su vez, se vio una mejora significativa en la dilatación arterial 6 meses después del tratamiento (Tonetti et al., 2007). Una revisión sistemática reciente concluye que los pacientes con periodontitis presentan como media valores de FDM de 5.1% menores que los pacientes periodontalmente sanos (95% CI 2.08 - 8.11), mientras que el tratamiento periodontal mejora el FMD en un 6.6% (95% CI 2.83 - 10.44), $p<0.0001$) (Orlandi et al., 2014). Un aspecto a tener en cuenta sobre los resultados de FMD es que están influidos y determinados por la metodología utilizada y una serie de factores fisiológicos y técnicos. Todos estos factores afectan la validez, reproducibilidad e interpretación de los resultados. Por otro lado, PWV se considera la medida “gold-standard” de rigidez arterial. La técnica habitualmente utilizada se basa en la medición de la velocidad de llegada de la onda del pulso en dos localizaciones distantes. Dicho parámetro se divide por la distancia existente entre las dos localizaciones donde se han realizado las mediciones. Hay una gran heterogeneidad

en la literatura periodontal en lo que respecta a PWV. Las investigaciones publicadas son limitadas, se utilizan definiciones diferentes, tanto de periodontitis como en los umbrales de PWV, muestras de pacientes con características distintas y los métodos para obtener PWV varían (en cuanto a aparatología utilizada, como en las localizaciones elegidas en el árbol arterial para realizar las mediciones). Asimismo, los resultados observados tampoco son homogéneos. Algunas publicaciones encontraron asociación entre PWV y periodontitis (Miyaki et al., 2006, Vieira et al., 2011, Hayashida et al., 2013, Shanker et al., 2013, Vidal et al., 2013, Jockel-Schneider et al., 2014, Kapellas et al., 2014a, Houcken et al., 2016), pero dicha asociación dejaba de ser estadísticamente significativa tras ajustar para posibles factores de confusión (Miyaki et al., 2006, Vieira et al., 2011), mientras que en otro estudio las diferencias prevalecían (Houcken et al., 2016). Otras publicaciones no encuentran diferencias estadísticamente significativas (Franek et al., 2009, Franek et al., 2012, Hanaoka et al., 2013). En el caso de otros estudios transversales, las diferencias se observan cuando se tienen en cuenta las formas más graves de enfermedad periodontal (Vieira et al., 2011, Kapellas et al., 2014a). Se han revisado tres estudios de intervención (Vidal et al., 2013, Kapellas et al., 2014b, Houcken et al., 2016), siendo los resultados obtenidos también heterogéneos (solo el primero, un estudio piloto prospectivo, encontró una reducción en el valor de PWV tras el tratamiento periodontal, estimándola en 0.9 m/s).

La evaluación de la onda del pulso mediante PWI (imagen de la onda de pulso) permite una evaluación de la elasticidad arterial cuantitativa y cualitativa. Dicha

técnica se ha desarrollado en el Laboratorio de Elasticidad, Ultrasonidos e Imagen del departamento del Bioingeniería de Columbia University. Otras ventajas que proporciona este método radican en el hecho de que todas las mediciones se realizan en un único lugar del árbol arterial, eliminando posibles inexactitudes y errores derivados del cálculo de la velocidad de onda en dos puntos anatómicos distantes, generalmente uno localizado en una arteria central y el otro en una periférica. Las principales razones de estas inexactitudes se basan en que es necesaria la determinación de la distancia exacta entre los citados dos puntos (midiéndose por encima de la piel y asumiendo que el recorrido de las arterias entre los dos puntos es una línea recta), y del hecho de que la velocidad y las propiedades anatómicas y funcionales de las arterias son particulares de un punto en concreto, siendo generalmente diferentes en los dos puntos donde se ha realizado las mediciones (arteria central y periférica). Este hecho es de gran importancia, y queda patente en una de nuestras publicaciones, cuando al comparar las presiones de pulso (PP) obtenidas en una arteria central –aorta- a las obtenidas en dos arterias periféricas –braquial y radial- en pacientes hipertensos, pre-hipertensos y normotensos (Li, 2017). Como se puede observar en los resultados, solo se observaron diferencias estadísticamente significativas al comparar los datos de PP observados en la arteria aorta de pacientes normotensos e hipertensos, siendo las diferencias obtenidas en las arterias braquial y radial no estadísticamente significativas. Por lo tanto, estos hallazgos resaltan la importancia de la obtención de medidas derivadas de arterias centrales (Li, 2017). Por otro lado, otras ventajas del método PWI serían la

disponibilidad de la aparatología, ya que se utiliza un aparato de ultrasonidos convencional, la facilidad en la realización de las mediciones y la comodidad para el paciente.

PWV en la carótida y la femoral ha sido propuesto por la Sociedad Europea de Cardiología como el índice a utilizar en el manejo del paciente hipertenso, y se ha sugerido que valores superiores a 12 m/s son indicadores de daño en los órganos. Por lo tanto, PWV es un buen índice en pacientes hipertenso, de edad avanzada o con factores de riesgo de enfermedad cardiovascular. El hecho de que PWI aporte información cualitativa, es decir, sobre la homogeneidad de la onda de pulso (coeficiente de correlación de la regresión linear (R^2)) lo convierte en un índice atractivo para el estudio del tipo de pacientes incluidos en nuestro estudio, ya que son pacientes relativamente sanos que no han sufrido accidentes cardiovasculares de gravedad ni diabetes mellitus y que, por lo tanto, pueden aún no mostrar signos de patología al analizar el PWV. Otra razón que hace R^2 de vital importancia es la escasez de estudios de equivalencia entre diferentes métodos y los umbrales utilizados para distinguir patología de no-patología. Es decir, R^2 constituye una variable de más fácil interpretación, ya que un valor elevado de R^2 significa homogeneidad en la transmisión y mayor elasticidad. En lo que respecta a la utilización de umbrales, R^2 nos permitió utilizar dos de ellos con la finalidad de analizar los datos desde dos puntos de vista diferentes. La idea de utilizar dos umbrales diferentes partió de la necesidad de distinguir mediciones que realmente reflejasen el estado de las arterias de posibles artefactos. El primer umbral utilizado (umbral A) abogaba por la eliminación de las

mediciones de R^2 y las mediciones de PWV correspondientes cuando R^2 era menor que 0.6 y se trata por lo tanto, de un umbral genérico, como la mayoría de umbrales utilizados en medicina (presión arterial, índice de masa corporal, etc.). En contraposición a éste, el umbral B se diseñó con la finalidad de tomar en consideración las mediciones individuales de cada paciente. Es, por lo tanto, un índice personalizado, que parte de la base de que valores menores de R^2 pueden deberse a la adquisición deficiente de las imágenes, pero también a arteriosclerosis (aunque debe tomarse en consideración que la muestra incluida en nuestro estudio tiene un reducido número de factores de riesgo de arteriosclerosis y, por lo tanto, un riesgo de arteriosclerosis relativamente reducido). El umbral B se aplicó al restar la desviación estándar (SD) a la media calculada a partir de todas las mediciones de R^2 obtenidas de la carótida derecha e izquierda, por separado (media –SD) (Sanz-Miralles et al., 2017)

En nuestro estudio incluimos una muestra de 40 individuos periodontalmente sanos y 40 pacientes con un grado moderado-grave de periodontitis. Con la finalidad de controlar posibles factores de confusión, se descartaron pacientes con diabetes mellitus y aquellos con historia de eventos cardiovasculares graves. No se excluyeron pacientes fumadores, aunque el número de individuos en nuestra muestra es reducido (correspondiéndose a la distribución de pacientes que acude generalmente al centro donde se realizó el estudio). El cálculo de muestra se basó en resultados de la variable resultado PWV de un estudio de diseño similar, dado que la varianza del R^2 en pacientes con periodontitis es desconocida. El cálculo de muestra se realizó basado en el estudio de Jockel-Schneider et al., 2014 (Jockel-Schneider et al., 2014), y se

determinó que con una n=31 parejas se detectaban diferencias entre ambos grupos, con una capacidad del 85% y un α=0.05 unilateral. Por lo tanto, conviene reconocer que la muestra de nuestro estudio era adecuada para detectar diferencias de 1.4 m/segundo entre pacientes periodontales y controles, sin embargo, la diferencia observada fue de 0.46 m/segundos al aplicar el umbral B (33 parejas incluidas) y de -0.13 m/segundos al aplicar el umbral A (30 parejas incluidas). Por otra parte, el método para calcular PWV utilizado en la investigación de Jockel-Schneider et al., 2014 tiene grandes diferencias respecto a PWI, ya que las mediciones se realizan mediante Atheriograph®, que basa el cálculo de PWV en la variacion de curvas de presión entre el brazo y la distancia entre una determinada parte de la yugular y la sínfisis (Sanz-Miralles et al., 2017)

En lo que respecta a la variable resultado R^2, se observó una clara inferioridad en la homogeneidad de la transmisión de la onda de pulso en el grupo de pacientes con periodontitis (al considerar el umbral B), indicando patología estructural subclínica compatible con rigidez arterial. En la literatura encontramos observaciones similares en cohortes de pacientes con historia de hipertensión y aneurisma de la aorta, para los cuales la forma de la onda cambia drásticamente en función de la severidad (Li et al., 2011).

Cabe destacar la importancia de la determinación de un umbral que permita: a) diferenciar los resultados que verdaderamente reflejan las condiciones de los vasos de posibles artefactos derivados de la medición y b) clasificar a los pacientes acorde a

su nivel real de patología. Hoy en día, todavía, la mayoría de umbrales utilizados siguen el patrón del umbral A; son umbrales genéricos que no tienen en cuenta la historia del paciente. Por lo tanto, creemos importante resaltar la importancia de umbrales individualizados que tienen en cuenta la situación clínica actual del paciente o su progresión y estabilidad a través del tiempo, como es el caso del umbral B. Al analizar los resultados obtenidos tras aplicar el umbral A no se encontraron asociaciones significativas entre periodontitis y las variables resultado PWV y R^2, aunque destaca la mayor dispersión en los resultados para ambas variables obtenida en el grupo de pacientes con periodontitis. Por otro lado, al utilizar el umbral B se observa una asociación estadísticamente significativa en cuanto a la variable resultado R^2 y la variable periodontitis y todas las variables asociadas con la condición periodontal. Dicha asociación prevalece en el modelo multivariante.

CONCLUSIONES

- Nuestros resultados confirman la asociación entre periodontitis y rigidez arterial, evaluada mediante una técnica nueva denominada Imagen de la Onda del Pulso (PWI)

- La información obtenida a partir de las mediciones en arterias centrales y periféricas puede no ser consistente

- En esta investigación se demuestra que la propagación del la onda del pulso a lo largo de las paredes arteriales de la carótida común ocurre de una manera menos homogénea en los pacientes periodontales, sugiriendo rigidez arterial o heterogeneidad en la transmisión

- PWI se presenta como una técnica más sencilla, técnicamente accesible y precisa para determinar el grado de rigidez arterial en un solo punto del árbol arterial

- Las diferencias metodológicas que existen entre la evidencia científica disponible impide la comparación y correlación de los resultados de la rigidez arterial. Dada la sustancial cantidad de evidencia que asocia la periodontitis crónica con la enfermedad cardiovascular, y la reducida y contradictoria literatura que ahonda en el estudio de la rigidez arterial en el paciente periodontal, se ha utilizado la técnica conocida como “Pulse Wave Imaging” para investigar su asociación

INTRODUCTION

1. INTRODUCTION

1.1. Relationship between periodontitis and systemic disease

1.1.1. Periodontitis and local and systemic inflammation

Periodontitis is a bacterially induced, localized, chronic inflammatory disease that destroys the connective tissue and bone that support the teeth (Friedewald et al., 2009). Commensal bacteria transition to pathogenic bacteria and this dysbiotic community targets specific mechanisms of the host's immune system. This leads to an uncontrolled inflammation of the periodontium and promotes an overall inflammatory response (Lamont and Hajishengallis, 2015). The host's response to the microbial plaque and genetic factors will determine the development of disease (Kinane, 2001, Nibali et al., 2014).

Recent data derived from the 2009-2010 cycle of the National Health and Nutrition Examination Survey (NHANES) describes a prevalence of periodontitis of 47.2%, while it reaches a prevalence of 70% when considering adults aged 65 years or older and all forms of periodontitis. These results are based on full-mouth results obtained from six sites per tooth and the definition of periodontitis used was the one proposed by the AAP/CDC (Eke et al., 2012).

Microorganisms quickly colonize the clean teeth surfaces after ceasing all oral hygiene procedures and the presence of the mentioned microorganisms and the release of

products will induce local tissue inflammation (Kinane, 2001). Consequently, antigens, a series of virulence factors, and in some cases invading bacteria constitute the microbial challenge. On the other hand, the host may influence the bacterial challenge with an immediate inflammatory and immune response (production of cytokines, eicosanoids, other inflammatory mediators such as kinins, complement activation products and matrix metalloproteinases). Depending on the host's susceptibility, this defensive response may perpetuate the inflammatory response and mediate connective tissue loss and bone destruction (Page and Kornman, 1997) and lead eventually to attachment and tooth loss. This susceptibility determines the shift from commensal bacteria to a pathogenic scenario, and dysbiosis (Lamont and Hajishengallis, 2015, Nibali et al., 2014)

1.1.2. Potential mechanisms linking periodontal infections and atherosclerosis

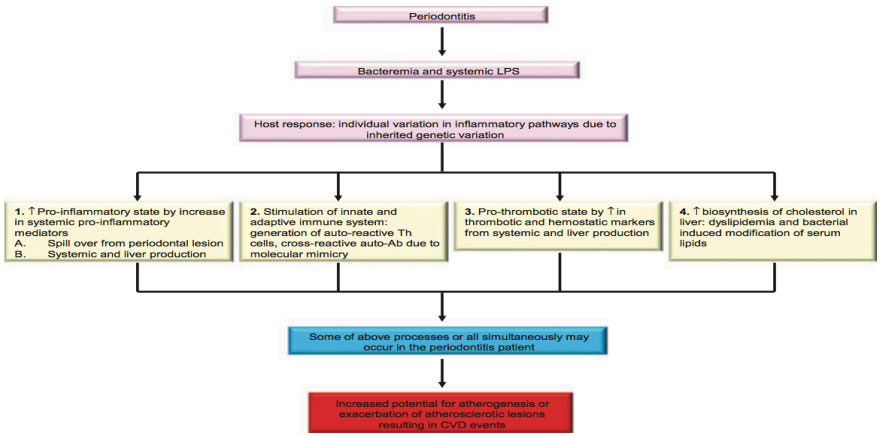


Fig 1. *Potential inflammatory mechanisms linking periodontitis to cardiovascular diseases (Schenkein 2013)*

Direct and indirect mechanisms have been shown to link periodontal disease (PD) and atherosclerotic vascular disease (ASVD).

Atherosclerosis is defined as a pathological condition of the intima, which is characterized by lipid accumulation, inflammatory cells, vascular smooth muscle cell migration, foam cell development, connective tissue fibers and calcium deposits (Cecelja and Chowienczyk, 2012). A large body of experimental and clinical research studies shows clearly that inflammation plays a central role in atherosclerosis (Faxon et al., 2004).

Periodontal infections result in low-grade bacteremia and endotoxemia, therefore it is biologically plausible that systemic effects on vascular physiology occur as a result of these exposures (Paquette and Williams, 2000). Bacteremia, defined as the presence of bacteria in the blood, is increased in periodontal patients after dental procedures (Kinane et al., 2005), oral hygiene procedures (Crasta et al., 2009) and also after chewing (Forner et al., 2006). Moreover, periodontal bacterial DNA has been identified in atherosclerotic lesions by different methods (Iwai, 2009).

Apart from bacteremia and the presence of periodontal bacteria in other regions far from the oral environment, periodontal inflammation is associated with an increase in systemic inflammatory markers, such as C-reactive protein (CRP), tumor necrosis factor –α (TNF-α, IL-1, -6, -8) and cellular and nuclear activation. Cellular and nuclear activation involves the expression or increase in the expression of cellular adhesion

molecules, toll-like receptors (TLR), matrix metalloproteinase (MMP), etc. and consequently, the activation of nuclear factor-κB (NF-κB) (Lockhart et al., 2012).

Regarding etiopathogenesis, four specific pathways have been proposed to explain the plausibility of a link between cardiovascular disease and periodontal infection. These pathways (acting independently or collectively) include direct bacterial effects on platelets, autoimmune responses, invasion and/or uptake of bacteria in endothelial cells and macrophages, and endocrine-like effects of pro-inflammatory mediators (Offenbacher et al., 2009). These four pathways are discussed in detail below:

- *P. gingivalis*, among other bacteria and microorganisms, can aggregate strongly, invade cells, enter the **platelets**, and survive. The two main effects observed on platelets are related to the reduction of bioactive nitrogen oxide (NO), which results in a reaction that has as a results the aggregation of the platelets. This event has been associated with **thrombosis**, and it also has a systemic effect that is strong enough to stimulate the secretion of various cytokines and of products such as serotonin, E-selectin, intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 (Iwai, 2009, Faxon et al., 2004).

- **Endothelial cells** suffer changes once exposed to bacteria or to the local and systemic inflammatory process provoked by bacteria. *P. gingivalis* may lead to **endothelial and macrophage apoptosis**. Moreover, toll-like receptors 2 (**TLR-**

2) are activated by fimbriated bacteria or lipopolysaccharides (LPS). This activation up-regulates adhesion molecules and creates a monocyte chemotactic protein-1 (MCP-1) gradient. This process will attract **monocytes** into the intima layer and also, will enable the transport of bacteria into the endothelial lesion (Kebschull et al., 2010). There are two main consequences of this event: the endothelial cells become activated and there is a migration of monocytes into the intima.

- The activation of endothelial cells is needed for **molecular mimicry**. Activated endothelial cells express heat-shock proteins (HSP60) that cross-react with antibodies against bacterial HSP60-related Gro-El (autoimmune mechanisms). The result of this event is the apoptosis of endothelial cells (Paquette and Williams, 2000, Kebschull et al., 2010).

- In susceptible locations in the vasculature, monocytes that migrate into the intima due to chemotactic cytokines will become dendritic cells. These cells will transform into **foam cells** after ingestion of oxidized low-density lipoproteins (LDL) (Schenkein and Loos, 2013, Kebschull et al., 2010). Foam cells will release inflammatory cytokines, chemo-attractants and metalloproteinases (MMPs) while macrophages will release the macrophage colony-stimulating factor that further enhances the inflammatory response in the lesion. Th1 cells are main components of the lesion and will amplify the inflammatory process by producing, among other mediators, INF-gamma

(Schenkein and Loos, 2013, Paquette and Williams, 2000).

- Periodontal pathogens may also help in **atherosclerotic plaque maturation**. Maturation entails migration of **smooth muscle cells** (SMCs) into the intima, with progressive fibrosis. MMPs and other proteases will degrade the extracellular matrix, promote SMC migration, and stimulate production of new collagen. Fibrosis of the plaque will be enhanced by TGF-beta, Th1 cytokines, up-regulation, and dysfunction of endothelial cells. The inflammatory status of the lesion will increase as a consequence of the establishment of a compensatory blood supply within the lesion, while thrombin will be generated in the event of injury (Schenkein and Loos, 2013, Kebschull et al., 2010).

Plaque rupture appears to be mediated by inflammatory cells. An increase in matrix degradation by MMP, a decrease in SMC production, presence of Th-1 cells and mast cells seem to compromise the matrix and the fibrous cap. This instability may lead to a process of fissuring of the atheroma, leaving pro-coagulant factors present in the necrotic and lipidic core , such as tissue factor and von Willebrand factor, exposed. These pro-coagulant factors together with the sub-endothelial collagen will determine the formation of a potential thrombus (Faxon et al., 2004, Schenkein and Loos, 2013, Kebschull et al., 2010).

1.1.3. Schematic overview of the potential mechanisms linking periodontal infections and atherosclerosis

The following figures explain the potential mechanism linking periodontal infections and endothelial dysfunction / incipient atherosclerosis (Fig. 2), fatty-streak formation/plaque maturation (Fig.3) and mature atherosclerotic plaques and plaque rupture (Fig.4) (Kebschull et al., 2010).

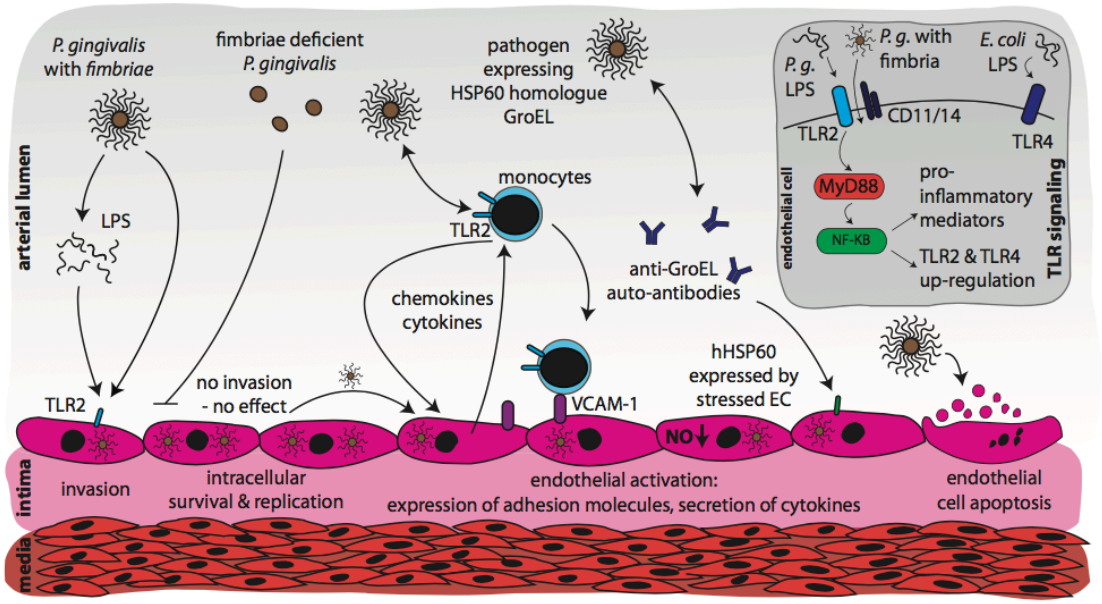


Fig 2. *P. gingivalis and other fimbriated bacteria affect the endothelial function. Cytokines and inflammatory mediators are released. Endothelial cells are activated and invaded. TLR-2 is expressed, adhesion molecules up regulated and monocytes recruited. Molecular mimicry: antibodies against bacterial HSP auto-react with mammalian HSP60 expressed by activated endothelium leading to cell destruction. P. gingivalis may induce apoptosis.*

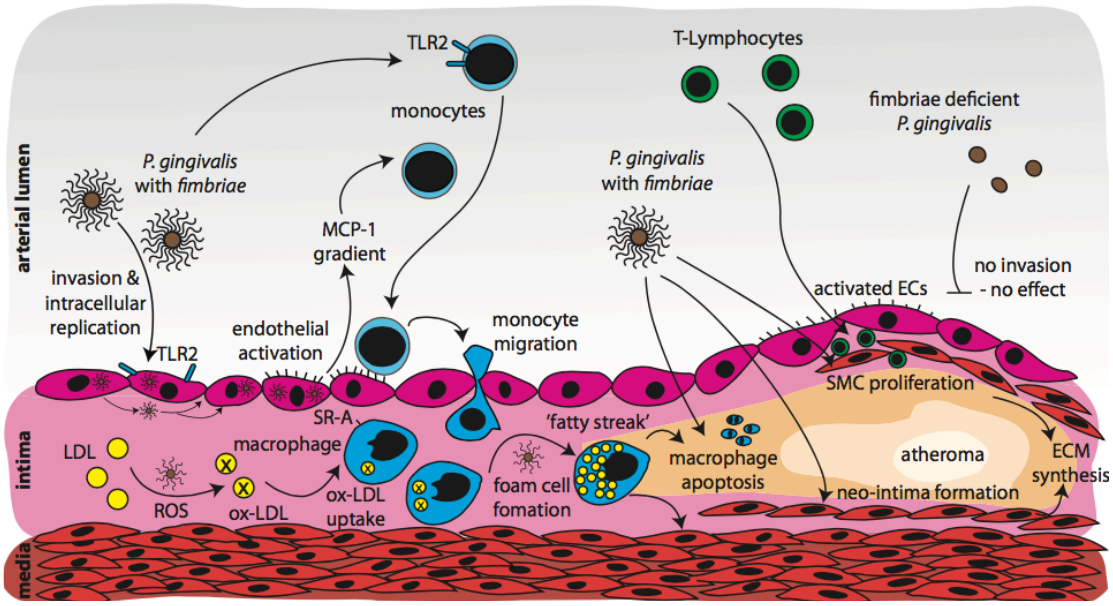


Fig 3. *Activated monocytes migrate into the sub-endothelial space, and transform into foam cells after uptake of oxidized LDL. Accumulation of lipids takes place when these cells suffer apoptosis. Cells of the smooth muscle proliferate in the intima and neo-intima and there is an extracellular matrix build-up and extravasation of T-cells that consummate the formation of a fibrous cap covering the plaque.*

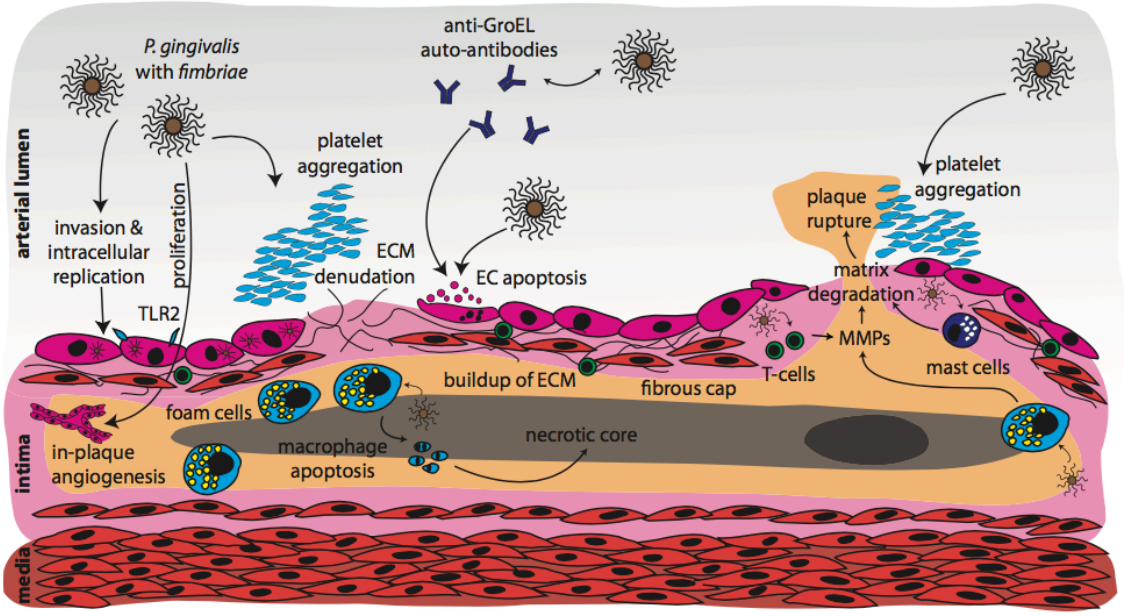


Fig 4. *Angiogenesis in the plaque. Endothelial cell apoptosis mediated by bacteria or by immunitary reaction (molecular mimicry) and extracellular matrix degradation by endothelial cells, plaque macrophages, T-cells, and plasma cells lead to denudation of the fibrous cap and release of pro-thrombotic factors and posterior occlusion.*

1.2. Endpoints and surrogate markers used to study the association between PD and CV

Coronary heart disease (CHD), coronary artery disease (CAD), atherosclerotic vascular disease (ASVD), myocardial infarction (MI), acute coronary syndrome (ACS), stroke and cerebral vascular accident (CVA) have been used as outcome variables in epidemiologic studies (Lockhart et al., 2012).

Surrogate markers for atherosclerosis have also been identified and used in mechanistic and epidemiologic studies. The surrogate markers are based on the identification of inflammatory markers and assessment of anatomical changes and functional aberrations in the vasculature or end organ impact (Lockhart et al., 2012).

Endothelial dysfunction is a surrogate marker that has been demonstrated in patients with risk factors for atherosclerosis even in the absence of atherosclerosis itself. The clinical relevance of endothelial dysfunction has been borne out in recent studies as its existence predicts the presence of significant coronary artery disease and provides prognostic information about the likelihood of events in patients with coronary artery disease (Faxon et al., 2004).

Methods to determine endothelial dysfunction and therefore subclinical atherosclerotic disease include (Jacobs and Crow, 2007):

- *Computed tomography (CT) of the coronary arteries* in order to assess calcium deposits in the endothelium and plaques (O'Leary et al., 1999)

- *Ultrasound of the carotid arteries* to assess presence of plaques and thickening of the intima and media (IMT) layers of the vessel wall

- *Echocardiography and magnetic resonance imaging (MRI)* as it allows the assessment of many parameters, including left ventricular size, wall motion abnormalities, arterial distensibility, and cardiac output

- *Applanation tonometry* that will allow the calculation of PWV and pulse pressure, commonly tested in the radial artery

On the other hand, various indices have been introduced to assess arterial function. These include concepts related to arterial distensibility, arterial compliance, Peterson's elastic modulus, Young's elastic modulus, stiffness index (β), etc. The indexes most widely used are:

- *Systolic and diastolic blood pressure.* The most popular measurement, it is calculated through central catheters of sphygmomanometry. It provides information about the arterial, hemodynamic and cardiac characteristics.

- *Ankle-brachial index*, which compares blood pressure in the arms versus legs as a

measure of potentially reduced peripheral blood flow, often related to atherosclerosis.

- *Microalbuminuria* and other biochemical measures of kidney dysfunction, as the kidney is a primarily vascular organ. Microalbuminuria assesses changes in endothelial cells and is predictive for hypertension and diabetes

- *Flow-mediated vasodilation (FMD)* of the brachial artery. This allows the assessment of the ability of the artery to recover after occlusion, which is presumably a function of the health of the endothelium and perhaps nitric oxide (NO) release

- *Pulse pressure (PP),* defined as the difference between the systolic and diastolic blood pressures (i.e. the pressure increase required to generate a pulse), has been recognized as a significant predictor of all-cause cardiovascular mortality and morbidity (Dart and Kingwell, 2001). PP can be assessed in peripheral arteries using cuff sphygmomanometry at the brachial artery and applanation tonometry at the radial or other sites (Filipovsky et al., 2000, Li, 2017). Measuring PP at the large central arteries remains challenging as the only method to obtain a direct measurement is by a catheter.

- *Augmentation pressure (AP*), is defined as the difference between the second and the first systolic peak (Weber et al., 2004). (The AP caused by pulse wave reflection will add additional pressure and stress to the left ventricle. The AP

together with the *augmentation index (Aix*), which is calculated by dividing the augmentation pressure by the pulse pressure, have been shown to be predictive for coronary artery disease (Jockel-Schneider et al., 2014) and premature coronary artery disease (Weber et al., 2004). In addition, pulse waveform analysis methods appear to be more repeatable than FDM.

- *Pulse wave velocity*, which will be explained in detail throughout this work.

1.3. Arterial stiffness in cardiovascular disease

In the literature, anatomical and functional changes in the vessels at subclinical stages of atherosclerosis and cardiovascular disease have been described. Aortic stiffness is an important subclinical functional indicator of arterial stiffness and has been proven to be a strong independent predictor of all-cause and cardiovascular mortality, primary coronary events, and fatal stroke in patients with hypertension (Laurent et al., 2001), end-stage renal disease, and type 2 diabetes, as well as in the general population (Luo et al., 2012).

Arterial stiffness refers to a reduction in the capability of an artery to expand and contract in response to pressure changes. As the aorta stiffens it leads to a range of linked pathophysiological changes within the circulation. These changes translate into the reduction in the ability to accommodate the volume of blood ejected by the left ventricle and as a consequence, an increase in the pressure during systole is observed

(Quinn et al., 2012). Another reasonable consequence of this increase in central blood pressure may be the increase of the left ventricular mass, which may be assessed through electrocardiograms (Franek et al., 2009)

When the arteries are stiffer, the pulse wave will also be reflected earlier than expected, impacting the central arteries and amplifying aortic and ventricular pressures during systole. As the central pressure increases, the gradient between the central and peripheral pressure diminishes and there is an increase in the peak- and end-systolic pressure in the aorta, resulting overall in the raise of the myocardial pressure load (ventricular hypertrophy) and oxygen consumption (Briet et al., 2012). Parameters that describe vessel stiffness include *compliance* and *distensibility*. *Compliance (C)* constitutes a measure of volume change (ΔV) in response to a change in blood pressure (ΔP), or $C = \Delta V/\Delta P$. *Distensibility (D)* is defined as compliance relative to initial volume ($D=\Delta V/\Delta P \times V$) and relates more closely to wall stiffness (Cecelja and Chowienczyk, 2012).

Parallel to the reduction in the capacity of distension of the artery, there is also a reduction in the aortic elastic recoil. This will lead to a fall in diastolic pressure and to widened pulse pressure (difference between systolic and diastolic blood pressure), which can be observed in pathological processes but also as a physiological consequence of age (Quinn et al., 2012).

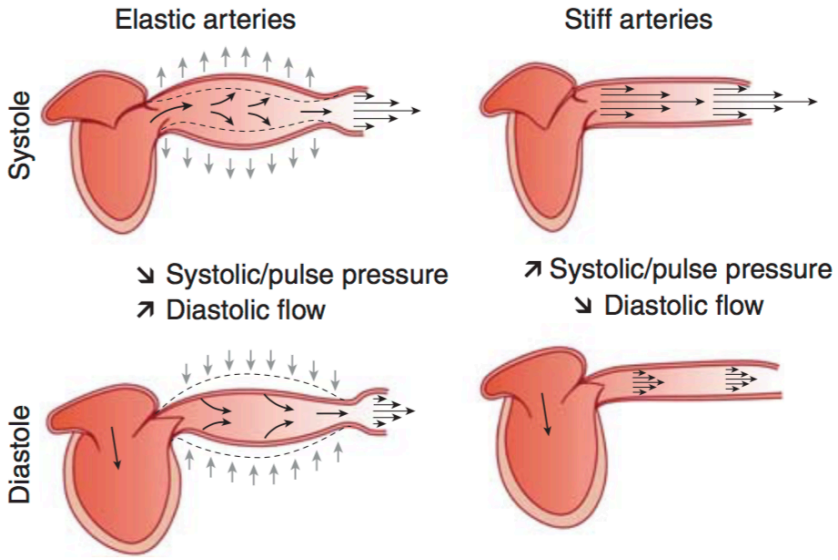


Fig 5. *Schematic representation of the role of arterial stiffness in assuring blood flow through the peripheral circulation (Briet et al., 2012)*

The relationship between age and microcirculation, end-oral damage, and arterial stiffness may be explained by the existence of reflection waves and stiffness gradient. Waves get reflected due to the reduction in diameter and local arterial branching of the vessels when advancing distally from the heart, the stiffness gradient (as peripheral arteries are stiffer than central arteries in healthier and younger individuals), and a pathological increase of stiffness. The mentioned reflection will amplify the forward-advancing wave and the effect on the vessels will be different depending on the area of the arterial tree where forward wave meets the reflected wave.

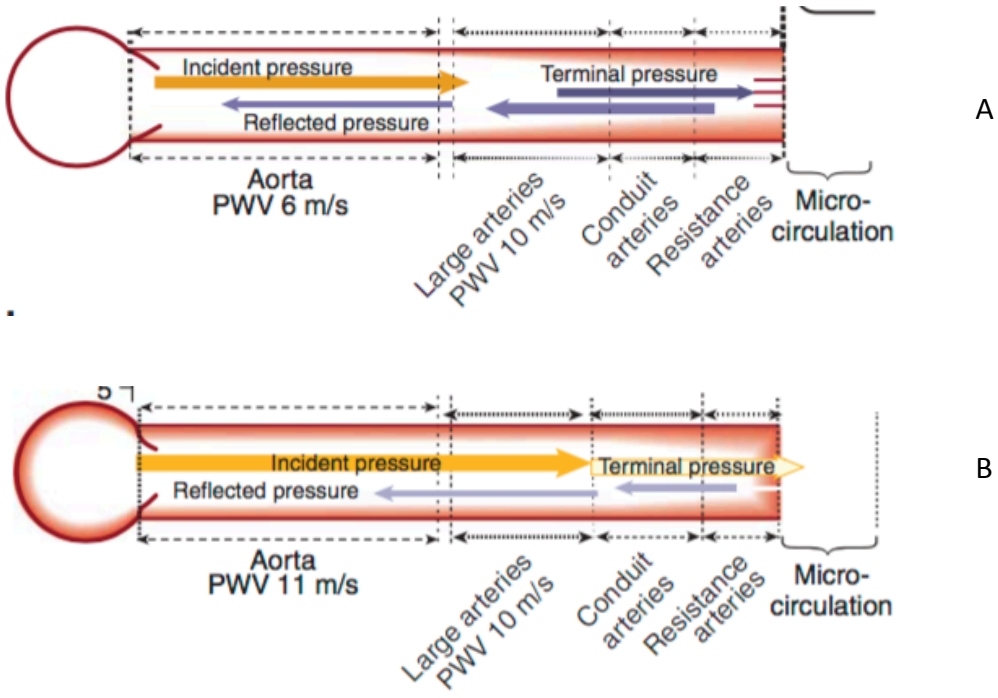


Fig 6. *A) When the gradient is present (aortic stiffness (PWV) < peripheral stiffness (PWV)), the reflection of the wave occurs far from the microcirculation and at low PWV in the area close to the aorta in diastole, maintaining the central-to-peripheral amplification and protecting the microcirculation. B) When the gradient disappears, the pulse pressure is not sufficiently dampened and it is transmitted, damaging the microcirculation.*

Various indices have been introduced to quantify arterial stiffness. These include arterial distensibility, arterial compliance, Peterson's elastic modulus, Young's elastic modulus, stiffness index (β), and pulse wave velocity (PWV). Among these, PWV is "the most hallowed (and still probably the best)" measure of arterial stiffness (Luo et al., 2012), although flow-mediated dilation (FMD), which is a functional index, and

intima media thickness (IMT), which is an anatomical index, have also been used extensively in the literature.

1.3.1. Flow-mediated dilation (FMD)

Flow-mediated dilation is assessed through the hyperemia provoked after the release of cuff-mediated occlusion. In order to differentiate endothelial from muscular mediated dilation, the assessment is performed in natural conditions and after taking nitroglycerin. The estimate of the distension of the vessel is performed often in a peripheral artery like the brachial artery and methods for its assessment include 2D ultrasound images, intra-arterial transducers, applanation tonometry and/or Doppler flow signals.

1.3.2. Intima media thickness (IMT)

Intima media thickening is thought to represent one of the earliest stages of atherosclerosis. It predicts atheromatous plaque development, characterized by the presence of vascular smooth muscle cells, elastin and proteoglycans, but not lipid deposits.

Holdis, 1998 determined in a university-based study that for each 0.03-mm increase per year in carotid arterial intima-media thickness, the relative risk for nonfatal myocardial infarction or coronary death was 2.2 (95% CI, 1.4 to 3.6) and the relative risk for any coronary event was 3.1 (CI, 2.1 to 4.5) ($P < 0.001$) (Hodis et al., 1998).

The association between IMT and arterial stiffness remains unclear. Although some studies have shown correlation between IMT and PWV or other measures of distensibility, such associations (when positive) have been relatively weak. On the other hand several studies have shown no relation, suggesting that wall hypertrophy does not contribute to intrinsic wall stiffness of peripheral arteries (Cecelja and Chowienczyk, 2012).

1.3.3. Pulse Wave Velocity (PWV)

Arterial stiffness, as measured by carotid-femoral PWV, is an independent predictor of cardiovascular morbidity and mortality in patients with hypertension, type 2 diabetes, and end-stage renal disease, and in elderly populations. Given the predictive power of PWV, identifying strategies that prevent or reduce stiffening may be of clinical relevance in the prevention of cardiovascular events (Luo et al., 2012).

PWV is calculated by measuring the time taken for a pressure pulse to travel between two set points (Figure 2) (Cecelja and Chowienczyk, 2012). In the conventional foot-to-foot method, the sites in which the temporal pulse profile is obtained are the carotid and femoral artery as they represent superficial arteries of easy access. The PWV is calculated as the distance between two measurement points divided by the time shift of the waveforms at the two points. By measuring the time delay Δt of the same point in the wave-form at the start of the wave (i.e. the foot of the wave) and by estimating the arterial distance ΔD between the two measurement locations, the PWV can be simply estimated as $PWV = \Delta D/\Delta t$ (Laurent et al., 2006). Increase in PWV

(faster velocity) corresponds to reduced compliance/distensibility and therefore translates into the existence of a stiffer artery (Luo et al., 2012).

A B

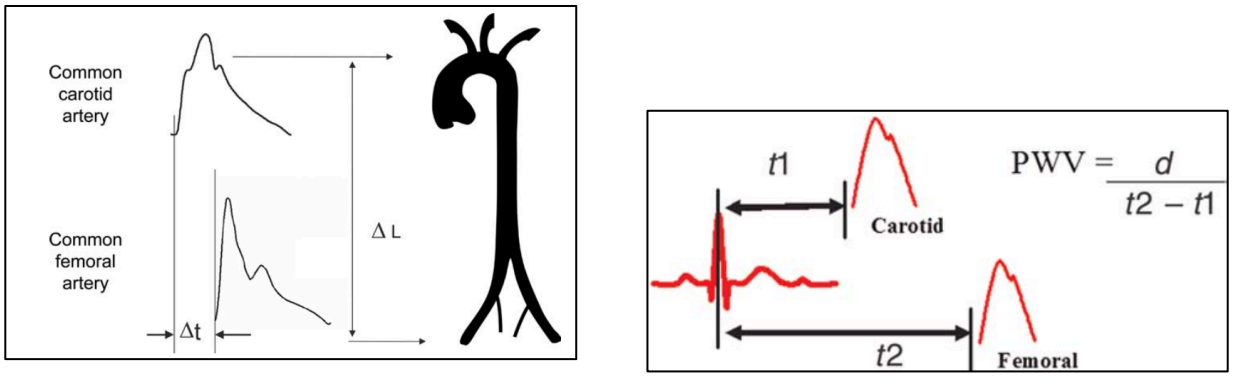


Fig 7. *PWV measurement through foot-to-foot method. Carotid-to-femoral PWV is calculated by dividing the distance (d) between the two arterial sites by the difference in time of pressure wave arrival between the carotid (t1) and femoral artery (t2) referenced to the R wave of the electrocardiogram A) (Laurent et al., 2006); B) (Cecelja and Chowienczyk, 2012)*

The vast majority of studies involving PWV of the aorta (aPWV) to date have used two techniques: Complior® and Sphygmocor®, both involving probes placed in the carotid and femoral arteries.

There is concern that values derived from these techniques may not accurately represent stiffness of the aorta, but are in part determined by local arterial

properties. There are several drawbacks described when measuring PWV through these techniques, including the following points.

- The accuracy of PWV measured from the foot-to-foot method may suffer from errors in the measurement of the distance between the two measuring sites. Another source of inexactness in the measurement is that the arteries are not necessarily uniform or straight, especially in aged subjects and patients with vascular disease.

- Errors may also derive from the difficulty of measuring small time shifts, as the pulse wave travels on the order of several meters per second. Measurements are therefore highly technique sensitive.

- There is a lack of uniformity in the elastic properties of the vessels along the vasculature. It is known that the stiffness of the arteries, and thus PWV, increases from the proximal to the distal regions. Therefore, there are concerns that these derived values may not accurately represent stiffness of the aorta, but are in part determined by local arterial properties of the arteries used for the measurements (Vappou et al., 2011b, Quinn et al., 2012).

Techniques that calculate regional PWV have been proposed in order to overcome some of these issues. They may offer better control of some of the aspects described above by calculating PWV throughout one location.

Examples are Arterio-graph® and Mobil-o-Graph® that calculate PWV from oscillometric measurements of the brachial artery waveform. These techniques are simpler and regional, and they make widespread clinical measurement of aPWV a realistic possibility in the near future (Quinn et al., 2012).

Between the factors to consider when calculating PWV it is important to outline:

- Age: brachial PWV changes little with age despite significantly increased blood pressure (Cecelja and Chowienczyk, 2012).

- Presence of atheromatous plaque: conflicting findings have been reported, probably related to differences in the composition of plaques. The Rotterdam Study found no association between mild plaque score and PWV although PWV was significantly increased in cases of more severe and calcified plaques (van Popele et al., 2001).

- Blood pressure: PWV depends upon the distending pressure, best represented by the mean arterial pressure (MAP) and the pulse pressure (PP). An increase in MAP corresponds to higher measurements of PWV and therefore, stiffer arteries. In longitudinal studies increased blood pressure determines progression of arterial stiffness (Quinn et al., 2012). Other studies did not find that blood pressure measured in a peripheral artery was related with PWV, although this relationship was present when considering central pulse pressure (PP) (Li, unpublished). Measuring central PP is a challenge, as its

direct measurement is only possible by an invasive catheter. Several authors have employed radial applanation tonometry to derive central PP in large populations of patients (Osmanski et al., 2015). However, an indirect method may not be used for evaluation on an individual patient basis.

1.4. Pulse Wave Imaging (PWI)

PWI is a novel technique developed at the Ultrasound and Elasticity Imaging Laboratory (UEIL), Department of Biomedical Engineering, Columbia University that facilitates the study of arterial wall displacements and PWV.

PWI is an ultrasound-based technique that allows the non-invasive quantification of the regional arterial stiffness by estimating the PWV. It also permits a qualitative visualization of the propagation of the pulse wave in real time (Fujikura et al., 2007) and an analysis of the arterial waveform and arterial wall displacements, allowing the calculation of the central pulse pressure (PP). This is a technique known as Pulse Wave Ultrasound Manometry (PWUM) and it is based in the incremental distension waveform observed in the scans and the application of the Laplace law and the Modified Moens-Korteweg equation (Vappou et al., 2011b, Li, 2017)

Several medical imaging-based methods have been previously developed for noninvasive measurements of regional PWV, mainly using magnetic resonance imaging (MRI) and ultrasound. On the other hand, catheterization has been used in

central arteries and is an invasive method. Applanation tonometry represents a non-invasive method to calculate PP in peripheral arteries, although questions are raised about reliability of the measurements obtained through tonometry in general (Vappou et al., 2011b).

One advantage of PWI over other PWV estimation methods is that the propagation patterns of the pulse wave can also be visualized. This information can be very useful for diagnosis of local vascular diseases such as abdominal aortic aneurysm (AAA), as previously demonstrated by this group. PWI has been validated with finite-element simulations, phantom experiments using mechanical testing, and healthy subjects using applanation tonometry (Luo et al., 2012).

Originally, the technique was developed using the longitudinal (long-axis) of the aorta artery (Fujikura et al., 2007) and has been validated in AAA (Nandlall et al., 2014), carotid of healthy patients (Luo et al., 2012) and in cases presenting with stenosis of the carotid (Li et al., 2013).

1.4.1. Data acquisition

The images are captured using a SonixTOUCH system (Ultrasonix Medical Corp., Burnaby, BC, Canada) and a L14–5/38 linear array operating at 10 MHz with the patient in supine position.

The image depth is fixed at a convenient distance for the visualization of the walls of the artery. In order to obtain the information required to calculate PWV, different

frame rates are applied for each parameter. Due to the high speed that the pulse wave travels (about 5m/s), a high frame rate (Hz) (approximately around 505Hz to 1127Hz) is necessary to visualize its propagation. Therefore, the beam density is reduced to values approximate between 16 to 32 beams for PWV acquisition. This acquisition takes 2.5 seconds and generally includes two cardiac cycles.

Subsequently, these parameters must be changed to obtain the images that will allow the segmentation of the anterior (closer to the transducer) wall. For these means, a high quality image is obtained by increasing the density of the beams to approximately 128-256 and reducing the frame rate to an approximate range of 63-140 Hz for 0.2 seconds.

A full PWI acquisition (combining both high and low beam densities) lasts about 3 seconds, in which the patient is asked to hold his/her breath. About 5-15 acquisitions are obtained per patient, including approximately 10-20 cardiac cycles. The total time of acquisition per patients is approximately 20 seconds to 1 minute (Fujikura et al., 2007, Li et al., 2011) (Fig. 8).

1.1.1. PWV calculation

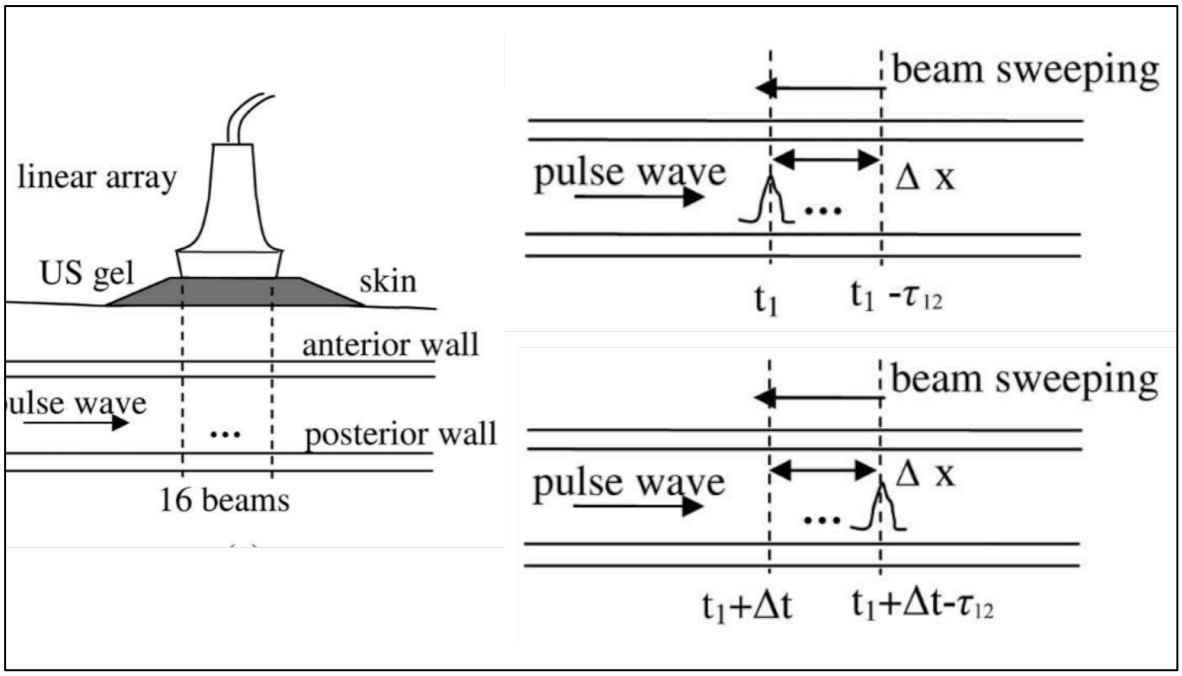


Fig 8. *Direction of beam sweeping and PWV calculation*

PWV calculation is based on the estimate of 1D radial incremental displacements of the arterial wall in a longitudinal view (Fujikura et al., 2007).

At time t_1, the pulse wave arrives at the leftmost beam. Several (N) frames later, the pulse wave arrives at the rightmost beam. The distance between the leftmost and rightmost beams is Δx. The time delay between these two frames is Δt, equal to N/(frame rate). The true propagation time of the pulse wave is $\Delta t - \tau_{12}$, and the true PWV is $\Delta x/(\Delta t - \tau_{12})$, where τ_{12} is the time delay between the leftmost and rightmost beams resulting from beam sweeping. Without correction, the propagation time of

the pulse wave is estimated as Δ*t* (i.e., overestimated), and the PWV is estimated as Δ*x*/Δ*t* (i.e., underestimated) (Luo et al., 2012) (Fig. 8).

The spatiotemporal variations of the displacement allow the visualization of the pulse wave propagation and the estimate of PWV. The wall velocities are color-coded and overlaid onto the B-mode image. Positive velocities (in red) represent upward motion (Fig. 9, Fig. 10 a, b, c)

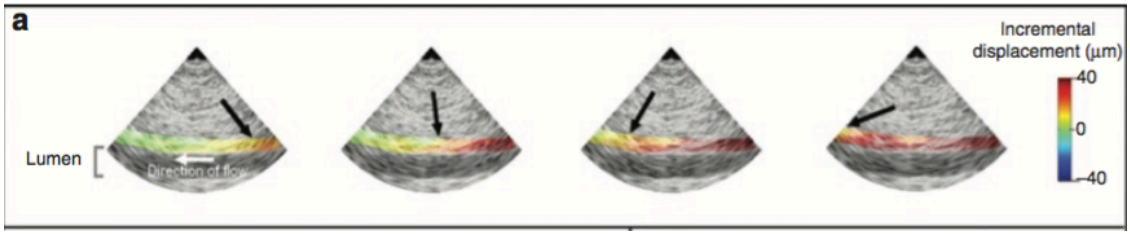


Fig 9. *Aortic wall displacement at four different frames (delay between two successive frames is 7.7 ms) overlaid onto B-mode image, showing clearly the propagation of the wave front (black arrow) from right to left. For clarity, the displacement is also represented on the surrounding tissue. The solid arrows indicate the propagation of the pulse wave from the proximal (left) to the distal (right) sites.*

The pulse wave propagation is represented in a spatio-temporal diagram (Fig. 10 d). The time when the peak velocity occurs is depicted for each longitudinal position of the aorta and both parameters are plotted. The linear regression of the pulse-wave propagation is performed to examine the relation between the distance (longitudinal position of the vessel) (independence variable) and the timing of the maximum wall velocity (time of the foot of the pulse wave) (dependent variable). The slope defined

indicates the regional PWV. Subsequently PWV is estimated as the inverse of the slope of the linear regression and is expressed in m/s (Fig. 10 f). The correlation coefficient of linear regression (R^2) will express the quality of the regression fit and will therefore provide the qualitative portion of the PWI index. Pulse pressure (PP) is obtained through the PWV measurement.

PWV results will consecutively be expressed as mean ± standard deviation (SD) from the measurements obtained for each cardiac cycle (Fujikura et al., 2007, Luo et al., 2009, Luo et al., 2012)

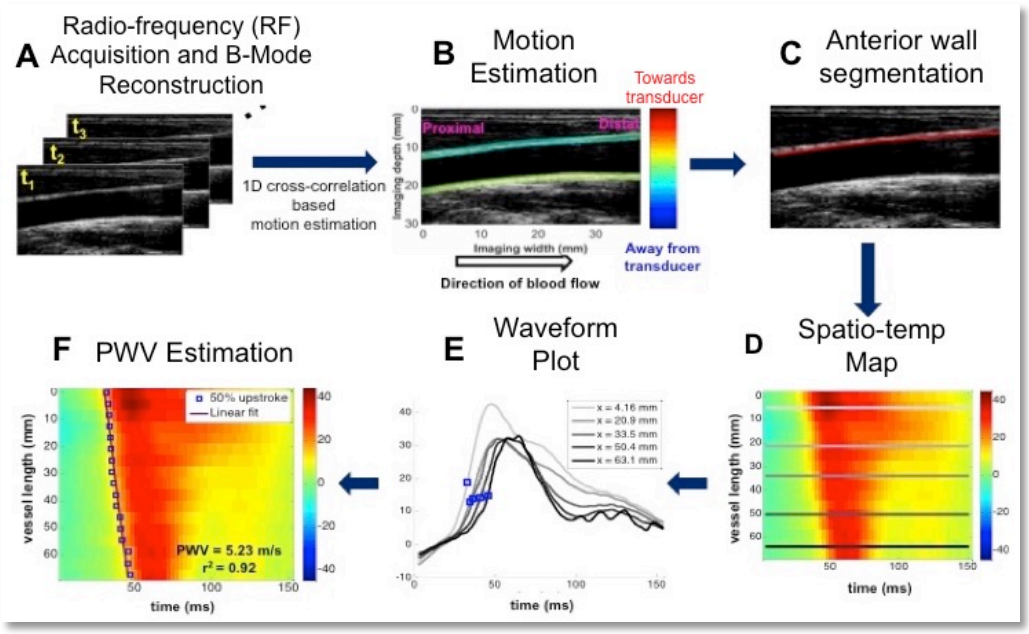


Fig 10. *Overview of PWI generation and PWV calculation*

1.5. Arterial stiffness and Periodontitis

Several peer-reviewed publications explore the relationship between arterial stiffness (a consequence of endothelial dysfunction) and periodontitis. The three methods used in these studies are:

- Periodontitis and flow mediated dilation (FMD) of the brachial artery
- Periodontitis and intima media thickness (IMT)
- Periodontitis and pulse wave velocity (PWV) of the peripheral arteries

It is difficult to reach a general conclusion from these studies as the criteria used to define periodontitis and the inclusion/exclusion criteria are not uniform and the methods and sites used to measure the elasticity of the vessels differ.

1.5.1. Periodontitis and flow mediated dilation (FMD) of the brachial artery

Amar 2003 tested FMD and nitroglycerin-mediated dilation of the brachial artery using vascular ultrasound and serum levels of C-reactive protein in a sample of 26 age- and gender-matched subjects with advanced periodontal disease and 29 periodontally healthy controls. Patients with risk factors for CVD were excluded. The results of this case-control study showed that periodontitis patients presented a higher level of C-reactive protein and a decreased FMD. This result was still valid when matching the patients for baseline vessel diameter, but it was not valid when

considering a subgroup of patients with mild periodontitis in which FMD and C-reactive protein results were comparable to controls (Amar et al., 2003).

Mercanoglu 2004 (Mercanoglu et al., 2004), Seinost 2005 (Seinost et al., 2005), and Blum 2007 (Blum et al., 2007) published cross-sectional case-control studies, also including longitudinal results for the periodontitis groups after treatment. Elter 2006 (Elter et al., 2006) and Tonetti 2007 (Tonetti et al., 2007) are longitudinal studies comparing a cohort of periodontal patients before and after periodontal treatment. The latter study constitutes an RCT in which two approaches of periodontal treatment were compared: intensive periodontal treatment vs. community-based periodontal care.

Higashi 2008 included patients with a CV risk factor and compared a cohort of case and control patients, before and after treatment, differentiating patients with high blood pressure from medically healthy periodontal patients (Higashi et al., 2008).

In spite of the differences between all these studies, there is a general trend towards impaired FMD in patients affected by periodontitis and improvement after periodontal treatment. Tonetti 2007 shows impairment in FMD in the first days after treatment followed by an improvement at the 6-month follow-up. The group that received community-based periodontal care (control) didn't show any change, proving a dose-dependent effect of the treatment (Tonetti et al., 2007).

Higashi 2008 demonstrated that periodontitis patients presented with impaired endothelium-dependent dilation. It was also shown that periodontitis therapy augmented Ach-induced vasodilation, possibly through an increase in NO production. This study also outlines the importance of presenting concomitantly with high blood pressure as in patients with hypertension the presence of periodontitis greatly increased the magnitude of endothelial dysfunction. These findings suggest that treatment of periodontitis is especially important in patients with high blood pressure (Higashi et al., 2008).

FMD	Type of study	Inclusion/ exclusion criteria based on medical history	n	Outcome variable	Method used to assess endothelial dysfunction
(Amar et al., 2003)	Case-control Observational gender- and age-matched study	Exclusion of hypercholesterolemia, diabetes mellitus, hypertension and history of smoking	26 patients with periodontitis 29 controls	FMD	FMD and nitroglycerin-mediated dilation (NMD) of the brachial artery. 2D ultrasound images and Doppler flow signals

(Mercanoglu et al., 2004)	Case-control study Observation and comparison before-after treatment Matched sample for medical history characteristics	All medically healthy or borderline for hyperlipidemia	28 patients with periodontitis 26 controls	FMD	FMD and NMD of the brachial artery. 2D ultrasound images
(Seinost et al., 2005)	Matched case-control study Observation and comparison before-after treatment	Excluded if history of CVD, DM, HBP or hypercholesterolemia, systemic illnesses, treated with CV or anti-inflammatory mediations, or antibiotics or antioxidant agents in the prior 3 months	30 patients with periodontitis 31 controls	FMD C-reactive protein	FMD and NMD of the brachial artery. 2D ultrasound images
(Elter et al., 2006)	Comparison of a cohort before and after treatment of periodontal disease	Excluded if pregnant or anticipated pregnancy during study, history of heart disease or stroke, DM, HBP or treatment with hypertensive mediations and any blood or bleeding disorders	10 patients with periodontitis	FMD C-reactive protein IL-6 Total cholesterol, HDL levels	FMD and NMD of the brachial artery. 2D ultrasound images
(Blum et al., 2007)	Age-matched case-control study Observation and comparison before-after treatment	Excluded if had any risks factors for atherosclerosis	22 patients with periodontitis 10 controls	FMD	FMD and NMD of the brachial artery. 2D ultrasound images and Doppler flow signals

(Tonetti et al., 2007)	RCT. Intensive periodontal treatment vs. community based periodontal care	Excluded if systemic disease, history or presence of any other acute or chronic infections and if systemic antibiotic treatment in the prior 3 months	61 patients 59 controls	FMD Inflammatory, coagulation and endothelial activation markers	FMD and NMD of the brachial artery. 2D ultrasound images and Doppler flow signals
(Higashi et al., 2008)	Case-control study Observation and comparison before-after treatment	Group with healthy patients and group with high blood pressure	No HBP: 32 patients with periodontitis and 20 controls HBP: 28 patients with periodontitis and 24 controls	Blood flow C-reactive protein IL-6	Intra-arterial pressure transducer after acetylcholine and nitroglycerin

Table 1. Periodontal-flood mediated dilation literature

FMD	Definition of periodontitis and controls
(Amar et al., 2003)	Advanced periodontitis. ≥ 6 teeth with PD>5mm and LOA of ≥3mm in 3 aspects of each involved tooth. Control: no PD ≥2mm and no LOA ≥3mm.
(Mercanoglu et al., 2004)	Chronic periodontitis
(Seinost et al., 2005)	Advanced periodontitis: Min 6 teeth with PD ≥ 5mm, LOA ≥ 3mm in 3 aspects of each involved tooth. Controls: No PD ≥2mm and no LOA ≥3mm.
(Elter et al., 2006)	Moderate to severe periodontitis. ≥1 tooth in at least 2 quadrants. Min 4 sites with PD ≥5mm in at least 2 quadrants and min 2 of the 4 sites with LOA ≥3mm.

(Blum et al., 2007)	Severe periodontitis
(Tonetti et al., 2007)	Severe periodontitis: PD >6mm and marginal alveolar bone loss >30% with 50% or more of their teeth affected
(Higashi et al., 2008)	Mild-to-moderate periodontitis

Table 2. Definition of periodontal disease used in the articles published on flow-mediated dilation

1.5.2. Periodontitis and intima media thickness (IMT)

Increase in the thickness of the IMT has been correlated with subclinical atherosclerosis (Beck and Offenbacher, 2001). The Atherosclerosis Risk in Communities (ARIC) study is a multicenter epidemiologic study on 6017 participants. In a multivariable logistic regression model, severe periodontitis was associated with IMT ≥1 mm (OR 1.31, CI 1.03 to 1.66), while adjusting for the other factors in the model. These results provided the first indication that periodontitis may play a role in the pathogenesis of atheroma formation, as well as in cardiovascular events.

Desvarieux 2005 in an epidemiologic study found a direct relationship between microbiological profiles associated with periodontal disease and subclinical atherosclerosis and that this relationship existed independent of C-reactive protein levels. Eleven periodontal bacteria were quantitatively assessed by checkerboard hybridization. Tertile groups corresponding to the presence of bacteria and risk of periodontitis associated to them were constructed. Adjusted mean IMT values across tertiles of bacterial dominance were 0.84, 0.85, and 0.88 (p=0.002). Similarly, white blood cell values increased across the mentioned tertiles from 5.57 to 6.09 and 6.03

cells x10^9/L (*P*=0.01). C-reactive protein values were unrelated to periodontal microbial status (*P*=0.82) (Desvarieux et al., 2005).

Piconi 2009 provided periodontal treatment to a cohort of 35 systemically healthy patients diagnosed with mild to moderate periodontal disease. A decrease in the bacterial load, an improvement in the inflammatory biomarkers and a reduction in the IMT were observed after treatment (Piconi et al., 2009).

IMT	Type of study	Inclusion/ exclusion criteria based on medical history	n	Outcome variables	Method to assess endothelial dysfunction
ARIC (Beck et al., 2001, Beck and Offenbacher, 2005)	Cross-sectional epidemiological study	No exclusion criteria applied	5953 subjects	IMT (cut-off established at 1mm) Serum biomarkers	B-mode ultrasound of carotid arteries
INVEST (Desvarieux et al., 2005)	Cross-sectional epidemiological study	Excluded if history of stroke or myocardial infarction	657 subjects	IMT Periodontal bacteria assessment Serum biomarkers	B-mode ultrasound of carotid artery

(Piconi et al., 2009)	Longitudinal prospective study No control All patients treated	Excluded if systemic disease, including presence of any CV risk factor	35 patients	IMT Serum biomarkers Microbiological assessment	Ultrasound scanner using electrocardiography-triggered images of the right and left carotid arteries at 1- and 2-cm points from bifurcation Echo-Doppler

Table 3. Periodontal- intima-media thickness literature

IMT	Definition of periodontitis and controls
ARIC (Beck et al., 2001)	None/mild (<10% of sites with AL≥ 3 mm; may include subjects with no clinical manifestations of periodontal disease, gingivitis, or slight periodontal disease), moderate (10% to <30% of sites with AL ≥3 mm), or severe (≥30% sites with AL ≥3 mm)
(Desvarieux et al., 2005)	Patients characterized by presence of 11 bacteria typically related to periodontal disease
(Piconi et al., 2009)	Mild to moderate periodontal disease

Table 4. Definition of periodontitis in the literature published on intima-media thickness

1.5.3. Periodontitis and pulse wave velocity (PWV) of the periphery arteries

Miyaki 2006 divided the sample of patients in “atherosclerosis positive” and “atherosclerosis negative” based on their PWV results. The cut-off applied was baPWV≥1400 (cm/sec) and the patients presented with mild periodontitis, classified using the CPITN index. The results show that the unadjusted odds ratio (OR) of atherosclerosis in relation to the CPITN score was 1.41 [95% CI: 1.16–1.73]. However,

after adjustment for age, systolic blood pressure and smoking, the CPITN score had no relationship with atherosclerosis (adjusted OR: 0.91 [0.68–1.20]) (Miyaki et al., 2006).

Vieira 2011 studied a cohort presenting heterozygous familial hypercholesterolemia (hFH). The individuals were divided in severe and non-severe periodontitis. It was noted that the group suffering from severe periodontitis had higher values of PWV, IMT and diastolic blood pressure than the non-severe group, suggesting that periodontitis may contribute to increased cardiovascular risk profile in that specific population (Vieira et al., 2011).

Franek 2012 presented a cross-sectional study in patients suffering from diabetes mellitus (DM) type 2. 121 patients were included. 16 were classified as periodontally healthy, 87 presented with gingivitis and 18 as having periodontitis with moderate bleeding. The IMT and blood pressure measurements of patients with gingivitis and periodontitis were observed to be higher than the healthy group. Lipid parameters and PWV were comparable in all groups, concluding that periodontal inflammation didn't lead to greater arterial stiffness in a cohort of patients with DM type 2 (Franek et al., 2012).

Hayashida 2013 published an epidemiological study in which IMT and CAV (cardio-ankle vascular index) were tested. CAV is an index that measures PWV and relates it with arterial pressure. After adjusting for confounders and risk factors of CV disease, it was observed that each 1-mm increase in mean periodontal pocket depth was associated with an increased risk of a maximal cIMT >1 mm (adjusted odds ratio [OR],

1.430; 95% confidence interval [CI], 1.067 - 1.918; P=0.017) and mean CAVI of ≥8 (OR, 1.323; 95% CI, 1.003 -1.743; P=0.047). A linear, dose-dependent relationship was found between periodontal pocket depth, cIMT, and arterial stiffness (Hayashida et al., 2013).

Kapellas 2014 tested the effects of periodontal treatment on arterial structure and function among a group of aboriginal Australians. The difference in IMT between patients treated periodontally and controls 12 months after treatment was statistically significant (-0.026 [95% CI, −0.048 to −0.003] mm; *P*=0.03), indicating changes in the structure after treatment. On the other hand, there were no changes in PWV (mean difference, 0.21 [95% CI, −0.01 to 0.43] m/s; *P*=0.062), indicating no functional improvement after treatment (Kapellas et al., 2014b).

PWV	Type of study	Exclusion (E)/inclusion (I) criteria based on medical history	n	Outcome variables	Method to assess endothelial dysfunction
(Miyaki et al., 2006)	Cross-sectional	No exclusion criteria applied	291 male subjects	Brachial-ankle PWV	Tonometry sensors (VP-2000/1000)
(Franek et al., 2009)	Cross-sectional	(I) Patients with essential hypertension, no history of cardiovascular events (E) DM, secondary hypertension, patients positive to certain blood work finding related to hepatitis cytomegalovirus and	99 subjects	Carotid-radial PWV Central blood pressure (CBP) Left ventricular mass index	Echocardiography (M mode used to calculate LVMI Sphygmocor, Atcor Medical, using a high- density applanation tonometer (SPT-304, Millar

		treatment with glucocorticoids		(LVMI)	Instruments) Blood pressure measured with a standard cuff
(Vieira et al., 2011)	Pilot study Cross-sectional	(I) Patients had heterozygous familial hypercholesterolemia (hFH) (E) Patients free from vascular disease manifestations.	79 subjects	Carotid-femoral PWV IMT Serum biomarkers BP	Complior Pulsate ultrasonographic echo-tracking
(Franek et al., 2012)	Cross sectional study	(I) DM II	121 subjects	Carotid-radial PWV Lipids, CRP IMT CRP	Sphygmocor®, Atcor Medical), using a high-fidelity applanation tonometer (SPT-304, Millar Instru-ments) Ultrasounds used for IMT
(Hanaoka et al., 2013)	Cross-sectional study cohort of patients with history of IDH	(I) All patients presented with ischemic heart disease (IHD) with angiographic documentation of >50% organic stenosis in one or more major coronary arteries, or had a history of percutaneous coronary intervention or coronary artery bypass grafting. (E) Patients with heart failure, valvular heart	127 patients	Antibody levels against P.gingivalis (ELISA) Brachial-ankle PWV BP	PWV/ ABI (ankle brachial index) device (Nippon Colin, Komaki, Japan). Approved by the FDA as VP-2000/1000 (able to monitor bilateral brachial and ankle pressure wave forms)

		disease, cardiomyopathy, severe arrhythmia and infection were excluded.			
(Hayashida et al., 2013)	Community-based cross-sectional study	(I) Subjects presented with 10 or more teeth (E) No exclusion criteria applied	1053 subjects	CAVI (cardio-ankle vascular index) IMT Blood biomarkers	Electrocardiographic data obtained by electrodes. Equation applied using blood pressure and PWV Ultrasonography of the right and left carotid arteries
(Shanker et al., 2013)	Case-control	(I) Presence of clinical asymptomatic for coronary artery disease confirmed by angiogram, history of percutaneous intervention, coronary bypass or myocardial infarction. Indian origin of the population	814 subjects	Right and left brachial PWV and carotid-radial PWV arterial stiffness index (ASI) ankle brachial index (ABI) Saliva tested for periodontal bacteria	Electrocardiography (ECG) Periscope test (M/S Genesis Medical Systems, India), an oscillometry-based blood pressure monitoring and PC-based acquisition and analysis
(Vidal et al., 2013)	Interventional study Prospective cohort pilot study All patients received non-surgical periodontal	(I) Adult patients were diagnosed with severe essential refractory hypertension with at least two years of anti-hypertensive treatment (E) Presence of systemic conditions that contra-indicate periodontal therapy or	26 patients	Carotid-femoral PWV Systolic and diastolic blood pressure levels Left	Complior® system (Complior®, Colson, Garges les Genosse, France) Blood pressure monitoring device (TM 2430", A&D, Santa Clara, CA,

	treatment (longitudinal analysis of the results)	that might affect the progression or treatment of periodontitis		ventricular mass Plasma inflammatory markers	USA) Doppler echocardiographic evaluation (CV70^{TM}, Siemens^{TM}, Munich, Germany) ELISA kits
(Jockel-Schneider et al., 2014)	Cross-sectional periodontitis–control matched study	(E) Patients that had periodontal treatment in the last 5 years, antibiotic medication within the last 6 months, atrial fibrillation or severe cardiac valve vitium	158 subjects	Aortic PWV AIx (augmentation index) PPA (pulse pressure amplification)	Arteriograph (analysis of the oscillometric pressure curves)
(Kapellas et al., 2014a)	Cross-sectional	(I) Aboriginal Australian population (E) Patients with previous history of CVD	269 subjects	Carotid-dorsalis pedis PWV IMT Inflammatory markers in blood	Applanation tonometry to measure PWV (SphygmoCor. Carotid-dorsalis pedis) Ultrasounds used for IMT ELISA
(Kapellas et al., 2014b)	RCT (Interventional study) Periodontal treatment vs. no treatment		273 subjects		
(Houcken et al., 2016)	Case-control and pilot interventional study (treated with SRP and some of them also with antibiotics vs. reference group)	(E) Patients with a history of any given disease or chronic medical condition (including diabetes mellitus, rheumatoid arthritis, bacterial infections, severe hypertension (>160 systolic and/or >110 diastolic mm Hg) and body mass index (BMI)	109 subjects for case-control and 105 for interventional part of the study (45 patients treated)	PWV measured at the aorta Blood pressure Mean arterial pressure (MAP)	Arteriograph

		435 kg m^{-2} . Patients under treatment with anti-inflammatories were also excluded			

Table 5. Periodontal-PWV literature. (I) Inclusion criteria; (E): Exclusion criteria

	Definition of periodontitis and controls
(Miyaki et al., 2006)	Community periodontal index of treatment needs score (CPITN) (Ainamo et al., 1982).
(Franek et al., 2009)	CPITN score used (Ainamo et al., 1982). Cases: Chronic periodontitis (CPITN score 3 and 4) ; Controls: absence of chronic periodontitis or diagnosis of moderate periodontitis (CPITN 0 to 2)
(Vieira et al., 2011)	Severe periodontitis group (SPG): Individuals with severe periodontitis had ≥3 sites, not on the same tooth, with clinical AL ≥7 mm and ≥1 interproximal sites with PDs ≥5 mm. The non-severe periodontitis group (NSPG) included individuals who did not meet the previous conditions
(Franek et al., 2012)	Patients divided in periodontally healthy (BGI-H), gingivitis (BGI-G) and periodontitis with moderate bleeding (BGI-P2) (Offenbacher and Beck, 2007)
(Hanaoka et al., 2013)	Patients divided between High Pg-IgG and Low Pg-IgG. Periodontal risk score based on vector score calculated(*)(Renvert et al., 2004) (*) This index takes into account BoP, number of sites with PD≥6.0mm, number of teeth lost in the past and deducted from a total of 28 teeth, proportion of mesial/distal sites with evidence of distance from the CEJ to bone level ≥half the tooth length and smoking status with regard to packs/year. Full mouth radiographic and clinical evaluation. At four sites/tooth presence of plaque, BoP, PD were calculated. Number of teeth with signs of gingival recession and number of remaining teeth also accounted for.
(Hayashida et al., 2013)	A periodontal examination was performed using the method modified from the Third National Health and Nutrition Examination Survey. Epidemiologic

	study. Mean probing depth and CAL per patient calculated
(Shanker et al., 2013)	Gingivitis: presence of swollen or bleeding gums and gum recession Periodontitis: presence of tooth mobility, tooth decay, bone loss or tooth loss
(Vidal et al., 2013)	All patients diagnosed generalized advanced chronic periodontitis (Armitage, 1999)
(Jockel-Schneider et al., 2014)	Untreated severe generalized chronic or aggressive periodontitis. Diagnosis based on AAP and CDC definitions (Armitage, 1999, Page and Eke, 2007). Inclusion criteria involved the presence of clinically detectable attachment loss ≥ 6mm in a minimum of two different sextants and a minimum of six interproximal sites on six interproximal teeth. Individuals exhibiting minor periodontal pockets ≤ 3mm were designated periodontally healthy controls.
(Kapellas et al., 2014a) (Kapellas et al., 2014b)	Periodontitis case status defined as the presence of at least 2 interproximal sites with clinical attachment loss (CAL) ≥4 mm, or at least two interproximal sites with probing depth (PD) ≥5 mm (Page and Eke, 2007). Extent and severity of periodontitis determined (Carlos et al., 1986). Patients stratified in quartiles of extent PD ≥4 mm. post-hoc analysis using the stratifications of periodontal disease as described in the analysis from the dental component of the Atherosclerosis Risk in Communities (ARIC) study (Beck and Offenbacher, 2001, Beck et al., 2001)
(Houcken et al., 2016)	Periodontitis subjects were included, when having at least the presence of proximal attachment loss of ⩾ 3 mm in ⩾ 2 non-adjacent teeth with bleeding on probing (Tonetti et al., 2005)

Table 6. Definition of periodontitis in the literature published on PWV

	Findings and results
(Miyaki et al., 2006)	Atherosclerosis was defined as baPWV ≥1400 (cm/sec) and "Atherosclerosis +" and "Atherosclerosis –" groups were compared finding statistically significant differences between both groups for all the periodontal variables considered (CPITN, average PD, gingival bleeding index and prevalence of severe periodontal disease). The calculation of the Odds Ratio for atherosclerosis for all the periodontal variables considered was significant when data were unadjusted. On the other hand, the odds were not significantly higher when performing the

<table>
<tr><td></td><td>appropriate adjustments</td></tr>
<tr><td>(Franek et al., 2009)</td><td>Statistically significant differences found between patients with CPITN score 0-2 and CPITN 3-4 with regards to left ventricular mass index (p<0.01), aortic systolic pressure (mm Hg) (<0.05) and aortic pulse pressure (mm Hg) (p<0.05).

Differences with regard to PWV (m/s) were not statistically significant (7.9 ± 1.2 vs. 8.3 ± 1.5 with a p = 0.15)</td></tr>
<tr><td>(Vieira et al., 2011)</td><td>The “severe periodontitis group” (SPG) showed significantly higher values of cholesterol-year scores, triglycerides, glucose, PWV, IMT, and diastolic blood pressure (DBP) (P <= 0.05) than the “non-severe periodontitis group”.

Differences with regard to SPG PWV (m/s) and the non-severe periodontitis group were statistically significant (9.67 ± 1.65 vs. 8.99 ± 1.16 p=0.03). After adjustment for traditional risk factors for atherosclerosis, only the association between severe periodontitis and DBP (odds ratio: 3.1; 95% CI: 1.1 to 8.5; P = 0.03) was confirmed (PWV OR: 1.5; CI 0.45 to 4.6; p = 0.512)</td></tr>
<tr><td>(Franek et al., 2012)</td><td>PWVs were comparable in all the groups (periodontally healthy (BGI-H); gingivitis (BGI-G); periodontitis with moderate bleeding (BGI-P2)).

<table><tr><td></td><td>BGI-H</td><td>BGI-G</td><td>BGI-P2</td><td>p (ANOVA or X^2 test)</td></tr><tr><td>PWV m/s</td><td>8.4 ± 1.0</td><td>8.7 ± 1.1</td><td>9.0 ± 1.4</td><td>NS</td></tr></table></td></tr>
<tr><td>(Hanaoka et al., 2013)</td><td>Groups “High PgIgG” vs. “Low PgIgG” were compared:

<table><tr><td></td><td>High PgIgG group</td><td>Low PgIgG</td><td>p</td></tr><tr><td>Mean PWV (cms $^{-1}$)</td><td>1760±359</td><td>1659±281</td><td>0.096</td></tr></table>
The composite periodontal risk score (p = 0.0003), systolic BP (p = 0.030), diastolic BP (p = 0.038), pulse pressure (p = 0.050) and mean BP (p = 0.055) were higher in the high Pg antibody group than in the low Pg antibody group.

Correlation with serum level of Pg-Ig-IgG before and after adjusting for age were not statistically significant. The composite periodontal risk score (r = 0.320, p = 0.0003), systolic BP (r = 0.212, p = 0.017), diastolic BP (r = 0.188, p = 0.035) and mean BP (r = 0.225, p = 0.011) correlated with the level of serum antibody against Pg, even after adjustment for age.</td></tr>
</table>

<table>
<tr><td></td><td>An elevated antibody level against Pg indicates advanced periodontal disease and suggests advancement of atherosclerosis and hypertension.</td></tr>
<tr><td>(Hayashida et al., 2013)</td><td>In a multiple linear regression analysis adjusted for age, sex, number of present teeth, and other confounders, each 1-mm increase in mean periodontal pocket depth corresponded to a 0.1 increase in mean CAVI (beta= 0.133; P=0.040). A multiple logistic regression analysis revealed that each 1-mm increase in mean periodontal pocket depth was associated with an increased mean CAVI ≥8 (OR, 1.323; 95% CI, 1.003 -1.743; P=0.047). Pearson's correlation coefficient between CAVI and mean probing depth was of r=0.15, while it was of 0.54 with respect to age

Conclusion: A linear, dose-dependent relationship was found between periodontal pocket depth, cIMT, and arterial stiffness.</td></tr>
<tr><td>(Shanker et al., 2013)</td><td>PWV and arterial stiffness index (ASI) were elevated in Periodontitis compared to Gingivitis cases ($p < 0.0001$) and in those with diabetes and hypertension.

PWV results from gingivitis vs. periodontitis individuals were:

- Right brachial (1435.13 ± 23.38 cm/s vs. 1300.52 ± 12.75 cm/s)

- Left brachial (1466.92 ± 26.56 cm/s vs. 1294.12 ± 12.90 cm/s

- Carotid-femoral PWV (975.66 ± 19.34 cm/s vs. 847.91 ± 9.74 cm/s)</td></tr>
<tr><td>(Vidal et al., 2013)</td><td>PWV (m/s) at baseline was quantified in 13.7 (±2.4) and it changed to 12.5 (±1.9) 6 months after treatment. Effective periodontal treatment reduced all cardiovascular risk markers, including PWV that significantly decreased by 0.9 m/s</td></tr>
<tr><td>(Jockel-Schneider et al., 2014)</td><td>Subjects suffering from severe chronic or aggressive periodontitis exhibited significantly higher PWV ($p = 0.00004$)

<table><tr><td></td><td>No/mild periodontitis</td><td>Severe chronic/aggressive periodontitis</td><td>p</td></tr><tr><td>PWV m/s</td><td>7.76 ± 1.9</td><td>9.16 ± 2.2</td><td>0.00004</td></tr></table></td></tr>
<tr><td>(Kapellas et al., 2014a)</td><td>While PWV increased monotonically with increasing extent of PD ≥4 mm, no consistent relationship was found concerning common carotid IMT at the univariate analysis level.

Arterial stiffness (PWV) significantly increased with increasing extent of periodontal pocketing (p trend = 0.001). By contrast, carotid IMT did not differ across quartiles.</td></tr>
</table>

<table>
<tr>
<td>(Kapellas et al., 2014b)</td>
<td>
IMT decreased significantly after 12 months in the intervention group (mean reduction=−0.023 [95% confidence interval (CI), −0.038 to −0.008] mm), but not in the control group (mean increase=0.002 [95% CI, −0.017 to 0.022] mm). The difference in intima-media thickness change between treatment groups was statistically significant (−0.026 [95% CI, −0.048 to −0.003] mm; P=0.03).
<table>
<tr><th>Participant Characteristics</th><th>Treatment (n=138)</th><th>Control (n=135)</th></tr>
<tr><td>Mean PWV, m/s</td><td>8.23 (1.22)</td><td>8.45 (1.27)</td></tr>
</table>
In contrast, there were no significant differences between treatment groups in PWV at 3 months (mean difference, 0.06 [95% CI, −0.17 to 0.29] m/s; P=0.594) or 12 months (mean difference, 0.21 [95% CI, −0.01 to 0.43] m/s; P=0.062).
<table>
<tr><td></td><td colspan="2">Treatment</td><td colspan="2">Control</td><td rowspan="2">ANCOVA</td><td rowspan="2">p-value</td></tr>
<tr><td></td><td>Baseline</td><td>12 Mo</td><td>Baseline</td><td>12 Mo</td></tr>
<tr><td>PWV, m/s</td><td>8.27 (1.30)</td><td>8.44 (0.92)</td><td>8.37 (1.36)</td><td>8.33 (1.04)</td><td>0.21 (−0.01-0.43)</td><td>0.06</td></tr>
</table>
ANCOVA: Least Squares Mean Δ (95% CI)

Periodontal therapy reduced subclinical arterial thickness but not function in Aboriginal Australians with periodontal disease, suggesting periodontal disease and atherosclerosis are significantly associated.
</td>
</tr>
<tr>
<td>(Houcken et al., 2016)</td>
<td>Analysis of a series of different models. The authors reached the following conclusions “periodontitis patients showed a significantly increased PWV compared with the reference group (8.01 ± 0.20 vs 7.36 ± 0.22 m s^{-1} respectively; P = 0.029) and this remained significant after adjustments for ACVD risk factors (P = 0.019). After periodontal therapy, no significant reduction in PWV was seen (8.00 ± 1.8 to 7.82 ± 1.6 m s^{-1}; P = 0.13), but systolic blood pressure (SBP) was significantly reduced (119.8 ± 14.6 to 116.9 ± 15.1 mm Hg; P = 0.040)”.</td>
</tr>
</table>

Table 7. Findings and results of the literature published on PWV

OBJECTIVES

2. OBJECTIVES

Carotid-femoral pulse wave velocity (PWV) is the established gold standard for assessing arterial stiffness. PWI is a novel ultrasound-based method developed at our institution. Using PWI to measure PWV has some advantages over the gold standard. It constitutes a regional measurement (PWV is estimated from an individual central artery (aorta or carotid)), enables visualization of the propagation of the pulse wave along the artery, and is a quick and less technically demanding method for measuring PWV for patients and professionals. Estimation of the regional stiffness of the carotid artery near the bifurcation allows repeatable measurements in a central artery. This holds great clinical interest due to its implication in CVD complications such as stroke.

Periodontitis is a risk factor for endothelial dysfunction and stiffness of the arteries, which are main contributors for atherosclerotic vascular disease (AVD). So far, few studies have explored the association between carotid-femoral PWV and periodontitis and none have explored the association between central PWV and periodontitis. The results of the literature published are not conclusive about the impact of periodontitis in the measurement of PWV.

In addition, blood pressure levels are routinely checked during dental appointments. Pulse pressure (PP) has been recognized by several studies as a significant predictor of call-cause cardiovascular mortality and morbidity. It is defined as the difference between systolic and the diastolic blood pressure. Moreover, it has been

demonstrated that central and peripheral blood pressures do not always correlate and that central blood pressure is key in the pathogenesis of cardiovascular disease. Pulse Wave Ultrasound Manometry (PWUM), an application of the ultrasound elasticity imaging described above, allows the calculation of central PP in a technically simple fashion. PWUM has been tested in healthy, normotensive patients, but has not yet been used to evaluate patients with elevated blood pressure.

Primary and secondary goals

The main goal of this research project is to investigate the association between periodontitis and arterial stiffness assessed in a central artery though PWI.

The secondary goal is to explore the relationship between the pulse pressure (PP) measurements obtained at three different sites of the arterial tree (brachial, radial and aorta arteries) using three different techniques (brachial sphygmomanometry, radial applanation tonometry and aortic PWI) in a subsample of patients categorized by their brachial blood pressure status. This will allow us to test the feasibility of a more complete assessment of PP variation though the arterial tree. The measurements were performed in a subsample of patients categorized by their systolic and diastolic blood pressure levels (measured through brachial sphygmomanometry).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Study design and statistical procedures

3.1.1. Overview

This is a cross-sectional study in which PWV results obtained through PWI were compared in a cohort of patients diagnosed with moderate to severe periodontal disease versus an age- and gender-matched periodontally healthy control group.

All participants underwent a comprehensive clinical evaluation of the periodontal tissues of all present teeth and were subjected to PWI examination of the right and left common carotid arteries. A subsample of the patients also underwent determination of pulse pressure (PP) at the infrarenal abdominal aorta and radial artery.

Patients with moderate to severe periodontitis were informed about their condition and the possibility of participating in the study. State-of-the-art periodontal therapy was offered as the treatment.

3.1.2. Power calculations and statistical analysis

The variance in PWI-assessed arterial tonometry outcomes in periodontitis is unknown. Based on an earlier study using a similar methodology in which PWV was determined with the Atheriograph® (a technique based on the analysis of the

oscillometric pressure curves registered on the upper arm with a single pressure cuff and the distance between the sternal notch (jugulum) and the symphysis), a sample size of 31 pairs of periodontitis patients/healthy controls provided 85% power to detect a difference in PWV of 1.4 m/sec between cases and controls with an one-sided α=0.05 (Jockel-Schneider et al., 2014),

In addition, based on prior experience with studies of vascular elasticity in periodontitis, it was determined that a minimum sample size of 30 case-control pairs (60 individuals in total) was likely sufficient to reveal meaningful differences between groups, should such differences exist. Parametric or non-parametric (depending on the distribution of the data) tests for paired observations were to be applied (paired t-test or Wilcoxon signed-rank test).

In case of significant discrepancies in exposure to confounders relevant to cardiovascular health between the two groups, additional adjustments were to be carried out, as needed.

Considering that PWI is a relatively novel technique, it was agreed to include a total of 80 participants in order to account for possible technical deficiencies in the acquisition of the PWI ultrasound (Sanz-Miralles et al., 2017).

A subsample of patients was selected in order to explore pulse pressure differences in three different sites. Only patients who exhibited a clear, unobstructed acoustic

window of an infrarenal abdominal aortic segment were included in this part of the study (Li, 2017).

3.2. Participants

3.2.1. Patient pool and recruitment

Subjects with moderate to severe periodontitis were recruited among patients referred for periodontal therapy to the Clinic for Post-doctoral Periodontics, Columbia University College of Dental Medicine between January 2013 and December 2015. Gender- and age-matched (within 5 year intervals) periodontally healthy subjects were recruited from other clinics at the College of Dental Medicine.

These facilities at the Columbia University Medical Center annually receive approximately 4,000 patient visits and 700 patient referrals.

The target sample was expected to include equal numbers of men and women and to comprise Hispanic patients at a frequency of approximately 60% and African Americans at approximately 30%, reflecting the demographics of the population served at the Medical Center. Children were not included in the study.

Assessing the referral records and the accompanying full-mouth series of intra-oral radiographs performed a preliminary screening for eligibility. Eligible prospective participants were contacted and inquired about their interest in enrolling the study.

3.2.2. Inclusion criteria

Inclusion criteria entailed a preliminary assessment of **moderate to severe periodontitis** made on the basis of radiographic bone loss (horizontal or vertical) affecting a minimum of two teeth per quadrant, followed by a brief clinical examination. Eligible patients had to (i) have moderate to severe periodontitis with at least 2 teeth per quadrant with a pocket depth of >5mm and concomitant attachment loss of >3mm and bleeding on probing at >30% of their tooth sites; (ii) be between 25 and 65 years old; (iii) have >20 teeth present; (iv) have received no systemic antibiotics for at least 3 months prior to enrollment; (v) not suffer from any of the systemic conditions or genetic disorders that entailed the diagnosis of periodontitis associated with systemic disease (1999) (Lindhe et al., 1999) (vi) not suffer from diabetes mellitus, (vii) not be pregnant, and (viii) not have malignancies or rheumatic diseases.

Gender- and age-matched (within 5 year intervals) **periodontally healthy controls** were defined as the patients that did not present pockets deeper than 4 mm and interproximal attachment loss greater than 2 mm.

3.2.3. Informed consent

The study objectives and procedures were approved by the Institutional Review Board of the Columbia University Medical Center (IRB# AAAL1851). Potential study participants were identified by the study investigators and written informed consent

was obtained prior to enrollment.

3.3. Data collection

All collected data remained confidential and study participants were assigned unique identifiers. These included self-reported race and ethnicity, medical and dental history, current medications and smoking status (current, former or never smoker). Height and weight measurements were obtained to calculate Body Mass Index (BMI). Systolic and diastolic blood pressure were measured in triplicate at both the right and left arm, with a participant in a lying position, using an automatic blood pressure monitoring device (Omron HEM-705CP), and the average of six measurements was used in all analyses.

3.3.1. Periodontal examination

A single examiner carried out all periodontal assessments (author ESM) that included full-mouth examination at six sites per tooth, using a manual periodontal probe (UNC-15).

The examination included dichotomous assessments of dental plaque and bleeding on probing (BoP), and linear measurements of probing depth (PD) and clinical attachment level (CAL) were recorded to the nearest millimeter. In addition, all

participants were screened for intra-oral presence of mucosal pathology, and presence of carious lesions.

The findings of the examination were communicated to the participants.

3.3.2. Ultrasound imaging

The arterial pulse wave was mapped by quantifying the pulse wave-induced motion of the anterior arterial wall using ultrasonic speckle tracking motion estimation methods (Fujikura et al., 2007, Luo et al., 2009, Vappou et al., 2010).

First, 2D RF data was obtained at the right and left common carotid arteries. In order to maximize consistency, all captures were performed at the area localized one centimeter away from the bifurcation (to external and internal carotid) using a commercially available ultrasound scanner equipped with a linear array and a curvilinear array transducer (SonixTouch, RP or MDP system, Ultrasonix Medical Corporation). The measurements were performed at the Ultrasound and Elasticity Imaging Laboratory, College of Physicians and Surgeons, Columbia University by an experienced examiner.

1D and 2D displacement estimates were obtained using ultrasonic speckle tracking motion estimation methods (Luo et al., 2009). The PWV and pulse pressure (PP) in the right and left common carotid were computed using previous methods (Fujikura et al., 2007, Luo et al., 2009, Luo et al., 2012, Vappou et al., 2010) on the displacement estimates.

In addition, color Doppler images were obtained to assess the arterial blood flow and the distension of the arterial wall, providing a qualitative assessment of the vessel characteristics and the arterial blood flow.

The image depth was adjusted in each case to better visualize the vessel. A regular beam density capture was utilized for vessel segmentation while a 2.5-second low beam density acquisition was used to visualize the propagation of the pulse wave of approximately two cardiac cycles. The number of captures obtained per individual was determined during the process of scanning, depending on the quality of the images and the consistency of the transmission of the wave through the vessel (Fig. 11, Fig. 12). Overall, between five and twelve captures per site were obtained for each participant. All cardiac cycles contained in the different captures were measured by a single calibrated examiner (author ESM).

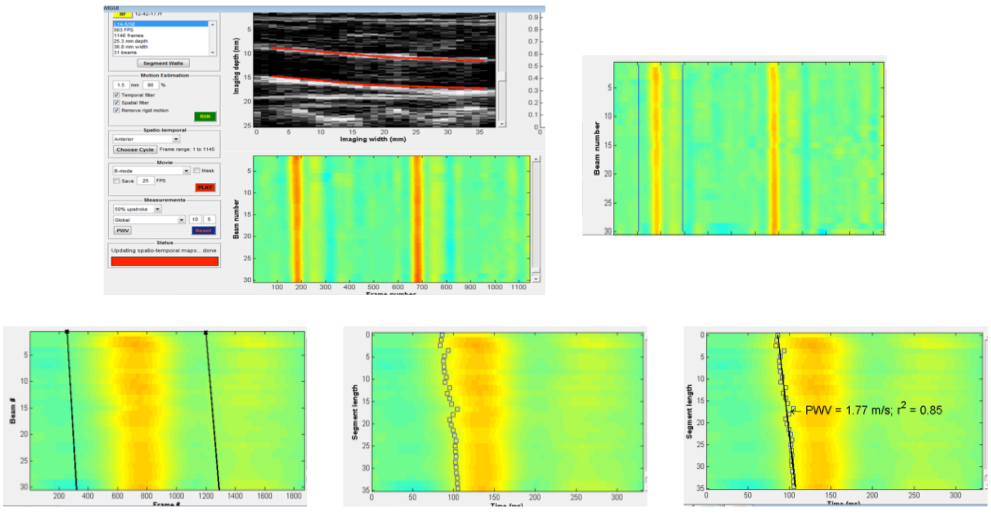


Fig 1. *High image quality. The vessel walls are easy to demarcate (ultrasound image) and motion corresponding to two cardiac cycles is obtained. Wave*

transmission (red line) is uniform as observed in all the color-coded images and high result of r^2.

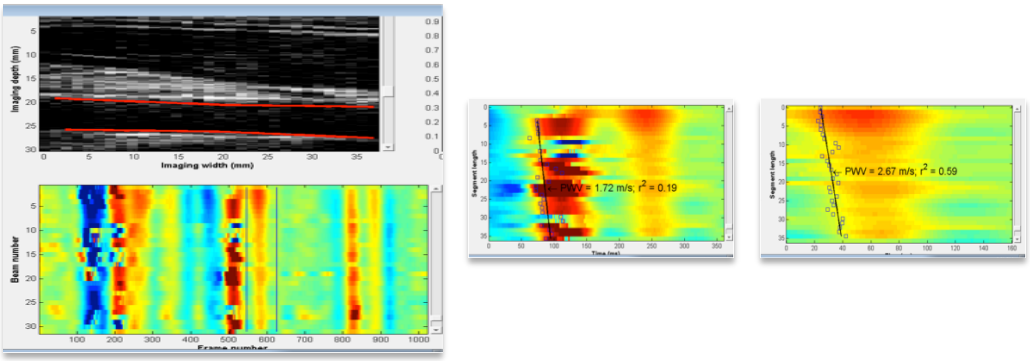


Fig 2. *Poor image quality. The vessel walls are not well demarcated. Cardiac cycles are not easy to determine and pulse wave doesn't seem to follow a harmonious propagation as shown in the color-coded map and r^2 results.*

Central pulse pressure (PP) was obtained through PWV at the infrarenal abdominal aortic segment of the aortic artery from the patients that allowed clear and unobstructed access and measurements. The abdominal aorta was chosen for the PWUM measurements due to its clinical significance and the fact that it is impossible to directly access it with other non-invasive methods such as brachial sphygmomanometry or applanation tonometry. A Sonic-TOUCH system and a 3.3 MHz curvilinear array transducer were used. A high frame was also used to capture the pulse wave, which travels at high velocity in the human abdominal aortas (4-12 m/s). The imaging depth was adjusted to the minimum depth so as to visualize both the

anterior and posterior aortic walls, and the lateral resolution was reduced to between 19 and 25 scan lines over the field of view. This resulted in imaging depths of 7-12 cm and frame rates of 222-351 Hz, which corresponded to maximum measurable PWVs of 14.9-23.2 m/s. RF frames were acquired over 2-second intervals during which the subjects were required to hold their breath in order to minimize rigid motion. One additional RF frame was acquired at a high line density (180) to provide a reference frame for accurate manual segmentation of the aortic walls. All RF samples were digitized at a sampling frequency of 40 MHz, and 5-7 acquisitions were performed for each subject in order to average the measurements over multiple cardiac cycles. Due to proximity of the spine to the posterior wall, the anterior wall was selected for the measurements and was segmented manually. Segmentation of the walls is performed using the high line density reference frame while the displacements at each point along the dynamic trace were mapped over time, generating a 2-D image depicting the spatio-temporal variation of the pulse wave propagation. Waveform tracking was performed by automatically identifying the 50% upstroke of each displacement waveform on the spatio-temporal map, and PWUM was estimated via linear regression of the 50% upstroke markers. The incremental distension curve obtained at the central scan line was used along with the PWV to derive the pulse pressure waveform based on the Laplace law and the Modified Moens-Korteweg equation. The use of these two equations implies the assumptions of a cylindrical geometry and an elastic behavior of the arterial wall in a linear way. This linear elasticity assumption denotes that the distension and pressure waveforms have temporal variations that

are in-phase. This is a commonly accepted hypothesis relying on the fact that the non-linear behavior of the arterial wall in vitro starts to happen at higher deformation than the physiological one (Li, 2017).

3.3.3. Applanation tonometry

Peripheral pulse pressure measurements were obtained through radial applanation tonometry. An applanation tonometry system was assembled and calibrated for the purpose of this study by connecting an SPT-301 noninvasive pulse tonometer to the input channel of a PCU-200 pressure control unit. The output channel of the control unit was connected to a USB digital I/O device for data acquisition. The I/O device was controlled by a MATLAB GUI (MathWorks, Natick, MA, USA), which displayed and saved the tonometer signal in real-time on the SonixTouch scanner. Once the pulse in the left radial artery was located by palpation, the tonometer was placed on the top of the artery to record 20 seconds of radial pressure waveforms. For each subject, radial pulse pressures were calculated from the respective waveforms as the amplitude difference between the peak and the beginning of the upstroke (i.e. foot) and averaged over 5-10 cycles (Li, 2017)

3.3.4. Brachial systolic and diastolic blood pressure and brachial pulse pressure

Blood pressures used for the calculation of pulse pressure were calculated as follows. Three brachial blood pressure measurements were performed on the left arm over a

15-minute period using a clinically recommended automatic digital blood pressure monitor (HEM-705CP, Omron Corp., Kyoto, Japan). The first measurement was excluded and the average of the latter two was used to classify each subject as pre-hypertensive (systolic blood pressure between 120 ad 139 mmHg) or hypertensive (systolic blood pressure > 140 mmHg). Brachial PP was then calculated as the difference between the systolic and diastolic pressures (Li, 2017).

Separately, blood pressure used as a co-variable was calculated as the average of six measurements – three measurements obtained in each arm with the same model of monitoring device as indicated above and with the patient in a supine position (Sanz-Miralles et al., 2017).

3.4. Data processing

The specific time of the wave arrival (foot of the wave) at each beam position, expressed in milliseconds, was plotted in an x-y diagram against the distance travelled, measured in millimeters, following which a linear regression fit was applied. PWV was calculated as the inverse of the slope of the linear regression fit, and R^2 as the determination coefficient of the linear regression fit, reflecting the goodness of fit and the harmony of wave propagation. In all instances, the tracing of the vessel and the calculation of PWV and R^2 were performed by a single calibrated examiner (author ESM). Reproducibility assessments involved duplicate assessments carried out on all

scans in all participants resulting in an intra-examiner kappa of 0.88 for PWV and 0.85 for R^2 measurements.

For the pulse pressure data management, a two-way ANOVA was performed using the Bonferroni method to evaluate statistical significance among the three subject groups.

3.4.1. Determination thresholds

PWI has the advantage of offering a qualitative and quantitative analysis of PWV. A wide range of results has been observed in the literature about arterial stiffness and PWV in the periodontal patient, and scarce literature has been published about PWI in relatively healthy patients. Therefore, we have classified the results and designed thresholds to characterize the patients according to qualitative information (homogeneity of the wave propagation) rather than quantitative (pulse wave velocity measured in m/s). This way of categorizing the results allowed us to differentiate measurements that properly reflected the status of the vessels from artifacts that were derived from poor image quality.

Thresholds were based on R^2 results. This decision was made based on previous research showing that R^2 gives a better understanding of the health of the vessels than PWV by itself in patients who don't have a history of major cardio-vascular events (Vappou et al., 2011a, Li et al., 2013, Ben-Shlomo et al., 2014). In addition, as there is consistency in the literature associating IMT and FMD with periodontal

disease (Orlandi et al., 2014), but no general agreement in the association of PWV and periodontal disease (Franek et al., 2009, Vieira et al., 2011, Franek et al., 2012, Hanaoka et al., 2013, Kapellas et al., 2014b, Miyaki et al., 2006), we decided to establish the threshold for R^2 results rather than PWV.

Two different thresholds were used in order to explore potential differences in the results. It is well known that the significance of the association is determined by the definition or the measurement used (Manau et al., 2008). Considering the novelty of the technique and the limited evidence on the use of PWI in patients without a history of major cardio-vascular events but with risk factors for CVD, two different thresholds were applied. Results obtained from applying these two thresholds were analyzed separately.

The first threshold applied was established at $R^2 \geq 0.6$. The decision to select this specific number was based on the fact that captures featuring lower R^2 results are prone to be the result of poor image acquisition. As indicated previously, there are no prior studies on the use of PWI on the periodontal patient. When considering methods other than PWI to assess atherosclerosis, there was only one publication that mentioned the cut-off applied in order to establish the presence of atherosclerosis. In this publication the threshold made reference to PWV and the goal was to classify the CV condition of patients. The same threshold was applied to all patients, not taking into account any individual characteristics or the individual results obtained (Miyaki et al., 2006). In contrast, the second threshold designed for our

study – $R^2 \geq$ mean minus one standard deviation (SD) – was established in an attempt to individualize the results and was set taking into account the individual measurements obtained for each patient.

Once the thresholds were applied for each analysis, separate estimates for PWV and R^2 at the right and left carotid were calculated. The values that represented maximum pathology, i.e., the highest PWV (because higher PWV signifies stiffer arteries) and the lowest R^2 (as lowest goodness of fit indicates less harmonious wave propagation) were selected to represent each particular patient.

3.4.1.1. **Threshold A**. Selection of results for $R^2 \geq 0.6$

It was considered that results with $R^2 < 0.6$ were highly likely artifacts and therefore, R^2 measurements < 0.6 and their associated PWV values were discarded. A mean for the right and a mean for the left carotid measurements were obtained separately.

As discussed previously, in order to represent maximum pathology, the lowest R^2 and highest PWV (from right and left measurements) were selected to represent the patient.

3.4.1.2. **Threshold B**. Selection of results for $R^2 \geq$ mean – one standard deviation (SD)

In order to create a threshold adapted to each specific patient and with the goal of being able to account for their individual clinical situation, a second threshold was applied.

To establish this threshold, an overall mean and standard deviation (SD) of the right and left R^2 assessments from all the results obtained for each individual were calculated. R^2 estimates (and consequently their associated PWV estimates) that were lower than the value obtained by subtracting a single standard deviation from the R^2 mean were disregarded.

Once the non-suitable measurements were disregarded, separate estimates for PWV and R^2 from the right and left carotid were calculated. The value that represented maximum pathology, i.e. the highest PWV (as higher PWV translates into stiffer arteries) and the lowest R^2 (as lower goodness of fit indicates a less harmonious wave propagation) from right and left measurements were selected to represent each particular participant.

RESULTS

4. RESULTS

4.1. Demographics

The sample was composed of a total of 80 volunteers (39% male, mean age 47.5 years, SD 11.6, range 24-78), including 40 patients with chronic periodontitis and 40 periodontally healthy controls. 64% of the individuals defined themselves as Hispanic – the distribution was 70% Hispanics in the periodontitis group and 57.5% in the control group.

The participants' demographic information and other characteristics as well as their clinical periodontal status are described in Table 8. Patients with diabetes mellitus were excluded. The two groups were largely comparable except for age and smoking status, while a trend for a higher use of anti-hypertensive medications was observed in the periodontitis group.

There were no significant differences between the two groups in regards to "body mass index", "systolic blood pressure" and "diastolic blood pressure".

***Table 8**. Demographics and other characteristics in the study participants*

	Total (n=80)	Periodontitis (n=40)	Controls (n=40)
Gender (male/female)	31/49	14/26	17/23
Age; mean (SD)	47.5 * (11.6)	50.1 (11.6)	44.8 (10.9)
Ethnicity n (%)			
Hispanic	51 (64%)	28 (70%)	23 (57.5%)
Non-Hispanic	29 (36%)	12 (30%)	17 (42.5%)
Body Mass Index; mean (SD)	26.4(4.2)	26.8 (4.3)	26.1 (4.1)
Current smokers; *n* (%)	7* (9%)	7 (18%)	0 (0%)
Taking blood pressure medication; *n* (%)	13 (16%)	10 (25%)	3 (8%)
Systolic blood pressure; mean (SD)	128 (18.5)	126 (16.9)	130 (20.0)
Diastolic blood pressure; mean (SD)	80 (10.0)	80 (11.0)	79 (9.7)

* *Age* (*p*=0.04, t-test for unpaired observations) *and % of smokers (p*=0.005, Fisher's exact test) *were statistically different between periodontitis and periodontally healthy controls.*

4.2. Periodontal status

Information regarding the periodontal status of the participants is presented in Table 9. Periodontal parameters were assessed at six sites (buccal, lingual/palatal, mesio- and disto-buccal and mesio- and disto-lingual/palatal), including all teeth present. Both bleeding on probing (BoP) and plaque scores were expressed as dichotomous variables (presence/absence). Mean probing depth (PD) and mean clinical attachment loss (CAL) were rounded up to the nearest millimeter. As reflected in Table 9, differences in all periodontal parameters were statistically significant.

***Table 9**. Clinical periodontal status in periodontitis patients and periodontally healthy controls*

	Periodontitis (n=40)	Controls (n=40)
No. of teeth (n ± SD)	26.1 ± 3.46	28.2 ± 2.12
% Plaque (mean ± SD) ¶	79 ± 0.20	18 ± 0.12
% BoP (mean ± SD) ¶	63 ± 0.04	9.0 ± 0.04
Mean PD (mean ± SD)	4.0 ± 0.72	2.2 ± 0.29
No. pockets ≥4 mm	83.3 ± 30.14	6.2 ± 6.26
% pockets ≥4 mm	53.1 ±0.18	3.6 ± 0.04
No. pockets ≥6 mm	31.0 ± 21.33	0.0 ± 0.00
% pockets ≥6 mm	19.7 ± 0.14	0.0 ± 0.00
Mean CAL (mean ± SD)	4.7 ± 1.22	1.3 ± 0.52
No. sites with CAL ≥4 mm	104.1 ± 28.40	5.3 ± 4.69
% sites with CAL ≥4 mm	67.0 ± 0.18	3.2 ± 0.03
No. sites with CAL ≥6 mm	49.0 ± 30.75	0.0 ± 0.16
% sites with CAL ≥6 mm	31.5 ± 0.20	0.0 ± 0.00

¶ Dichotomous variable (0= absence and 1= presence)
BoP: bleeding on probing; PD: probing depth; CAL: clinical attachment loss

4.3. Pulse Wave Velocity (PWV) and uniformity in the wave propagation (R^2 results)

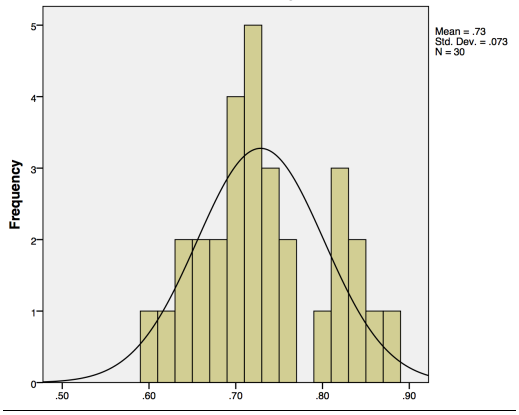
In order to ensure consistency, the right and left common carotid arteries were scanned one centimeter away from the bifurcation. From the 80 participants who were scanned, PWV and R^2 values for 78 participants were obtained. Two scans, one from a periodontitis patient and one from a control were not readable, and therefore PWV and R^2 results were not obtained for these two subjects.

4.3.1. **Threshold A**. Selection of PWV and R^2 values when $R^2 \geq 0.6$

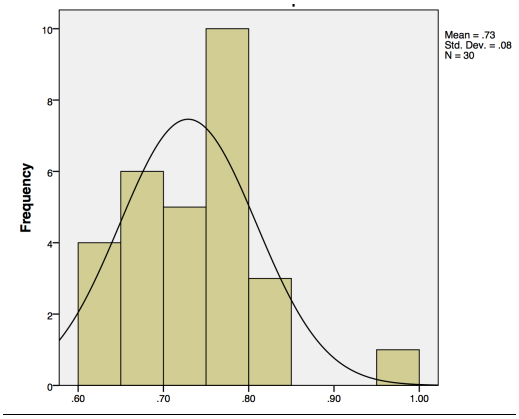
After applying “threshold A” to all the data available from the measurement of the 78 available scans, PWV and R^2 results for 73 subjects were available as five individuals presented with all R^2 results lower than the desired cut-off of 0.6. Subsequently, 30 pairs of age- and gender-matched subjects were formed and analyzed from the sample of 73 patients.

4.3.1.3. Normality

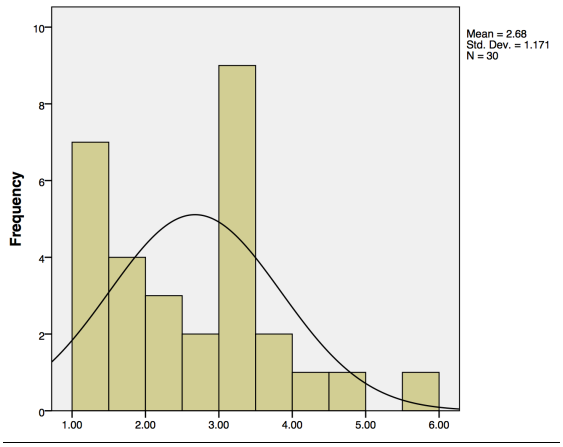
The distribution of the outcome variable PWV and R^2 of the 30 pairs of subjects was checked with histograms. As can be observed below, the data didn’t follow a normal distribution.



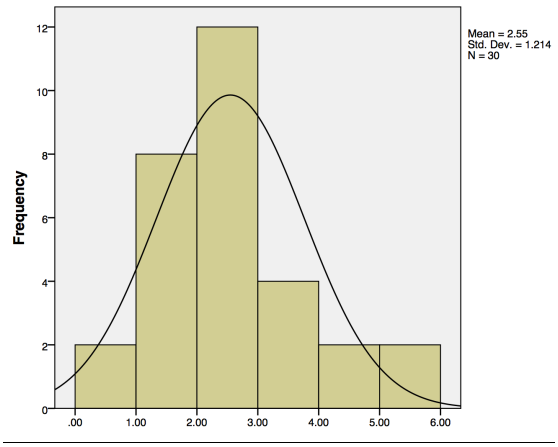
Histogram 1: Distribution of R^2 values in the control group



Histogram 2: Distribution of R^2 values in periodontitis patients



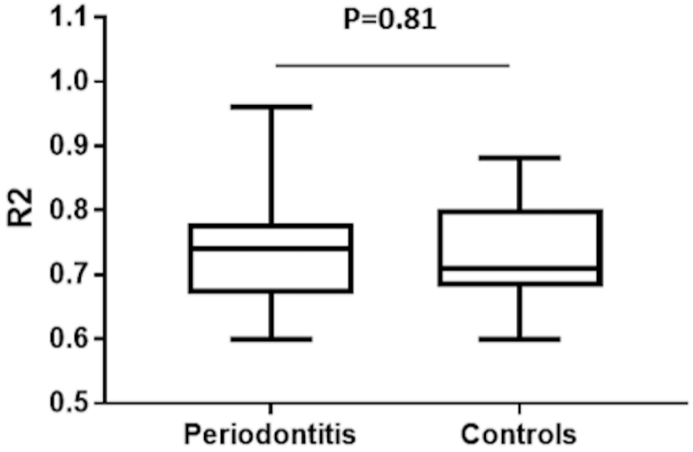
Histogram 3: Distribution of PWV values in the control group



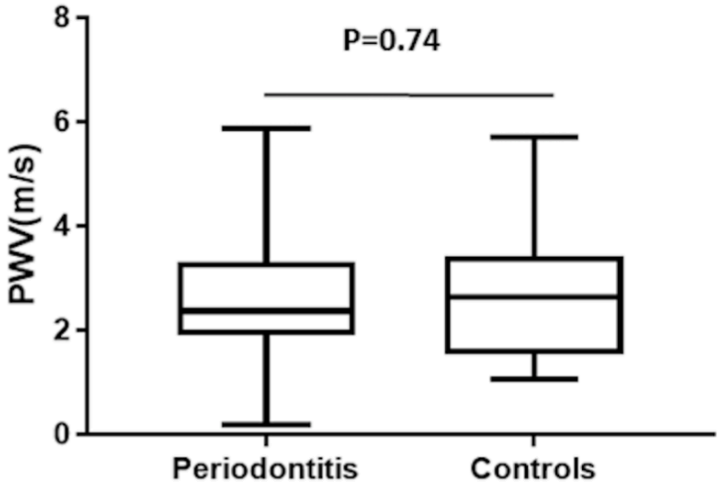
Histogram 4: Distribution of PWV values in periodontitis patients

4.3.1.4. Statistical analysis comparing matched pairs

Median values and interquartile ranges for PWV and R^2 values in periodontitis patients and controls for the 30 matched pairs are illustrated in Boxplot 1 and 2. Due to the fact that the results were not normally distributed, the Wilcoxon signed rank test was applied in order to explore differences between the groups. No significant differences were observed between the groups for either PWV (median PWV 2.37 m/sec vs. 2.64 m/sec in periodontitis and periodontally healthy controls, respectively, Wilcoxon signed rank test p=0.74), or R^2 results (0.74 vs. 0.71, respectively, p=0.81).



Boxplot 1: Distribution of R^2 values in cases and controls



Boxplot 2: Distribution of PWV values in cases and controls

4.3.1.5. Univariate and multivariate analysis

Univariate linear regression analysis based on values from the 73 patients for whom there were results available didn't show significant association for either PWV or R^2 or any of the explanatory variables considered. Negative associations were found between the uniformity in wave propagation (R^2) and presence of periodontitis

(*p*=0.60), age (*p*=0.87), systolic blood pressure (p=0.62), diastolic blood pressure (p= 0.42), as well as all continuous measures related to periodontitis (percentage of sites with BoP (*p*=0.54), percentage of sites with dental plaque (*p*=0.56), percentage of sites with PD ≥4mm (*p*=0.32), PD ≥6mm (*p*=0.34), CAL ≥4mm (*p*=0.47) and CAL ≥6mm (*p*=0.35). PWV also showed negative associations with the variables periodontitis (p=0.35), gender (p=0.78), taking blood pressure medication (p=0.47) and all the continuous variables related with the presence of periodontitis (Table 10).

Table 10. *Univariate linear regression analysis of the association between explanatory variables and the main outcomes (PWV and R2) in all participants with obtainable PWI data (n=78)*

	Outcome variable PWV		**Outcome variable R^2**	
Variable	**Coefficient (95% CI)**	***p*-value**	**Coefficient (95% CI)**	***p* - value**
Periodontitis	-0.27 (-0.85, 0.31)	0.35	-0.009 (-0.04, 0.02)	0.60
Gender	-0.08 (-0.77, 0.51)	0.78	0.03 (-0.006, 0.06)	0.10
Age	0.01 (-0.01, 0.03)	0.43	-0.0002 (-0.001, 0.001)	0.87
Ethnicity	0.09 (-0.52, 0.70)	0.76	0.002 (-0.03, 0.04)	0.90
BMI	0.04 (-0.02, 0.10)	0.24	0.002 (-0.001, 0.006)	0.20
Smoking ¶	0.03 (-1.12, 1.19,)	0.95	0.01 (-0.62, 0.08)	0.77
BP medication ¶	-0.28 (-1.06, 0.50)	0.47	0.02 (-0.02, 0.07)	0.29
BP systolic	0.004 (-0.01, 0.02)	0.56	-0.0002 (-0.001, 0.0007)	0.62
BP diastolic	0.004 (-0.02, 0.03)	0.78	-0.0007 (-0.002, 0.001)	0.42
Number of teeth	-0.05 (-0.14, 0.03)	0.25	0.002 (-0.003, 0.008)	0.44
% sites PD ≥4mm	-0.73 (-1.77, 0.31)	0.16	-0.03 (-0.09, 0.03)	0.32
% sites CAL ≥4mm	-0.54 (-1.41, 0.31)	0.20	-0.01 (-0.07, 0.03)	0.47
% sites PD ≥6mm	-1.9 (-4.09, 0.18)	0.07	-0.06 (-0.20, 0.07)	0.34
% sites CAL ≥6mm	-1.33 (-2.77, 0.10)	0.06	-0.04 (-0.13, 0.04)	0.35
% sites with plaque¶	-0.47 (-1.32, 0.36)	0.26	-0.01 (-0.06, 0.03)	0.56
% sites with BoP ¶	-0.74 (-1.69, 0.19)	0.11	-0.01 (-0.07, 0.04)	0.54

¶Dichotomous variable (0= absence; 1= presence)

Gender: 0 male, 1 female; Ethnicity: 0 Hispanic, 1 non-Hispanic; BoP: bleeding on probing; BMI: body mass index; BP: blood pressure; PD: probing depth; CAL: clinical attachment loss

In the multivariate linear regression analysis, based again on all participants with obtainable PWI data, after adjusting for gender, age, ethnicity, BMI, smoking, systolic and diastolic blood pressure, blood pressure mediation, and number of teeth present, the only statistically significant association was between the outcome variable R^2 and the explanatory variable gender (p=0.04) (Table 11).

Table 11. *Multivariate linear regression analysis of the association between explanatory variables and the main outcomes (PWV and R2) in all participants with obtainable PWI data (n=78)*

	Outcome variable PWV		**Outcome variable R^2**	
Variable	**Coefficient (95% CI)**	***p*-value**	**Coefficient (95% CI)**	***p* -value**
Periodontitis¶	-0.56 (-1.30, 0.18)	0.13	-0.003 (-0.04, 0.04)	0.88
Gender	-0.13 (-0.86, 0.60)	0.72	0.04 (0.0001, 0.09)	0.04*
Age	0.001 (-0.03, 0.03)	0.93	-0.0003 (-0.002, 0.001)	0.78
Ethnicity	0.15 (-0.61, 0.91)	0.69	0.03 (-0.01, 0.07)	0.19
BMI	0.05 (-0.02, 0.13)	0.20	0.003 (0.0009, 0.008)	0.11
Smoking ¶	-0.07 (-1.31, 1.21)	0.90	-0.01 (-0.09, 0.06)	0.62
BP medication¶	-0.36 (-1.42, 0.70,)	0.49	0.004 (-0.06, 0.07)	0.89
BP systolic	-0.004 (-0.03, 0.02)	0.74	-0.0001 (-0.001, 0.001)	0.84
BP diastolic	-0.0004 (-0.05, 0.05)	0.98	0.0008 (-0.003, 0.002)	0.59
Number of teeth	-0.06 (-0.19, 0.06)	0.32	0.002 (-0.006, 0.01)	0.62

¶Dichotomous variable (0= absence; 1= presence);
Gender: 0 male, 1 female; Ethnicity: 0 Hispanic, 1 non-Hispanic; BoP: bleeding on probing; BMI: body mass index; BP: blood pressure; PD: probing depth; CAL: clinical attachment loss

In addition, a multivariate linear regression analysis was performed with the periodontal explanatory variables percentage of sites CAL≥4mm (Supplementary table 1), percentage of sites CAL≥6mm (Supplementary table 2), percentage of sites

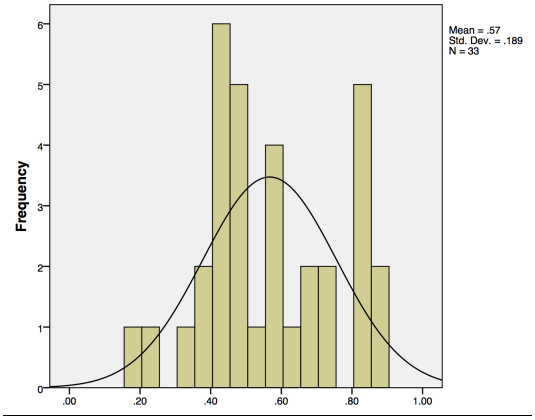
presenting BOP (Supplementary table 3) and the outcome variables PWV and R^2. Apart than controlling for these periodontal explanatory variables, the models were adjusted for the variables of gender, age, ethnicity, BMI, smoking, whether the patient was taking blood pressure medication, systolic and diastolic blood pressure, and number of teeth. In regards to the first model the results show that after controlling for all the covariates, sites with CAL≥4mm (p=0.03) have significant association with the PWV. When considering the outcome variable R^2 and after controlling for all covariates, gender showed a significant association with R^2 (p=0.04). Specifically R^2 was significantly higher in women (Supplementary table 1). These results were consistent throughout all the models designed using various periodontal when considering the different periodontal explanatory variables. In the second model, the p value for the association percentage of sites CAL≥6mm and the outcome variable PWV was of 0.008. In regards to the outcome variable R^2 the association with gender was almost statistically significant (p=0.05) being as found in the previous model, higher in women (Supplementary table 2). In regards to the third model, the association between % of sites presenting BOP and PWV (p=0.03) and gender and the outcome measure R^2 (p=0.04) were significant (Supplementary table 3). It should be pointed out that the association between the periodontal variables and PWV is negative while the association between R^2 and gender is positive.

4.3.2. **Threshold B**. Selection of results for R^2 ≥ mean – one standard deviation (SD) (mean and SD obtained from all results available)

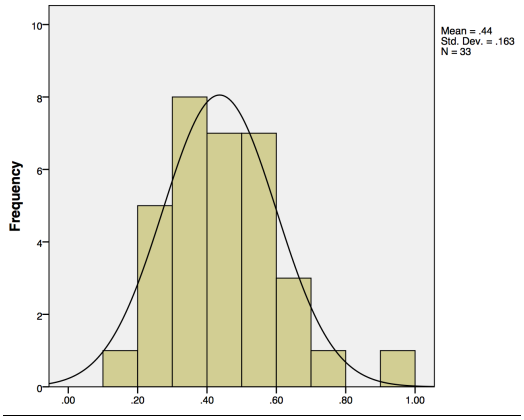
After applying threshold B to all the measurements obtained from the scans, PWV and R^2 results from 78 subjects were available. 33 pairs of age- and gender-matched subjects were formed and analyzed.

4.3.2.6. Normality

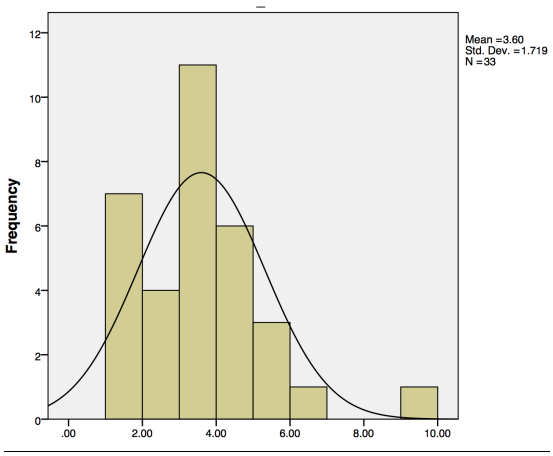
Distribution of the outcome variables of the 33 pairs of subjects checked with histograms.



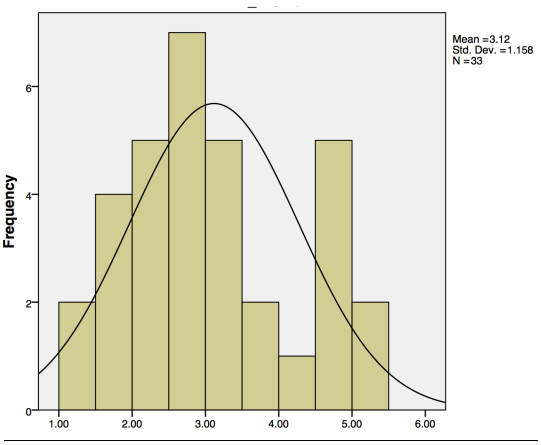
Histogram 5: Distribution of R^2 values in the control group



Histogram 6: Distribution of R^2 values in periodontitis patients



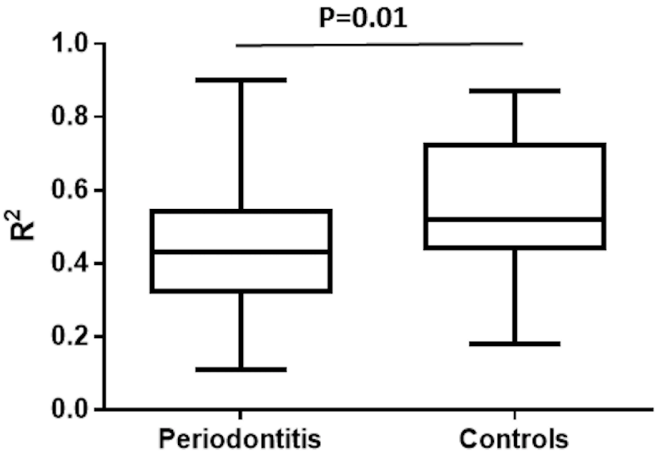
Histogram 7: Distribution of PWV values in the control group



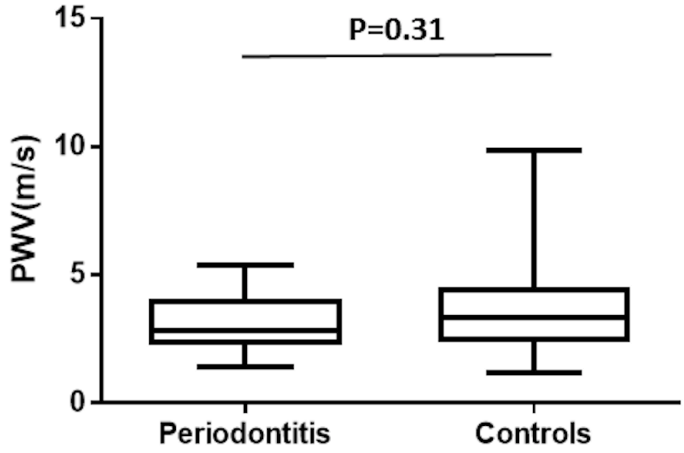
Histogram 8: Distribution of PWV values in periodontitis patients

4.3.2.7. Statistical analysis comparing matched pairs

Median values and interquartile ranges for PWV and R^2 values in periodontitis patients and controls for the 33 matched pairs are illustrated in Boxplot 3 and 4. Due to the fact that the results were not normally distributed, the Wilcoxon signed rank test was applied in order to explore differences between the groups. No significant differences were observed between the groups for PWV (median PWV 2.81 m/sec vs. 3.35 m/sec in periodontitis and periodontally healthy controls, respectively, Wilcoxon signed rank test p=0.31), but statistically significantly lower median R^2 values were observed in periodontitis patients than in controls (0.43 vs. 0.52, respectively, p=0.01).



Boxplot 3: Distribution of R^2 values in cases and controls



Boxplot 4: Distribution of R^2 values in cases and controls

4.3.2.8. Univariate and multivariate analysis:

Univariate linear regression analysis based on values from the 78 patients for whom there were results available (Table 12) showed a significant, negative association between the uniformity in wave propagation (R^2) and presence of periodontitis (*p*=0.004), current smoking (*p*=0.02) as well as continuous measures of periodontitis including percentage of sites with BoP (*p*=0.02), percentage of sites with dental plaque (*p*=0.03), percentage of sites with PD ≥4mm (*p*=0.008), PD ≥6mm (*p*=0.02), CAL

≥4mm (*p*=0.003) and CAL ≥6mm (*p*=0.002). On the other hand, PWV did not show statistically significant associations with any of the variables studied apart from the percentage of sites with BoP (*p*=0.04).

***Table 12**. Univariate linear regression analysis of the association between explanatory variables and the main outcomes (PWV and R2) in all participants with obtainable PWI data (n=78)*

	Outcome variable PWV		Outcome variable R^2	
Variable	**Coefficient (95% CI)**	***p*-value**	**Coefficient (95% CI)**	***p* -value**
Periodontitis¶	-0.37(-1.03, 0.30)	0.27	-0.12(-0.20, -0.04)	0.004*
Gender	-0.27 (-0.92, 0.38)	0.41	-0.0005(-0.09, 0.8)	0.99
Age	-0.01(-0.04, 0.02)	0.49	-0.004(-0.004, 0.003)	0.80
Ethnicity	0.53(-0.15, 1.21)	0.13	-0.008(-0.10, 0.08)	0.83
BMI	-0.02 (-0.10, 0.06)	0.57	0.009(-0.0008, 0.02)	0.07
Smoking ¶	0.16(-0.92, 1.23)	0.77	-0.16(-0.29, -0.02)	0.02*
BP medication ¶	0.18 (-0.62, 0.98)	0.66	-0.06(-0.16, 0.4)	0.23
BP systolic	0.001(-0.01, 0.02)	0.91	0.003(-0.002, 0.003)	0.76
BP diastolic	0.001(-0.31, 0.03)	0.94	0.0007(-0.003, 0.004)	0.73
Number of teeth	0.008(-0.10,0.12)	0.89	0.007(-0.007, 0.02)	0.36
% sites PD ≥4mm	-0.92(-2.08, 0.25)	0.12	-0.20(-0.35, -0.05)	0.008*
% sites CAL ≥4mm	-0.79(-1.74, 0.17)	0.11	-0.18(-0.30, -0.06)	0.003*
% sites PD ≥6mm	-1.62(-3.98, 0.74)	0.18	-0.34(-0.65, -0.5)	0.02*
% sites CAL ≥6mm	-0.92(-2.46, 0.62)	0.24	-0.30(-0.50, -0.11)	0.002*
% sites with plaque ¶	-0.72(-1.67, 0.22)	0.13	-0.14(-0.26, -0.02)	0.03*
% sites with BoP ¶	-1.14(-2.20, -0.9)	0.04*	-0.16(-0.30, -0.3)	0.02*

¶Dichotomous variable (0= absence; 1= presence)
Gender: 0 male, 1 female; Ethnicity: 0 Hispanic, 1 non-Hispanic; BoP: bleeding on probing; BMI: body mass index; BP: blood pressure; PD: probing depth; CAL: clinical attachment loss

In the multivariate linear regression analysis, the only remaining statistically significantly association with R^2 was the presence of periodontitis (*p*=0.01) after

adjusting for gender, age, ethnicity, BMI, smoking, blood pressure medication, systolic and diastolic blood pressure, and number of teeth (Table 13).

***Table 13**. Multivariate linear regression analysis of the association between explanatory variables and the main outcomes (PWV and R2) in all participants with obtainable PWI data (n=78)*

	Outcome variable PWV		Outcome variable R^2	
Variable	**Coefficient (95% CI)**	***p*-value**	**Coefficient (95% CI)**	***p* -value**
Periodontitis	-0.49 (-1.30, 0.32)	0.24	-0.12 (-0.22, -0.02)	0.01*
Gender	0.56 (-0.78, 0.90)	0.88	-0.02 (-0.12, 0.07)	0.61
Age	-0.009 (-0.04, 0.03)	0.63	0.001 (-0.003, 0.006)	0.60
Ethnicity	0.56(-0.31, 1.44)	0.20	-0.0002 (-0.10, 0.10)	0.99
BMI	0.002 (-0.08, 0.09)	0.95	0.009 (-0.002, 0.02)	0.10
Smoking ¶	0.34 (-0.97, 1.66)	0.60	-0.04 (-0.20, 0.12)	0.61
BP medication ¶	0.27 (-0.67, 1.22)	0.56	-0.03 (-0.15, 0.07)	0.50
BP systolic	-0.005 (-0.03, 0.02)	0.73	-0.001 (-0.005, 0.002)	0.45
BP diastolic	0.006 (-0.04, 0.05)	0.80	0.002 (-0.003, 0.008)	0.44
Number of teeth	-0.03 (-0.17, 0.10)	0.62	0.001 (-0.01, 0.01)	0.89

¶Dichotomous variable (0= absence; 1= presence)
Gender: 0 male, 1 female; Ethnicity: 0 Hispanic, 1 non-Hispanic
BoP: bleeding on probing; BMI: body mass index; BP: blood pressure; PD: probing depth; CAL: clinical attachment loss

In addition, a multivariate analysis was performed with the periodontal explanatory variables % sites CAL≥4mm (Supplementary table 4), % sites CAL≥6mm (Supplementary table 5), % sites BOP (Supplementary table 6) and the outcome variables PWV and R^2. In the results, a significant negative association can be observed for the variable % sites CAL≥4mm and the outcome measure PWV (p=0.04) and R^2 (p=0.03) (Supplementary table 4), % sites CAL≥6mm and the outcome variable

R^2 (p=0.04) (Supplementary table 5) and % sites BOP and the outcome variable PWV (p=0.02) (Supplementary table 6), after adjusting for the periodontal variables and the variables gender, age, ethnicity, BMI, smoking, whether the patients are taking blood pressure medication, systolic and diastolic blood pressure and number of teeth.

4.4. Pulse pressure (PP)

Measurements were obtained for a sample of 9 pre-hypertensive (5 male, 4 females, mean age 37.8 ± 8.4 years old), 5 hypertensive (3 male, 2 female, mean age 54.6 ± 2.7 years old) and 5 normotensive subjects (3 male, 2 female, mean age 27.2 ± 3.2 years old), yielding a total subject population of 19 individuals. Results revealed a higher PWV measurement in hypertensive patients, followed by pre-hypertensive and normotensive, suggesting that the aortas of the hypertensive patients were stiffer. The ANOVA two-sided test and Bonferroni's correction was used to explore differences among the three subject groups (normotensive, pre-hypertensive and hypertensive) in relation to the 5 different measurements (systolic and diastolic blood pressure, brachial PP, radial PP and aortic PP. The results revealed a significantly higher PP in the hypertensive group, detected only at the aortic site, while the radial and brachial PP were not significantly different among the three subject groups. In addition, a relatively strong correlation was found between aortic PP and both radial and brachial PP, but only in hypertensive subjects.

DISCUSSION

5. DISCUSSION

In this study, we detected differences in the elastic properties of the common carotid artery between a group of patients with periodontitis and gender- and age-matched periodontally healthy controls, none of which presented with a history of established cardiovascular disease or diabetes mellitus. In the multivariate analyses, after controlling for demographic variables, BMI, smoking, and blood pressure, it was shown that presence of periodontitis was associated with a less uniform propagation of the pulse wave along the carotid arteries, indicating arterial stiffness. Our results are in line with the body of literature that has associated periodontitis with systemic inflammation, endothelial dysfunction and adverse cardiovascular outcomes.

Both the systemic effect and the link between cardiovascular (CDV) and periodontal disease have been studied extensively for more than 20 years. Arterial stiffness constitutes a strong, independent subclinical indicator of cardiovascular disease (CVD) and is a hallmark of atherosclerosis (Cecelja and Chowienczyk, 2012). The biological basis of this association resides in the role of inflammation and its systemic effects (Khader et al., 2004, Kebschull et al., 2010). The mechanisms proposed to explain the link between cardiovascular disease and periodontal disease can be divided into direct (such as the inflammatory process *per se* and molecular mimicry) and indirect (bacteremia and vascular infection by periodontal pathogens) (Lockhart et al., 2012). Periodontitis is a chronic condition associated with both local and systemic inflammation, the result of a bacterial challenge in a susceptible host. The chronic

inflammatory status that it constitutes leads to a series of events that may lead to endothelial dysfunction.

Although it would be adventurous to talk about a causal relationship between periodontitis and atherosclerotic vascular disease, it has been widely documented that there is an association between these two conditions. They share risk factors such as cigarette smoking, age, and diabetes mellitus, making the determination of their relationship more complex (Lockhart et al., 2012). As stated by Kebschull in 2010, "although the strength of the reported associations is modest, the consistency of the data across diverse populations and a variety of exposure and outcome variables suggests that the findings are not spurious or attributable only to the effects of confounders. Evidence from epidemiologic studies suggests that periodontal infections are independently associated with subclinical and clinical atherosclerotic vascular disease" (Kebschull et al., 2010).

The association between arterial stiffness and periodontitis has been studied using two major methodologies: those examining vessel anatomy – such as intima-media thickness (IMT) (Beck and Offenbacher, 2001, Beck and Offenbacher, 2005, Southerland et al., 2012) – and those assessing the vessel functionality – such as flow-mediated dilation (FMD) (Amar et al., 2003, Mercanoglu et al., 2004, Seinost et al., 2005, Elter et al., 2006, Blum et al., 2007, Tonetti et al., 2007, Higashi et al., 2008) and foot-to-foot PWV (Miyaki et al., 2006, Franek et al., 2009, Vieira et al., 2011, Franek et al., 2012, Hanaoka et al., 2013, Hayashida et al., 2013, Shanker et al., 2013, Vidal et

al., 2013, Jockel-Schneider et al., 2014, Kapellas et al., 2014a, Kapellas et al., 2014b).

IMT has been shown in large epidemiological studies to be positively associated with periodontitis (Beck et al., 2001, Desvarieux et al., 2005), although its actual association with arterial stiffness remains unclear (Cecelja and Chowienczyk, 2012). Importantly, IMT increases physiologically with age (O'Leary et al., 1999), which may confound its association with periodontitis.

Flow-mediated dilatation (FMD) has also been used to test the functional capacity of the endothelium and its impairment after a systemic inflammatory stimulus. Commonly, FMD is compared to nitroglycerin-mediated dilation (NMD), in order to differentiate it from endothelium-independent vasodilation, and is assessed through two-dimensional ultrasound imaging of the brachial artery after occlusion-induced reactive hyperemia. Case-control and cross-sectional observations (Amar et al., 2003, Mercanoglu et al., 2004, Seinost et al., 2005, Elter et al., 2006, Blum et al., 2007, Tonetti et al., 2007, Higashi et al., 2008), single-arm intervention studies (Mercanoglu et al., 2004, Seinost et al., 2005, Elter et al., 2006, Blum et al., 2007, Higashi et al., 2008) and a single randomized controlled trial (Tonetti et al., 2007) have all demonstrated an impaired FMD in periodontitis, a temporary deterioration in FMD immediately after periodontal treatment due to the massive bacterial inoculation that occurs in conjunction with soft tissue instrumentation, but substantial improvement six months after completed periodontal therapy. A recent systematic review and meta-analysis concluded that individuals with periodontitis presented with an average

FMD that was 5.1% lower (95% CI 2.08 - 8.11) than that of periodontally healthy controls, while periodontal treatment was estimated to result in approximately 6.6% improvement in FMD (95% CI 2.83 - 10.44), $p<0.0001$). Nevertheless, the assessment of endothelial function with FMD is impacted by methodological, physiological and technical factors all of which can influence the validity, reproducibility and interpretation of results in clinical research (Orlandi et al., 2014).

Carotid-femoral PWV is considered the 'gold-standard' measurement of arterial stiffness. The relationship between PWV and elasticity of the vessel is based on the fact that propagation of the pressure wave along the arterial tree (PWV) is directly related to the intrinsic elasticity of the arterial wall (Laurent et al., 2006). The established method of calculating PWV is based on the speed of propagation of the pulse wave between two different sites, commonly the aorta and the femoral arteries. Compared to the other methods that assess arterial stiffness, PWV potentially constitutes a superior method. In regards to IMT, the association between IMT and arterial stiffness remains unclear (Cecelja and Chowienczyk, 2012). PWV is also considered superior to FMD; one of the reasons being that there is a risk of introducing measurement errors through FMD.

Although a significant association has been observed between periodontitis and higher values of PWV (Miyaki et al., 2006, Vieira et al., 2011, Hayashida et al., 2013, Shanker et al., 2013, Vidal et al., 2013, Jockel-Schneider et al., 2014, Kapellas et al., 2014a, Houcken et al., 2016), this association was attenuated or not detected in

several studies after adjusting for age, systolic blood pressure, smoking and other common risk factors of CVD (Miyaki et al., 2006, Vieira et al., 2011). In another study, differences remained after adjusting for ACVD risk factors (Houcken et al., 2016). Interestingly, no differences in PWV between periodontitis patients and controls were detected in a number of studies (Franek et al., 2009, Franek et al., 2012, Hanaoka et al., 2013). Other cross-sectional studies observed differences when considering the most severe forms of periodontitis (Vieira et al., 2011, Kapellas et al., 2014a). Regarding the three interventional studies found in our literature search, one is a prospective cohort pilot study in which all patients received non-surgical treatment (Vidal et al., 2013). The second is an RCT in which periodontal treatment was compared to no treatment and the population chosen was very specific, presenting with an increased risk of mortality due to CVD (Kapellas et al., 2014b). The third and most recently published investigation constitutes a pilot interventional study in which the treatment group was treated with SRP with or without antibiotics while the control group was formed by a reference group (Houcken et al., 2016). Overall, in the interventional studies that assessed PWV before and after periodontal therapy, there are notable differences in their findings, with some studies suggesting a reduction in PWV post-treatment (Vidal et al., 2013, Houcken et al., 2016). Vidal et al., 2013 is a cohort study in which it is concluded that effective periodontal treatment reduces PWV by 0.9 m/s (Vidal et al., 2013). However, Kapellas et al., 2014b recently published an RCT measuring PWV and IMT, in which they did not find significant changes in the function of the vessel as measured by PWV, but differences were

found in IMT, suggesting a change in the vessel structure after periodontal treatment (Kapellas et al., 2014b). It is important to highlight that the periodontal improvement of these patients after treatment was modest. As mentioned previously, there is further periodontal literature supporting an improvement in IMT after treatment (Tonetti et al., 2007), although in this study the level of severity of periodontal disease was higher than in most of the studies.

Lastly, the most recent publication regarding PWV could not demonstrate an effect of periodontal treatment on PWV (Houcken et al., 2016), while a recent systematic review published by Schmitt et al., 2015 concluded that patients with periodontitis have increased arterial stiffness compared to controls (higher mean PWV by 0.85 m/s; 95% CI: 0.53-1.16; $p<0.00001$) (Schmitt et al., 2015). On the other hand, the two interventional studies included in our literature review (Vidal et al., 2013, (Kapellas et al., 2014b) showed inconsistent effects of periodontal treatment on PWV, as concluded by Schmitt et al., 2015 in their systematic review (Schmitt et al., 2015).

Pulse Wave Imaging (PWI) is a novel methodology for the assessment of PWV that has been developed at the department of Bioengineering at Columbia University (Laboratory of Elasticity, Ultrasounds and Imaging). PWI holds several advantages compared to other means of assessing the elastic properties of the vessel and determining PWV.

Traditional methods used to determine PWV are global rather than regional, i.e., the calculation of PWV is carried out at two distant sites of the arterial tree (Miyaki et al.,

2006, Franek et al., 2009, Vieira et al., 2011, Franek et al., 2012, Hanaoka et al., 2013, Hayashida et al., 2013, Shanker et al., 2013, Vidal et al., 2013, Kapellas et al., 2014a, Kapellas et al., 2014b), such as carotid-femoral (Vieira et al., 2011, Vidal et al., 2013), carotid-radial (Franek et al., 2009, Franek et al., 2012) and less commonly, carotid-dorsalis pedis (Kapellas et al., 2014a, Kapellas et al., 2014b) and brachial-ankle (Miyaki et al., 2006, Hanaoka et al., 2013), among others (Hayashida et al., 2013, Shanker et al., 2013, Jockel-Schneider et al., 2014, Houcken et al., 2016). There are a series of challenges derived from these methods, for example difficulties in determining small time shifts (as the wave travels fast), difficulties in obtaining an accurate measurement of the distance between the two sites, and dependence on the assumption that the waves travel along a straight and uniform pathway between the two loci. These issues are especially important in older cohorts and patients that have suffered CV events or present with other CV risk factors (Luo et al., 2012). Only two studies obtained their PWV measurements from a single central artery (Jockel-Schneider et al., 2014, Houcken et al., 2016) by using an Arteriograph® and measuring oscillations at the aorta. It is important to note that typically the Arteriograph® has been used in the brachial artery and when compared to commonly used methods that perform measurements at two distant sites in the arterial tree (Compilor®, Sphygmocor®), the results of the three methods are not uniform, probably due to differences in calculating the distance travelled by the wave (Rajzer et al., 2008). While the equivalency between PWV results obtained by using different methodologies has not been thoroughly established, results are compared

throughout the literature.

PWI provides quantitative (PWV) and qualitative (R^2) information with regard to the elastic properties of the arteries and the uniformity of wave transmission. The European Society of Cardiology concluded that carotid-femoral PWV measurements higher than 12 m/s in hypertensive patients were an estimate of subclinical organ damage (Schmieder, 2007). Accordingly, PWV may be considered a good indicator for atherosclerosis in patients with hypertension or other CV risk factors, older populations and patients that have already suffered organ damage (presence of calcified atheromatous plaques).

In PWI, the PWV measurement obtained is accompanied by the correlation coefficient of the linear regression (R^2, which is a measurement of homogeneity of the wave transmission). This is especially important when considering a "healthier" cohort like the one included in this project as PWV may still show results within normal limits. Another reason that R^2 is a valuable piece of information is the fact that, as discussed in our literature review, there is a lack of consistency between arterial stiffness and PWV and periodontal disease. As with other methods, there is limited information available about the equivalency of the PWV obtained through this method and the "gold standard". Even more importantly, evidence of the accuracy of the "gold standard" as compared with more invasive methods that measure arterial stiffness directly from the aorta, which is one of the first and most important arteries to be affected by atherosclerosis, is scarce and lacks consistency. In our study, taking into

account the characteristics of our population and the evidence available about PWV in general and PWI in particular, R^2 appeared to be a more sensitive measurement for analyzing the data. R^2 also allowed us to analyze the data from two different points of view, as reflected in the two thresholds established.

The idea of establishing two different thresholds came from the desire to distinguish between real measurements, which reflect the elastic capacity of the arteries, and artifacts, which result from poor acquisition of the ultrasound. Threshold A is generic – all measurements associated to an R^2 measurement inferior to 0.6 were dismissed. Although generic thresholds are the most commonly used (e.g. blood pressure, body mass index, etc.), a second threshold was designed to take into account the individual variability (Threshold B). Threshold B accounts for all the R^2 results obtained for each patient (cut-off point is not static as in Threshold A in which low results under the threshold are discarded), which may represent an advantage as low R^2 measurements could correspond to poor acquisition of the scan, but also to severe atherosclerosis (although in these cases this was not expected considering their medical history). Creating a personalized cut-off adapted to each patient's results may be of a higher value considering the low cardiovascular risk profile of our population.

In our study, we observed no statistically significant differences in PWV between periodontitis patients and controls, likely due to the fact that the recruited cohort did not have a history of established CVD, diabetes, obesity, or uncontrolled blood pressure, and the frequency of current smokers was low. The mean age of the total

sample was of 47.5 ± 11.6 years old and there was a statistically significant higher number of smokers and patients taking high blood pressure medication in the periodontitis group. None of the patients had any signs of atheromatous plaque observed in the ultrasound scan and the mean blood pressure levels were determined as normal or pre-hypertensive. The groups were age- and gender-matched in order to minimize the effect of confounders.

It is difficult to compare the results of this and other published studies that also assess PWV or other variables related to arterial stiffness in periodontitis, due to differences in methodological aspects including the definition of cases and controls, differences in the CV status of the participants [presence of essential hypertension (Franek et al., 2009, Vidal et al., 2013), heterozygous familial hypercholesterolemia (Vieira et al., 2011), type II diabetes (Franek et al., 2012), coronary artery disease (Shanker et al., 2013), ischemic heart disease (Hanaoka et al., 2013); or differences in race/ethnicity and accompanying levels of susceptibility to CVD (Kapellas et al., 2014b)].

Power calculations were based on PWV results, as the variance in R^2 in periodontitis patients is unknown. Our sample calculation was based on a study with a similar design (Jockel-Schneider et al., 2014). It was concluded that with a sample of n=31 of matched pairs it was possible to detect differences of 1.4 m/sec between the periodontitis and control groups, with a power of 85% (with unilateral α=0.05). Admittedly, our study was adequately powered to detect a difference of 1.4 m/sec between periodontitis patients and periodontally healthy controls, while the actual

observed difference in median PWV between the two groups amounted to only 0.46 m/sec when applying threshold B (33 matched pairs included) and -0.13 m/sec when applying threshold A (30 pairs included). On the other hand, the method used in Jockel-Schneider et al., 2014 to calculate PWV has little similarities with PWI as the measures are done with the Atheriograph®, which is a technique based on the analysis of the oscillometric pressure curves registered on the upper arm with a single pressure cuff and the distance between the sternal notch (jugulum) and the symphysis.

An important observation is that our study identified a clearly inferior uniformity in the transmission of the pulse wave though the aortic wall in periodontitis (when considering threshold B), indicating a level of subclinical structural pathology compatible with arterial stiffness. Similar observations were made in a cohort of patients with history of abdominal aortic aneurysm and hypertension, in whom the shape of the waveform was drastically changed in the most severe cases (Li et al., 2011).

It is important to use a threshold that allows us to: a) differentiate the results that truly reflect the status and health of the vessels from artifacts derived from the method used and b) classify the patients based on their level of pathology. Nowadays, generic thresholds such as threshold A are still the most commonly used. These thresholds don't take into account the current status of the patient or their fluctuations or stability over time. Therefore, it is important to note the importance of

individualized thresholds, based on the patient's measurements and status, as threshold B does. No association was found between periodontitis and controls after analyzing the data under threshold A, while a significant association was found beween R^2 and periodontitis and all periodontally related variables. This association was maintained in the multivariate model (Sanz-Miralles et al., 2017).

Although the primary objective of this thesis was to explore the results of PWV obtained through PWI in the periodontal patient, a comparison between the pulse pressure obtained in three different ways in normotensive, pre-hypertensive and hypertensive patients was also made. Due to reduced sample size it is not possible to establish relationships in PP among different subject groups, but it is possible to evaluate the feasibility of noninvasively obtaining direct measurements of PP at different arterial locations. The central PP in large arteries such as the aorta has been shown to play a more significant role in the pathogenesis of cardiovascular disease, but peripheral pressures obtained for example in the brachial and radial arteries are more widely extended and studied due to easier access to the arteries and use of a more extended technology. Therefore, routine assessment and monitoring of hypertension is currently based on peripheral blood pressure due to a lack of noninvasive and individualized methods for central PP measurement. In this study PP was determined from blood pressure measurements obtained through sphygmomanometry at the brachial artery, applanation tonometry at the radial artery and pulse wave manometry (PWUM) at the aorta, which is a measurement obtained with the same technology and principles as calculation of PWV through PWI. This

study was designed using a subsample of patients presenting a high quality vision of the aorta, with the goal of establishing the feasibility of obtaining direct PP measurements.

In the results it is observed that patients with hypertension presented with higher measurements of PP measured through all three approaches, although the results were statistically significant just for the aortic PWUM measurement ($p < 0.01$). This may suggest that changes in overall blood pressure may occur in the central arteries before they are apparent at peripheral sites and that changes throughout the arterial tree may not be uniform in relatively compliant arteries. A positive correlation between PP and age was observed at all three measurement sites, but not necessarily within each subject group, although this may be attributed to the fact that the subjects also represented three significantly different age groups (pre-hypertensive patients were significantly older ($p < 0.05$) than normal and hypertensive patients were significantly older than the pre-hypertensive ($p < 0.01$). Thus, the effects of aging are also reflected in the PP variations at the arterial sites. The effect of the stiffening of the arteries may have a burdensome effect as when the waves are reflected their pressure adds to the systolic pressure. The reflection of the wave in an artery with a lower degree of stiffness might actually not be so harmful and actually yield better PP measurements. The reason is that when the wave is reflected within a more elastic artery (present in normotensive or pre-hypertensive patients and generally younger patients) the amplitude of the reflection will be lower and usually it will not add more pressure to the current systolic pressure. As a result, the increase in PP that happens

in stiffer arteries will not be observed here.

In regards to the correlation between central and peripheral PP results, no strong correlation was observed in the normal and pre-hypertensive groups ($R^2 < 0.45$), while a relatively strong correlation was observed in the hypertensive group ($R^2 = 0.68$ for the radial and $R^2 = 0.87$ for the brachial). This last correlation is probably due to the cumulative effect of age-related stiffening of the arteries and prolonged effects of hypertension, especially at the peripheral locations. This is a finding that will not always be observed in hypertensive patients, for example as occurred in two of our patients in which the PP that was obtained was considered normal. This was due to the fact that the diastolic blood pressure measurements were high, meaning that the difference between systolic and diastolic blood pressures resulted in a PP of normal value. In this specific scenario, the fact that the diastolic blood pressure is high constitutes a CV disease indicator by itself.

An important aspect of the PWUM methodology is that it is based on equations that are based on assumptions that include the presence of cylindrical geometry and linear elasticity of the arterial wall. However, it is unclear how well this model translates to human arteries in vivo, as the non-linear behavior of the arterial wall may infer a more complex relationship between distension and pressure (Li, 2017).

CONCLUSIONS

6. CONCLUSIONS

- Our findings confirm an association between poor periodontal status and arterial stiffness, assessed through a novel pulse wave imaging technique.
- Our study demonstrated that the propagation of the pulse wave along the aortic wall occurs in a non-homogeneous fashion in periodontitis patients, suggesting an element of structural pathology that results in arterial stiffening and/or inhomogeneity.
- Pulse wave imaging may represent an easier, less technique-sensitive and more accurate method for assessing stiffness at a regional central arterial site.
- The methodological differences that exist between the scientific evidence available prevent the comparison and correlation of the results.
- Pulse wave ultrasound manometry is a technique that can be used to measure the PP in any artery accessible by ultrasound. Increases in pulse pressure may not be uniform throughout the arterial tree in relatively compliant arteries; therefore, the determination of pulse pressure at central arteries may provide valuable information about the cardiovascular status of the patient. Future studies are aimed at applying the PWUM method at other imaging sites such as the carotid and brachial arteries.

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ANNEXES

7. ANNEX 1. INSTITUTIONAL BOARD REVIEW DOCUMENT

Columbia University Human Subjects Protocol Data Sheet

General Information

Protocol:	AAAL1851(M00Y04)	**Protocol Status:**	Approved
Effective Date:	10/13/2015	**Expiration Date:**	10/12/2016
Originating Department Code:		CDM Periodontics (7920202)	
Principal Investigator:		Papapanou, Panos (pp192)	
From what Columbia campus does this research originate:		Medical Center	
Title:	Arterial Pulse Wave Imaging in Periodontitis		
Protocol Version #:		**Abbreviated Title:**	PWI in periodontitis
Was this protocol previously assigned a number by an IRB:			No

Is the purpose of this submission to obtain a "Not Human Subjects Research" determination?
No

IRB Expedited Determination

2. Collection of blood samples by finger stick, heel stick, ear stick, or venipuncture.
4. Collection of Data through noninvasive procedures (not involving general anesthesia or sedation) routinely employed in clinical practice, excluding procedures involving x-rays or microwaves.

Renewal Information

Enrollment status:
Closed to further enrollment: remaining research activities are limited to data analysis only
Provide any additional information necessary to explain the study status:
Since the last renewal:
Have there been any changes in the relevant literature that would affect the study design or procedures?
No
Have there been any interim findings associated with this study?
No
Have there been any publications resulting from this study?
No
Have any participants been enrolled using the Short Form process?
No
Is there a Data Monitoring Committee (DMC), Data Safety Monitoring Board (DSMB), or other monitoring entity for this study?
No
Is an annual Progress Report required by the funding organization or coordinating center for this study?
No
Does this submission include a modification?
Yes
Provide a description of, and explanation for, all changes being proposed in this submission:

Iya Gabre and Ronny Li are no longer at Columbia, so they have been removed from the protocol.

Indicate which sections of the Rascal submission are affected by the proposed modification. Each marked section must be revised as part of this submission:

[ ]General Information
[ ]Attributes
[ ]Funding
[ ]Research Aims and Abstracts
[ ]Locations
[ ]Data Security and Privacy
[ ]Informed Consent/Recruitment
[ ]No revisions to submission content required
[ ]Exempt and Expedited
[x]Personnel
[ ]Background
[ ]Procedures
[ ]Subjects
[ ]Risks/Benefits/Monitoring
[ ]Attachments (including Rascal-generated attachments)

Does this submission include a report of a protocol violation?

No

Attributes

Special review type: Check all that apply or check "None of the Above" box.

[ ]Review for 45 CFR 46.118 Determination (involvement of human subjects is anticipated but is not yet defined)
[ ]Funding review for Administrative IRB approval (such as for Center or Training Grants)
[x]None of the above

IRB of record information: Will a Columbia IRB be the IRB that is responsible for providing review, approval, and oversight for this study?

Yes

Select the most appropriate response:

Columbia will be the IRB of record for the study procedures conducted by Columbia researchers (Note: this response will apply to most submissions).

Is this research part of a multicenter study?

No

Please indicate if any of the following University resources are utilized:

[ ] Cancer Center Clinical Protocol Data Management Compliance Core (CPDM)
[ ] CTSA-Irving Institute Clinical Research Resource (CRR)
[ ] CTSA- Irving Institute Columbia Community Partnership for Health (CCPH)
[x] None of the above

Background

Abbreviated Submission:

The IRB has an abbreviated submission process for multicenter studies supported by industry or NIH cooperative groups (e.g., ACTG, HVTN, NCI oncology group studies, etc.), and other studies that have a complete stand-alone protocol. The process requires completion of all Rascal fields that provide information regarding local implementation of the study. However, entering study information into all of the relevant Rascal fields is not required, as the Columbia IRBs will rely on the attached stand-alone (e.g., sponsor's) protocol for review of the

overall objectives.
If you select the Abbreviated Submission checkbox and a section is not covered by the attached stand-alone protocol, you will need to go back and provide this information in your submission.

Study Purpose and Rationale:
Provide pertinent background description with references that are related to the need to conduct this study. If this is a clinical trial, the background should include both preclinical and clinical data. Be brief and to the point.

[x] Abbreviated Submission - This information is included in an attached stand-alone protocol. Proceed to the next question

---

Study Design:
Describe the methodology that will be used in this study, covering such factors as retrospective vs. prospective data collection, interventional vs. non-interventional, randomized vs. non-randomized, observational, experimental, ethnography, etc.

[x] Abbreviated Submission - This information is included in an attached stand-alone protocol. Proceed to the next question

---

Statistical Procedures:
Provide sufficient details so that the adequacy of the statistical procedures can be evaluated including power calculations to justify the number of participants to be enrolled into the study. Definitions of subject terms such as enrolled and accrued as used for Rascal submissions can be found in the Subjects section.

[x] Abbreviated Submission - This information is included in an attached stand-alone protocol. Proceed to the next question

Exempt and Expedited

Is the purpose of this submission to obtain an exemption determination, in accordance with 45CFR46.101(b):
No

Is the purpose of this submission to seek expedited review , as per the federal categories referenced in 45CFR46.110?
No

Funding

Is there any external funding or support that is applied for or awarded, or are you the recipient of a gift, for this project?
Yes

Award Type	Funding Source Name	Status	Award # or Application Date	Federal/State/Local Government Direct or Subcontract	What is the award covering?	Rascal PT Number
Internal	Divisional funds					

Locations

Location Type	Facility Name	Domestic or International	Geographic Location	Local IRB Ethics Approval	Local Site Approval
Columbia/CUMC	Dr. Konofagou's Imaging Laboratory				
Columbia/CUMC	Vanderbilt Clinic				

Personnel

UNI	Name	Role	Department	Edit/View	Obtaining Informed Consent
pp192	Papapanou, Panos	Principal Investigator	CDM Periodontics (7920202)	Edit	Y
	Roles and Experience: Principal Investigator				
ecs2181	Miralles Sanz, Elena	Other Engaged Personnel	CDM Periodontics (7920202)	Edit	Y
	Roles and Experience: Former fellow, will be soon employed as faculty member				
ek2191	Konofagou, Elisa	Investigator	ENG Biomedical Engineering (521800X)	View	N
	Roles and Experience: PWI expert				
rsc1	Celenti, Romanita	Coordinator	CDM Periodontics (7920202)	Edit	N
	Roles and Experience: Study coordinator				

Training and COI

The PI must ensure that each individual that is added as personnel has met the training requirements for this study (http://www.cumc.columbia.edu/dept/irb/education/index.html). For help identifying which research compliance trainings you may be required to take, visit the Research Compliance Training Finder.

UNI	Name	COI	HIPAA	HSP (CITI)	Research with Minors (CITI)	FDA-Regulated Research (CITI)	S-I	CRC
pp192	Papapanou, Panos	07/08/2015	12/07/2003	11/16/2014	03/08/2014	11/16/2014	04/29/2011	
ecs2181	Miralles Sanz, Elena	11/05/2014	12/22/2012	12/22/2012				
ek2191	Konofagou, Elisa	01/14/2015	09/13/2004	03/30/2011	10/16/2009	03/01/2012		
rsc1	Celenti, Romanita	10/08/2015	07/13/2004	07/20/2015	07/20/2015	07/20/2015		12/21/2011

Departmental Approvers

Electronic Signature: Elena Miralles Sanz (7920202) - Other Engaged Personnel	Date: 10/07/2015
Electronic Signature: Elisa Konofagou (521800X) - Investigator	Date: 10/02/2015
Electronic Signature: Romanita Celenti (7920202) - Coordinator	Date: 10/08/2015
Electronic Signature: Panos Papapanou (7920202) - Principal Investigator	Date: 10/08/2015

Privacy & Data Security

Indicate the methods by which data/research records will be maintained or stored (select all that apply):

[ ]Hardcopy (i.e., paper)

[x]Electronic

Where will the data be stored?

Y

[ ]On a System

[x]On an Endpoint

Identify what type of endpoint will be used (select all that apply):

[x]Desktop Computer

[ ]Laptop Computer

[ ]Mobile Device

[ ]Other

Does this study involve the receipt or collection of Sensitive Data?

Yes

If any Sensitive Data is lost or stolen as part of your research protocol, you must inform both the IRB and the appropriate IT Security Office (CUMC IT Security if at CUMC; CUIT if at any other University campus).

What type of Sensitive Data will be obtained or collected? Select all that apply:

[ ]Personally Identifiable Information (PII), including Social Security Numbers (SSN)

Will Social Security Numbers (SSNs) be collected for any purpose?

[x]Protected Health Information (PHI), including a Limited Data Set (LDS)

If any PHI is lost or stolen, you must inform both the IRB and the Office of HIPAA Compliance.

Indicate plans for secure storage of electronic sensitive data: check all that apply

[ ]Sensitive data will not be stored in electronic format

[ ]Sensitive data will be stored on a multi-user system

[x]Sensitive data will be stored on an encrypted endpoint

By Selecting an Endpoint Device and approving this protocol for submission to the IRB, the PI is attesting that the device and any removable media that may be used have been or will be registered and/or will be maintained in compliance with the University's Information Security Charter and all related policies. It is important that this information is updated, during the course of the study, as new devices are added.

Provide a description of how the confidentiality of study data will be ensured, addressing concerns or protections that specifically relate to the data storage elements identified above (e.g. hard copy, electronic, system, and/or endpoint):
All data are stored in a CUMC-certified (system ID 34_3959), secure server that is accessible only to the study investigators through password protection.

Is there or will there be a Certificate of Confidentiality (CoC) for this research?
No

Provide a description of the protections in place to safeguard participants' privacy while information is being collected:
Study participants are assigned study IDs; signed consents are stored in a locked cabinet by the study coordinator; all stored data (see above) use the study ID as the identifier.

Procedures

Is this project a clinical trial?
No
Is this project associated with, or an extension of, an existing Rascal protocol?
No
Do study procedures involve any of the following?
Analysis of existing data and/or prospective record review
No
Audio and/or video recording of research subjects
No
Biological specimens (collection or use of)
Yes
Cancer-related research
No
Drugs or Biologics
No
Future use of data and/or specimens
No
Genetic research
No
Human embryos or human embryonic stem cells
No
Imaging procedures or radiation
Yes
Medical Devices
No
Surgical procedures that would not otherwise be conducted or are beyond standard of care
No
Will any of the following qualitative research methods be used?
Survey/interview/questionnaire

No

Systematic observation of public or group behavior

No

Program evaluation

No

Will any of the following tests or evaluations be used?

Cognitive testing

No

Educational testing

No

Non-invasive physical measurements

Yes

Taste testing

No

Is there an external protocol that describes ALL procedures in this study?

Yes

[x]Check here if all procedures being conducted by Columbia researchers are detailed in the stand-alone protocol, or provide a detailed description of which procedures are being conducted by Columbia researchers.

Biological Specimens

Add an individual entry for each human specimen type that will be collected or utilized for the proposed study. For each specimen type, indicate the source or sources from which you will obtain the specimens.

The use of specimens for research purposes may require that informed consent (or a waiver, if applicable) and HIPAA Authorization (or a waiver, if applicable) be obtained from subjects.

Type:

Blood

Source:

[x] From Columbia and/or NYP Subjects/Patients or Repositories managed by Columbia

Select all that apply to this specimen type:

[x] Specimens will be prospectively collected specifically for this research.

[ ] Residual specimens from clinical care that would otherwise be discarded have been or will be collected.

[ ] Specimens to be analyzed will be (or have been) collected from a commercial source.

[ ] Specimens to be analyzed will be (or have been) collected under a separate CU IRB-approved protocol; this could be an approved research repository protocol.

[ ] From Non-Columbia/NYP Subjects/Patients or Repositories not managed by Columbia

Description of Specimen and Method of Obtaining:

Blood sample obtained by phlebotomy

Indicate the manner in which the specimens will be labeled:

[ ] Specimens will be labeled with direct identifiers

[x] Specimens will be labeled with a code and the research team has the key and can link specimens to direct identifiers. This code would be considered an indirect identifier

[ ] The identifiers will be removed prior to the receipt of the specimens by Columbia researchers and no link will remain

[ ] Specimens were originally collected without identifiers

If specimens are collected or received at any point in time as with direct or indirect identifiers by the current researchers, then the specimens are considered to be identifiable, and the requirements for Informed Consent (or a waiver, if applicable) and HIPAA Authorization (or a waiver, if applicable) apply. The necessary information will need to be included in the respective sections of this Rascal submission.

Imaging Procedures/Radiation Therapy

Will a contrast agent (e.g. gadolinium) be used in conjunction with radiation exposure that goes beyond the parameters established for the applicable standard of care (SOC), or will a contrast agent be administered for research purposes only?
No

For each type of radiation exposure (e.g., ionizing: CT, X-ray; non-ionizing: MRI), identify the procedure and whether the administration (e.g., radiation dosage, number or type of scans) is clinically indicated and in accordance with the parameters established for the applicable standard of care (SOC), or is "beyond" these parameters (i.e., includes procedures or exposure for research purposes only).

Procedure(s) Involving Ionizing Radiation
No data to display

Procedure(s) Involving Non-Ionizing Radiation

Procedure	The exposure to:
Ultrasound	As established for the applicable SOC

Recruitment And Consent

Recruitment:

Describe how participants will be recruited:
Subjects with moderate to severe periodontitis will be recruited among patients referred for periodontal therapy to the Clinic for Post-doctoral Periodontics, Columbia University College of Dental Medicine. Eligible prospective participants will be contacted in person.

Select all methods by which participants will be recruited:
[ ] Study does not involve recruitment procedures
[x] Person to Person

[ ] Radio
[ ] Newspapers
[ ] Direct Mail
[ ] Website
[ ] Email
[ ] Television
[ ] Telephone
[ ] Flyer/Handout
[ ] Newsletter/Magazine/Journal
[ ] ResearchMatch
[ ] CUMC RecruitMe

Informed Consent Process:

Informed Consent Process, Waiver or Exemption: Select all that apply

[x] Informed consent with written documentation will be obtained from the research participant or appropriate representative.

Documentation of informed consent is applicable to:
The study in its entirety

Identify the portion of the study (e.g., prospective portion, focus groups, substudy 2) or subject population for which documentation of consent will be obtained::

Documentation of participation will be obtained from::
[x] Adult participants
[ ] Parent providing permission for a child's involvement
[ ] Legally Authorized Representatives (LARs)

Describe how participants' written consent will be obtained:
Consent is obtained by a periodontist/investigator (Dr. Elena Sanz-Mirales) at the Clinic of Post-doctoral Periodontics at the same date as the screening takes place. No vulnerable persons are enrolled. The study protocol and procedures are straightforward and are easily explained verbally to each prospective participant.

Informed consent is not required for exempt research but is recommended for such research when there will be interaction with research participants for the purpose of the research.

[ ] Informed consent will be obtained but a waiver of written documentation of consent (i.e., agreement to participate in the research without a signature on a consent document) is requested.

[ ] A waiver of some or all elements of informed consent (45 CFR 46.116) is requested.

[ ] Planned Emergency Research with an exception from informed consent as per 21 CFR 50.24.

[ ] Informed consent is not required; this is exempt research.

Subject Language

Enrollment of non-English speaking subjects is not expected.

During the course of the study, if non-English speaking subjects are encountered, refer to the IRB's policy on the Enrollment of Non-English Speaking Subjects in Research for further details (http://www.cumc.columbia.edu/dept/irb/policies/documents/Nonenglishspeakingsubjects.Revised.FINALDRAFT.111909.website.doc

Capacity to Provide Consent:

Do you anticipate using surrogate consent or is research being done in a population where capacity to consent may be questionable?

No

Research Aims & Abstracts

Research Question(s)/Hypothesis(es):

In this study, we propose to use non-invasive pulse wave imaging technology to study the effects of periodontitis on vascular health, and examine how these effects relate to periodontitis-induced systemic inflammation.We hypothesize that periodontitis is associated with higher pulse wave image velocity that will be reduced after successful periodontal therapy.

Scientific Abstract:

Several intervention studies suggest that periodontal treatment may reduce the level of systemic inflammation and improve endothelial function. Pulse waving imaging (PWI) has been used to locally map the arterial pulse wave in normal humans by quantifying the pulse wave-induced motion in the arterial walls. Therefore, PWI constitutes a unique tool for the detection and characterization of cardiovascular pathologies, reflecting the underlying changes in arterial mechanical properties. In this study, we propose to use PWI to assess (i) the arterial health status in periodontitis patients and periodontally healthy controls, and (ii) the changes of arterial health status attributed to periodontal therapy-induced reduction in systemic inflammation.

Lay Abstract:

This research project examines the impact of periodontitis (gum disease) on the health status of th eblood vessels. Previous studies have shown that gum disease may contribute to the process of atherosclerosis (blood vessel disease). In this study, we intend to use pulse wave imaging (PWI) , a novel, non-invasive imaging methodology, to associate features related to the elasticity of blood vessels with the extent and severity of periodontitis. Patients with gum disease and control individuals with healthy gums will receive an initial gum evaluation, a PWI assessment of their blood vessels and will donate a blood sample. Patients that require periodontal treatment will be invited to participate in the treatment part of the study. They will receive (i) deep cleaning for the gum disease that will be completed within a 4-week period, (ii) additional gum surgery, if necessary, and (iii) monthly dental cleanings over a 6-month period to help them to maintain a

good standard of oral hygiene. At 2 months and 6 months after the start of the study, their gums will be re-examined, a new blood vessel assessment will be carried out, and a new blood sample will be obtained.

Risks, Benefits & Monitoring

Abbreviated Submission:
The IRB has an abbreviated submission process for multicenter studies supported by industry or NIH cooperative groups (e.g., ACTG, HVTN, NCI oncology group studies, etc.), and other studies that have a complete stand-alone protocol. The process requires completion of all Rascal fields that provide information regarding local implementation of the study. However, entering study information into all of the relevant Rascal fields is not required, as the Columbia IRBs will rely on the attached stand-alone (e.g., sponsor's) protocol for review of the overall objectives. .
If you select the Abbreviated Submission checkbox and a section is not covered by the attached stand-alone protocol, you will need to go back and provide this information in your submission.

Potential Risks:
Provide information regarding all risks to participants that are directly related to participation in this protocol, including any potential for a breach of confidentiality. Risks associated with any of the items described in the Procedures section of this submission should be outlined here if they are not captured in a stand-alone protocol. Risks of procedures that individuals would be exposed to regardless of whether they choose to participate in this research need not be detailed in this section, unless evaluation of those risks is the focus of this research. When applicable, the likelihood of certain risks should be explained and data on risks that have been encountered in past studies should be provided.

[x] Abbreviated Submission - This information is included in an attached stand-alone protocol. Proceed to the next question

---

Potential Benefits:
Provide information regarding any anticipated benefits of participating in this research. There should be a rational description of why such benefits are expected based on current knowledge. If there is unlikely to be direct benefit to participants/subjects, describe benefits to society. Please note that elements of participation such as compensation, access to medical care, receiving study results, etc. are not considered benefits of research participation.

[x] Abbreviated Submission - This information is included in an attached stand-alone protocol. Proceed to the next question

---

Alternatives:
If this research involves an intervention that presents greater than minimal risk to participants, describe available alternative interventions and provide data to support their efficacy and/or availability. Note, participants always have the option not to participate in research.

[x] Abbreviated Submission - This information is included in an attached stand-alone protocol. Proceed to the next question

---

Data and Safety Monitoring:
Describe how data and safety will be monitored locally and, if this is a multi-center study, how data and safety will be monitored across sites as well.

[x] Abbreviated Submission - This information is included in an attached stand-alone protocol. Proceed to the next question

Subjects

Unless otherwise noted, the information entered in this section should reflect the number of subjects enrolled or accrued under the purview of Columbia researchers, whether at Columbia or elsewhere.

Target enrollment:

80

Number enrolled to date:

80

Number enrolled since the last renewal or, if this is the first renewal, since the initial approval:

20

Number anticipated to be enrolled in the next approval period:

0

Does this study involve screening/assessment procedures to determine subject eligibility?

No

Of the number of subjects enrolled, or the number accrued for interventional studies with a screening process:

How many remain on the study?

0

How many are off study?

80

How many completed the study?

64

Have any withdrawn of their own initiative?

No

Have any been removed by PI?

No

Have any been lost to follow-up?

Yes

How many?

16

Please explain:

Patients who elected not to receive periodontal therapy after the initial examination, or who failed to attend the scheduled follow-up visits are considered as "lost to follow up".

Have any died while on study?

No

Have any subject complaints been received?

No

Is this a multi-center study?

No

Does this study have one or more components that apply to a subset of the overall study population (e.g. Phase 1/2, sub-studies)?

No

Of the number enrolled, or the number accrued for interventional studies with a screening process, indicate:

Population Gender

Females	Males	Non Specific
61%	39%	0%

Population Age

0-7	8-17	18-65	>65	Non Specific
0%	0%	95%	5%	0%

Population Race

American Indian/Alaskan Native	Asian	Native Hawaiian or Other Pacific Islander	Black or African American	White	More than One Race	Non-Specific
1%	6%	0%	11%	18%	64%	0%

Population Ethnicity

Hispanic or Latino	Not Hispanic or Latino	Non-Specific
64%	36%	0%

Vulnerable Populations as per 45 CFR 46:

Will children/minors be enrolled

No

Will pregnant women/fetuses/neonates be targeted for enrollment?

No

Will prisoners be targeted for enrollment?

No

Other Vulnerable Populations:

[ ]Individuals lacking capacity to provide consent

[x]CU/NYPH Employees/Residents/Fellows/Interns/Students

Please ensure that a plan for avoiding elements of coercion or undue influence of these populations is addressed on the Informed Consent page.

[ ]Economically disadvantaged

[ ]Educationally disadvantaged

[ ]Non-English speaking

[ ]Other Vulnerable populations

[ ]None of the Populations listed above will be targeted for Enrollment

Subject Population Justification:

Please see attached protocol

Does this study involve compensation or reimbursement to subjects?

Yes

Describe and justify reimbursement/compensation:

Comprehensive periodontal examination will be offered to them at no cost. Periodontitis patients involved in the treatment part of the study receive one periodontal surgical procedure free of charge, as compensation for the time dedicated to the additional imaging examinations that will be carried out solely for research purposes.

Are subjects eligible for compensation of $600 or more in a calendar year?

No

Attached Attestation

Attached HIPAA Forms

Number	Type	Title	Status
AAAL2550	A	PWI NOV 2014	Approve
AAAI1302	D	Arterial Pulse Wave Imaging in Periodontitis	Approve

Attached Consent Forms

Number	Copied From	Form Type	Title	Active/InActive	Initiator
AAAS3544	AAAQ3400	Consent	Arterial Pulse Wave Imaging in Periodontitis	Active	Elena Miralles Sanz (ecs2181)

Documents

Archived	Document Identifier	Document Type	File Name	Active	Stamped	Date Attached	CreatedBy
No	HIPAA-AAAL1851 (1)	Other	HIPAA-AAAL1851 (1).jpg	Y		11/20/2014	Elena Miralles Sanz (ecs2181)
No	HIPAA-AAAL1851 (2)	Other	HIPAA-AAAL1851 (2).jpg	Y		11/20/2014	Elena Miralles Sanz (ecs2181)
No	Standalone protocol	Standalone/Sponsor's Protocol	Standalone protocol PWI study.docx	Y		10/02/2015	Panos Papapanou (pp192)

8. ANNEX 2: SUPPLEMENTAL TABLES STATISTICAL ANALYSIS USING THRESHOLD A

SUPPLEMENTARY TABLE 1: Multivariate linear regression analysis of the association between outcome variables (PWV and R2) and percentage of sites with clinical attachment loss (CAL) equal or greater than 4mm

	Outcome variable PWV		**Outcome variable R^2**	
Variable	**Coefficient (95% CI)**	***p*-value**	**Coefficient (95% CI)**	***p* -value**
% sites CAL≥4mm	-1.22 (-2.39, -0.06)	0.03**	-0.009 (-0.08, 0.06)	0.79
Gender*	-0.10 (-0.83, 0.62)	0.76	0.04 (0.0006, 0.09)	0.04**
Age	0.006 (-0.02, 0.04)	0.71	-0.0002 (0.002, 0.001)	0.82
Ethnicity*	0.14 (-0.60, 0.89)	0.69	0.03 (-0.01, 0.07)	0.19
BMI+	0.03 (-0.04, 0.11)	0.34	0.003 (-0.001, 0.008)	0.16
Smoking *	0.34 (- 0.97,1.66)	0.59	0.02 (-0.06,0.10)	0.60
BP£ medication*	0.32 (-0.47,1.13)	0.41	-0.004 (-0.05, 0.04)	0.86
BP systolic	-0.006 (-0.03, 0.02)	0.63	-0.0001 (-0.002, 0.001)	0.83
BP diastolic	0.005 (-0.04, 0.05)	0.82	-0.0007 (-0.003, 0.002)	0.63
Number of teeth	-0.08 (-0.21, 0.04)	0.18	0.001 (-0.006, 0.009)	0.66

+BMI: body mass index; £ BP: blood pressure; CI: Confidence interval; *Gender: 0 male, 1 female; Ethnicity: 0 Hispanic, 1 non-Hispanic; Smoking:0 no, 1 yes; BP Medication: 0 no, 1 yes; **Indicates statistically significant difference with p-value less than 0.05

SUPPLEMENTARY TABLE 2: Multivariate linear regression analysis of the association between outcome variables (PWV and R2) and percentage of sites with clinical attachment loss (CAL) equal of greater than 6mm

	Outcome variable PWV		Outcome variable R^2	
Variable	**Coefficient (95% CI)**	***p*-value**	**Coefficient (95% CI)**	***p* -value**
% sites CAL≥6mm	-2.50 (-4.33, -0.66)	0.008**	- 0.02 (-0.14, 0.09)	0.67
Gender*	0.008 (-0.02, 0.04)	0.63	0.04 (-0.0006, 0.09)	0.05
Age	0.008 (-0.02, 0.04)	0.55	-0.0002 (-0.002, 0.002)	0.67
Ethnicity*	0.17 (-0.56, 0.90)	0.64	0.03 (-0.01, 0.07)	0.18
BMI+	0.03 (-0.04, 0.11)	0.37	0.003 (-0.001, 0.008)	0.17
Smoking *	0.58 (-0.73, 1.90)	0.38	0.02 (-0.05, 0.10)	0.55
BP£ medication*	0.31 (-0.46,1.10)	0.41	-0.004 (-0.05, 0.04)	0.86
BP systolic	-0.005 (-0.03, 0.02)	0.69	-0.0002 (-0.001, 0.001)	0.81
BP diastolic	0.0001 (-0.04, 0.04)	0.41	-0.0007 (-0.003, 0.002)	0.61
Number of teeth	-0.08 (-0.10, 0.03)	0.16	0.001 (-0.006, 0.009)	0.67

+BMI: body mass index; £ BP: blood pressure; CI: Confidence interval; *Gender: 0 male, 1 female; Ethnicity: 0 Hispanic, 1 non-Hispanic; Smoking:0 no, 1 yes; BP Medication: 0 no, 1 yes; **Indicates statistically significant difference with p-value less than 0.05

SUPPLEMENTARY TABLE 3: Multivariate linear regression analysis of the association between outcome variables (PWV and R2) and percentage of sites with bleeding on probing (BOP)

	Outcome variable PWV		Outcome variable R^2	
Variable	**Coefficient (95% CI)**	***p*-value**	**Coefficient (95% CI)**	***p* -value**
% sites BOP	-1.31 (-2.51, -0.11)	0.03**	-0.01 (-0.08, 0.06)	0.79
Gender*	-0.12 (-0.84, 0.60)	0.73	0.04 (0.0005, 0.09)	0.04**
Age	0.003 (-0.03, 0.03)	0.82	0.0002 (-0.002, 0.001)	0.81
Ethnicity*	0.04 (-0.71, 0.80)	0.91	0.03 (-0.01, 0.07)	0.21
BMI+	0.04 (-0.03, 0.12)	0.30	0.003 (-0.001, 0.008)	0.16
Smoking *	0.30 (-0.99, 1.6)	0.64	0.02 (-0.06, 0.10)	0.60
BP£ medication*	0.35 (-0.44, 1.16)	0.37	-0.004 (-0.05, 0.04,)	0.87
BP systolic	-0.006 (-0.03, 0.02)	0.66	-0.0001 (-0.001, 0.001)	0.83
BP diastolic	0.001 (-0.04, 0.05)	0.94	-0.0007 (-0.003, 0.002)	0.61
Number of teeth	-0.07 (-0.19, 0.05)	0.24	0.001 (-0.005, 0.009)	0.64

+BMI: body mass index; £ BP: blood pressure; CI: Confidence interval; *Gender: 0 male, 1 female; Ethnicity: 0 Hispanic, 1 non-Hispanic; Smoking:0 no, 1 yes; BP Medication: 0 no, 1 yes; **Indicates statistically significant difference with p-value less than 0.05

9. ANNEX 3: SUPPLEMENTAL TABLES STATISTICAL ANALYSIS USING THRESHOLD B

SUPPLEMENTARY TABLE 4: Multivariate linear regression analysis of the association between outcome variables (PWV and R2) and percentage of sites with clinical attachment loss (CAL) equal or greater than 4mm

	Outcome variable PWV		**Outcome variable R^2**	
Variable	**Coefficient (95% CI)**	***p*-value**	**Coefficient (95% CI)**	***p* -value**
% sites CAL≥4mm	-1.32 (-2.61, -0.03)	0.04**	-0.17 (-0.33, -0.01)	0.03**
Gender*	0.09 (-0.70, 0.89)	0.81	-0.01 (-0.11, 0.08)	0.78
Age	-0.005 (-0.04, 0.03)	0.79	0.001 (-0.003, 0.006)	0.54
Ethnicity*	0.59 (-0.26, 1.45)	0.17	0.01 (-0.09, 0.11)	0.84
BMI+	-0.0006 (-0.09, 0.08)	0.98	0.008 (-0.002, 0.01)	0.13
Smoking *	0.69 (- 0.60, 2.00)	0.29	-0.06(-0.22, 0.10)	0.45
BP£ medication*	0.23 (-0.69,1.17)	0.61	-0.04 (-0.15,0.07)	0.49
BP systolic	-0.009 (-0.04, 0.02)	0.56	-0.001 (-0.005, 0.002)	0.45
BP diastolic	0.01 (-0.03, 0.06)	0.55	0.002 (-0.004, 0.009)	0.44
Number of teeth	-0.06 (-0.20, 0.07)	0.38	0.0001 (-0.01, 0.01)	0.98

+BMI: body mass index; £ BP: blood pressure; CI: Confidence interval; *Gender: 0 male, 1 female; Ethnicity: 0 Hispanic, 1 non-Hispanic; Smoking:0 no, 1 yes; BP Medication: 0 no, 1 yes; **Indicates statistically significant difference with p-value less than 0.05

SUPPLEMENTARY TABLE 5: Multivariate linear regression analysis of the association between outcome variables (PWV and R2) and percentage of sites with clinical attachment loss (CAL) equal of greater than 6mm

	Outcome variable PWV		**Outcome variable R^2**	
Variable	**Coefficient (95% CI)**	***p*-value**	**Coefficient (95% CI)**	***p* -value**
% sites CAL≥6mm	-1.67 (-3.72, 0.37)	0.10	-0.25 (-0.50, -0.001)	0.04**
Gender*	0.04 (-0.76, 0.85)	0.91	-0.01 (-0.11, 0.07)	0.69
Age	-0.007 (-0.04, 0.03)	0.69	0.001 (-0.003, 0.006)	0.60
Ethnicity*	0.61 (-0.26, 1,48)	0.16	0.01 (-0.09, 0.12)	0.79
BMI+	-0.005 (-2.00, 0.71)	0.34	0.007 (-0.003, 0.01)	0.17
Smoking *	0.64 (-0.71,2.00,)	0.34	-0.05 (- 0.22,0.11)	0.51
BP£ medication*	0.19 (-0.74,1.14)	0.68	-0.04 (-0.16, 0.07)	0.43
BP systolic	-0.004 (-0.03, 0.02)	0.76	-0.001 (-0.004, 0.002)	0.60
BP diastolic	0.007 (-0.04, 0.05)	0.77	0.001 (-0.004, 0.008)	0.62
Number of teeth	-0.04 (-0.19, 0.09)	0.49	0.001 (-0.01, 0.01)	0.87

+BMI: body mass index; £ BP: blood pressure; CI: Confidence interval; *Gender: 0 male, 1 female; Ethnicity: 0 Hispanic, 1 non-Hispanic; Smoking:0 no, 1 yes; BP Medication: 0 no, 1 yes; **Indicates statistically significant difference with p-value less than 0.05

SUPPLEMENTARY TABLE 6: Multivariate linear regression analysis of the association between outcome variables (PWV and R2) and percentage of sites with bleeding on probing (BOP)

	Outcome variable PWV		**Outcome variable R^2**	
Variable	**Coefficient (95% CI)**	***p*-value**	**Coefficient (95% CI)**	***p* -value**
% sites BOP	-1.50 (-2.85, -0.15)	0.02**	-0.13 (-0.30, 0.03)	0.11
Gender*	0.03 (-0.77, 0.84)	0.92	-0.02 (-0.12, 0.08)	0.66
Age	-0.008 (-0.04, 0.03)	0.66	0.0008 (-0.003, 0.005)	0.71
Ethnicity*	0.46 (-0.40, 1.33)	0.28	-0.002 (-0.11, 0.10)	0.96
BMI+	-0.001 (-0.09, 0.09)	0.97	0.008 (-0.003, 0.01)	0.15
Smoking *	0.59 (-0.66,1.8)	0.35	-0.09 (-0.25, 0.06)	0.23
BP£ medication*	0.23 (- 0.70,1.17)	0.62	-0.04 (-0.16, 0.07)	0.48
BP systolic	-0.01 (-0.04, 0.02)	0.51	-0.001 (-0.005, 0.002)	0.52
BP diastolic	0.01 (-0.03, 0.07)	0.50	0.002 (-0.004, 0.008)	0.54
Number of teeth	-0.05 (-0.20, 0.08)	0.41	0.001 (-0.01, 0.01)	0.84

+BMI: body mass index; £ BP: blood pressure; CI: Confidence interval; *Gender: 0 male, 1 female; Ethnicity: 0 Hispanic, 1 non-Hispanic; Smoking:0 no, 1 yes; BP Medication: 0 no, 1 yes; **Indicates statistically significant difference with p-value less than 0.05

PUBLICATIONS

10. PUBLICATION 1: ASSESSMENT OF ARTERIAL STIFFNESS IN PERIODONTITIS USING A NOVEL PULSE WAVE IMAGING METHODOLOGY

10.1. Reference: Sanz-Miralles, E. C., Li, R., Momen-Heravi, F., Mendieta, C., Konofagou, E. E. & Papapanou, P. N. (2017) Assessment of arterial stiffness in periodontitis using a novel pulse wave imaging methodology. *J Clin Periodontol* 00, 1-9. https://doi.org/10.1111/jcpe.12717

10.1.1. Acceptance letter

01-Mar-2017

Dear Dr. Elena Sanz-Miralles,

It is a pleasure to accept your manuscript entitled "ASSESSMENT OF ARTERIAL STIFFNESS IN PERIODONTITIS USING A NOVEL PULSE WAVE IMAGING METHODOLOGY" in its current form for publication in Journal of Clinical Periodontology. The comments, if any, of the reviewer(s) who refereed your manuscript are included at the foot of this letter.

The manuscript now becomes the copyright of this journal. In accordance with accepted practice, the research findings reported in this paper must not appear in another publication before its publication in this journal.

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Thank you for your fine contribution. On behalf of the Editors of Journal of Clinical Periodontology, we look forward to your continued contributions to the Journal.

Yours sincerely,
Maurizio Tonetti
Editor in Chief, Journal of Clinical Periodontology

Thank you for your revisions, this paper is now acceptable for publication.

10.1.2. Publication

Accepted: 1 March 2017

DOI: 10.1111/jcpe.12717

EPIDEMIOLOGY (COHORT STUDY OR CASE–CONTROL STUDY)

WILEY Journal of Clinical Periodontology

Assessment of arterial stiffness in periodontitis using a novel pulse wave imaging methodology

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Funding information
The study was supported by the Division of Periodontics, Section of Oral, Diagnostic and Rehabilitation Sciences, and the Ultrasound Elasticity Imaging Laboratory, Columbia University Medical Center, New York, NY.

Abstract

Aim: We investigated the cross-sectional relationship between periodontal status and arterial stiffness, assessed through a novel Pulse Wave Imaging methodology.

Methods: Eighty volunteers were enrolled (39% male, age range 24–78 years) and 33 pairs were formed of periodontitis patients/periodontally healthy controls, matched by age and gender. A full-mouth periodontal examination was performed and the degree of stiffness of the right and left carotid arteries was assessed by measuring pulse wave velocity (PWV) and the uniformity in pulse wave propagation (R^2). Wilcoxon signed-rank tests for paired observations were used to compare periodontitis patients and healthy controls. Univariate and multivariate analyses were performed to analyze the association between PWV and R^2 and potential explanatory variables.

Results: Patients with periodontitis had a statistically significantly lower uniformity in wave propagation (R^2) than controls (p = .01), but PWV did not differ between the two groups. Univariate analysis showed a significant negative association between R^2 and periodontitis, body mass index and smoking; periodontitis remained statistically associated with R^2 in the multivariate analyses.

Conclusions: Patients with periodontitis and no established cardiovascular disease presented with lower degree of uniformity in the transmission of the pulse wave through the carotid arteries, suggesting an association between periodontitis and arterial stiffness/functional alterations.

KEYWORDS
atherosclerosis, cardiovascular, periodontal, pulse wave velocity

1 | INTRODUCTION

Intima-media thickness (IMT) (Beck & Offenbacher, 2001, 2005; Desvarieux et al., 2005; Franek et al., 2012; Piconi et al., 2009) and flow-mediated dilatation (FMD) (Amar et al., 2003; Blum et al., 2007; Elter et al., 2006; Higashi et al., 2008; Mercanoglu et al., 2004; Seinost et al., 2005; Tonetti et al., 2007) are commonly used measures of vascular structure and function that serve as surrogate markers for atherosclerosis, and have been used in association studies linking periodontitis and atherosclerotic vascular disease (Kebschull, Demmer & Papapanou, 2010).

Carotid-femoral pulse wave velocity (PWV), a global measurement obtained from two distant sites in the arterial tree, is considered the gold standard measurement of aortic stiffness (Vappou, Luo, Okajima, Di Tullio & Konofagou, 2011) and is generally accepted to be a direct, simple, non-invasive and reproducible method (Vappou, Luo, Okajima, Tullio & Konofagou, 2011). PWV has been widely used in epidemiologic studies to predict risk for subsequent cardiovascular (CV) events (Laurent et al., 2006) in patients with intermediate-level risk. A recent systematic review (Ben-Shlomo et al., 2014) reported a strong predictive value of PWV for CV events and mortality in younger individuals that was not modified by hypertension, smoking, sex, diabetes, or

kidney disease. Moreover, consideration of PWV in addition to standard risk factors enabled better identification of high-risk populations that might benefit from more aggressive CV risk factor management. In another systematic review and meta-analysis, an increase in aortic PWV by 1 m/s corresponded to an approximately 15% increase in the risk of CV events (Vlachopoulos, Aznaouridis & Stefanadis, 2010). PWV has also been used to explore arterial stiffness in periodontitis, although the results from these studies are not consistent (Franek et al., 2009, 2012; Hanaoka et al., 2013; Hayashida et al., 2013; Houcken et al., 2016; Jockel-Schneider et al., 2014; Kapellas et al., 2014; Miyaki et al., 2006; Shanker et al., 2013; Vieira et al., 2011). This is likely partly due to inherent methodological difficulties in the assessment of PWV, but also due to additional factors including the way periodontitis was assessed in the various studies, as well as actual differences in the level of periodontitis extent and severity across samples.

Pulse wave velocity can be measured at different sites of the vascular system. Its measurement requires little technical expertise (Laurent et al., 2006) and is usually computed using applanation tonometry (Vappou, Luo, Okajima, Di Tullio et al., 2011) by dividing the distance between two loci in the vasculature by the time shift of the waveforms at these two points. The major drawback of this technique is that it is influenced by errors derived from the measurement of distance, small time shifts, as well as the fact that stiffness of the arteries is non-uniform along the vasculature (Luo, Li & Konofagou, 2012; Vappou, Luo, Okajima, Di Tullio et al., 2011; Vappou, Luo, Okajima, Tullio et al., 2011).

To overcome a number of these limitations and provide more valid assessments than the traditional PWV foot-to-foot method, a novel, ultrasound-based technique termed Pulse Wave Imaging (PWI) was developed by members of our team (Fujikura et al., 2007; Li et al., 2013; Luo, Fujikura, Tyrie, Tilson & Konofagou, 2009). PWI allows direct visualization of the interior of the vessel and the wave propagation, estimates the regional PWV and provides both quantitative (PWV) and qualitative information (R^2, i.e. a measure of goodness of fit of the distribution of PWV that reflects the uniformity of the pulse wave propagation through the arteries). PWI has been validated in both in vitro (Huang, Ren & Luo, 2014) and in vivo studies, although the latter have generally included samples of limited size or cohorts of patients with manifest cardiovascular pathology (Li et al., 2011).

The aim of this cross-sectional study was to investigate the association between periodontitis and arterial stiffness assessed through PWI in a cohort of patients with periodontitis but no established cardiovascular disease (CVD), and in gender- and age-matched periodontally healthy controls. We hypothesized that PWI would reveal presence of increased arterial stiffening and/or inhomogeneity in wave propagation in periodontitis patients.

Clinical Relevance

Scientific rationale for the study: Periodontitis has been linked with cardiovascular disease, which is associated with arterial stiffness. We employed Pulse Wave Imaging (PWI), a novel, ultrasound-based technique to assess Pulse Wave Velocity (PWV) in periodontitis patients and age- and gender-matched periodontally healthy controls.

Principal findings: The propagation of the pulse wave along the carotid arteries was less uniform in patients with periodontitis, indicating presence of arterial stiffness.

Practical implications: PWI revealed that periodontitis is associated with subclinical arterial stiffness among individuals free of established cardiovascular disease.

2 | MATERIAL AND METHODS

2.1 | Participants

Patients with chronic periodontitis were recruited consecutively among individuals that attended the Clinic for Postgraduate Periodontics at the College of Dental Medicine Columbia University between April 2013 and March 2015. Periodontally healthy controls, matched for gender and age (± 5-years) were recruited among patients seeking general dental care at the same institution.

Periodontitis patients had at least two teeth per quadrant with pocket depth >5 mm and concomitant attachment loss >3 mm, and showed bleeding on probing at >30% of their tooth sites. Additional inclusion criteria were presence of more than 20 teeth; no treatment with systemic antibiotics within the preceding 3 months; and no presence of any systemic conditions or genetic disorders that entailed the diagnosis of periodontitis associated with systemic disease, according to the 1999 World Workshop for the Classification of Periodontal Diseases and Conditions (Lindhe et al., 1999). Pregnant women, patients with self-reported diabetes mellitus, malignancies or rheumatic diseases, and individuals with self-reported CVD, other than controlled hypertension were excluded. Periodontally healthy controls had no probing pocket depths >4 mm and no interproximal attachment loss >2 mm.

The study objectives and procedures were approved by the Institutional Review Board of the Columbia University Medical Center (IRB# AAAL1851). Potential study participants were identified after preliminary assessment of their electronic health care records and were contacted by the study investigators. Written informed consent was obtained prior to enrolment.

2.2 | Data collection

All collected data remained confidential and study participants were assigned unique identifiers. These included self-reported race and ethnicity, medical history, current medications and smoking status (current, former or never smoker). Height and weights measurements were obtained to calculate Body Mass Index (BMI). Systolic and diastolic blood pressure were measured in triplicate at both the right and left arm, with a participant in a lying position,

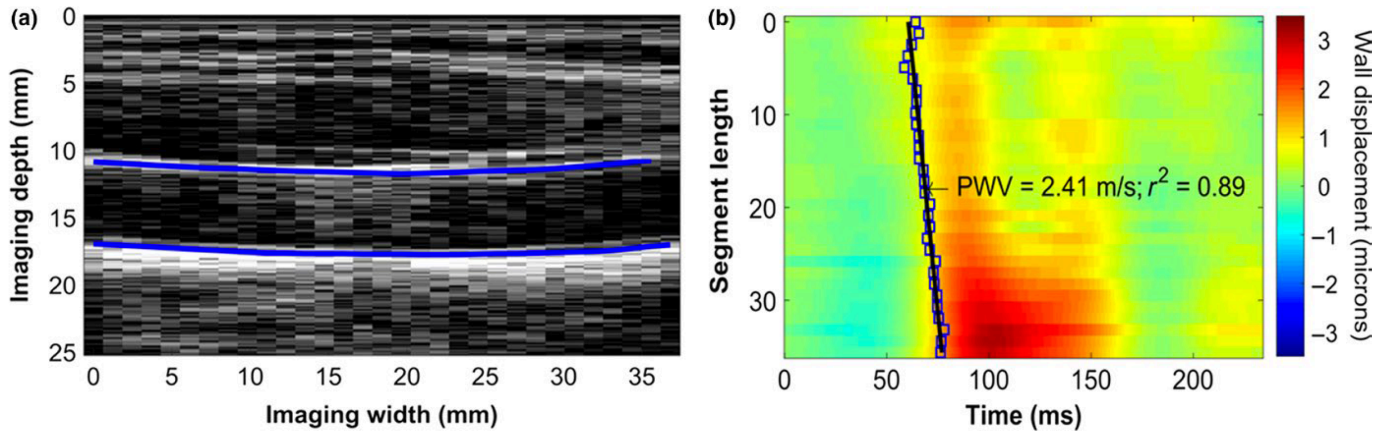

FIGURE 1 (a) Ultrasound tracing of a representative high quality capture of the carotid artery. The vessel walls are well-defined and easy to demarcate. (b) Spatio-temporal map from a vessel with homogenous transmission of the pulse wave

using an automatic blood pressure monitoring device (Omron HEM-705CP), and the average of six measurements was used in all analyses.

2.2.1 | Periodontal status

All participants underwent a full-mouth periodontal examination at six sites per tooth by a single calibrated examiner (author ESM) using a manual UNC-15 periodontal probe.

Presence of dental plaque and bleeding on probing (BoP) were recorded dichotomously, while probing depth (PD) and clinical attachment level (CAL) were recorded to the nearest millimeter.

2.2.2 | Pulse Wave Imaging

The arterial pulse wave was mapped by quantifying the pulse wave-induced motion of the anterior arterial wall using ultrasound-based speckle tracking motion estimation methods (Fujikura et al., 2007; Luo et al., 2009; Vappou, Luo & Konofagou, 2010). In addition, colour Doppler images were obtained to assess the arterial blood flow and the distension of the arterial wall, providing a qualitative assessment of the vessel characteristics. All scans were performed using a commercially available ultrasound scanner equipped with a linear array and a curvilinear array transducer (SonixTouch, RP or MDP system; Ultrasonix Medical Corporation), available at the Ultrasound and Elasticity Imaging Laboratory at Columbia University. Radio-frequency (RF) frames and B-mode images were acquired by a single experienced examiner (author RL). In order to maximize consistency, scans were performed at the site located one centimetre away from the right and left common carotid bifurcation into the external and internal carotid artery.

The image depth was adjusted in each case to better visualize the vessel. A regular beam density capture was utilized for vessel segmentation while a 2.5-s low beam density acquisition was used to visualize the propagation of the pulse wave of approximately two cardiac cycles during the process of scanning, depending on the quality of the images and the consistency of the transmission of the wave through the vessel. Overall, between five and twelve captures per site were obtained for each participant.

The specific time of the wave arrival (foot of the wave) at each beam position, expressed in milliseconds, was plotted in an x-y diagram against the distance travelled, measured in millimetres, following which a linear regression fit was applied. PWV was calculated as the inverse of the slope of the linear regression fit, and R^2 as the determination coefficient of the linear regression fit, reflecting the goodness of fit and the harmony of wave propagation. In all instances, the tracing of the vessel and the calculation of PWV and R^2 (main outcome variables) were performed by a single calibrated examiner (author ESM). Reproducibility assessments involved duplicate assessments carried out on all scans in all participants resulting in an intra-examiner kappa of .88 for PWV and .85 for R^2 measurements. Figure 1 illustrates an ultrasound tracing (Figure 1a) and a spatio-temporal map (Figure 1b), respectively, of a representative high quality capture of the carotid artery that indicates high homogeneity of the wave propagation.

In order to exclude artefacts derived from poor quality of capture, a threshold was established based on R^2 rather than on PWV, as the former has been shown to better reflect the elastic properties of arteries in cohorts with no established cardiovascular pathology (Ben-Shlomo et al., 2014; Li et al., 2013; Vappou, Luo, Okajima, Di Tullio et al., 2011). To establish this threshold, an overall mean and standard deviation (*SD*) of the right and left R^2 assessments for each individual were calculated. R^2 estimates (and their associated PWV estimates) that were lower than the value obtained by subtracting a single standard deviation from the R^2 mean were disregarded.

For each participant, separate estimates for PWV and R^2 at the right and left carotid were calculated, and the value that represented maximum pathology, i.e. the highest PWV (because higher PWV signifies stiffer arteries) and the lowest R^2 (because lowest goodness of fit indicates a less harmonious wave propagation) was selected to repre-

TABLE 1 Demographics and other characteristics in the study participants

	Total (*n* = 80)	Periodontitis (*n* = 40)	Controls (*n* = 40)
Gender (male/female)	31/49	14/26	17/23
Age; mean (*SD*)	47.5 (11.6)	50.1 (11.6)	44.8 (10.9)
Ethnicity *n* (%)			
Hispanic	51 (64)	28 (70)	23 (57.5)
Non-Hispanic	29 (36)	12 (30)	17 (42.5)
Race			
American Indian/Alaskan Native	1	1	0
Asian	5	1	4
Native Hawaiian/Other Pacific Islander	0	0	0
Black/African American	9	6	3
White	14	5	9
Other	50	26	24
Middle Eastern	1	1	0
Body Mass Index; mean (*SD*)	26.4 (4.2)	26.8 (4.3)	26.1 (4.1)
Current smokers; *n* (%)	7* (9)	7 (18)	0 (0)
Taking blood pressure medication; *n* (%)	13 (16)	10 (25)	3 (8)
Systolic blood pressure (mmHg); mean (*SD*)	128 (18.5)	126 (16.9)	130 (20.0)
Diastolic blood pressure (mmHg); mean (*SD*)	80 (10.0)	80 (11.0)	79 (9.7)

*Age (p = .04, t-test for unpaired observations) and % of smokers (p = .005, Fisher's exact test) were statistically different between periodontitis and periodontally healthy controls.

2.3 | Determination of sample size

Based on an earlier study that assessed PWV in periodontitis but did not use PWI assessments (Jockel-Schneider et al., 2014), a sample size of 31 pairs of periodontitis patients/healthy controls provided 85% power to detect a difference in PWV of 1.4 m/s between cases and controls with an one-sided α = .05. A total sample of 80 participants was recruited to account for attrition and/or inability to match according to the stipulated criteria.

2.4 | Statistical analysis

Demographic data and other characteristics of patients with periodontitis and periodontally healthy controls were compared using t-tests for unpaired observations for quantitative variables and Fisher's exact test for categorical variables.

Histograms of the distributions of the primary outcomes (PWV and R^2) revealed that they were both skewed, consequently hypothesis testing was carried out using non-parametric tests for paired observations (two-tailed Wilcoxon signed-rank test). Data were thus expressed using median and interquartile differences.

Univariate linear regression analysis was carried out having each of the two primary outcomes as the dependent variable and using presence of periodontitis, age, gender, ethnicity, BMI, smoking, mean systolic and diastolic blood pressure, and treatment with antihypertensive medications as the explanatory variables. Multivariable linear regression models were carried out. In addition, alternative models were undertaken with different variables related to periodontal disease. All analyses were performed using STATA SE 14.1 (STATA Corp., Texas, USA).

3 | RESULTS

3.1 | Demographics, other characteristics, and periodontal status

A total of 80 volunteers were recruited (31 male and 49 female, mean age 47.5 years, *SD* 11.6, range 24–78 years), including 40 patients with chronic periodontitis and 40 periodontally healthy controls. 64% of the individuals defined themselves as Hispanic; 70% of the periodontitis group and 57.5% of the control group. The participants' demographic information and other characteristics as well as their clinical periodontal status are described in Table 1. The two groups were largely comparable except for age and smoking status, while a trend for a higher use of antihypertensive medications was observed in the periodontitis group. Information regarding the periodontal status of the participants is presented in Table 2. Out of 80 participants who underwent PWI, valid PWV and R^2 values were obtained for 78 participants, as two scans, one from a periodontitis patient and another from a periodontally healthy control, were not readable. Of the 78 patients scanned, 66 could be matched with respect to gender and age into

TABLE 2 Clinical periodontal status in periodontitis patients and periodontally healthy controls

	Periodontitis (n = 40)	Controls (n = 40)
No. of teeth (mean ± *SD*)	26.1 ± 3.46	28.2 ± 2.12
% Plaque (mean ± *SD*)[a]	79 ± 0.20	18 ± 0.12
% BoP (mean ± *SD*)[a]	63 ± 0.04	9.0 ± 0.04
PD (mm; mean ± *SD*)	4.0 ± 0.72	2.2 ± 0.29
No. pockets ≥4 mm	83.3 ± 30.14	6.2 ± 6.26
% pockets ≥4 mm	53.1 ± 0.18	3.6 ± 0.04
No. pockets ≥6 mm	31.0 ± 21.33	0.0 ± 0.00
% pockets ≥6 mm	19.7 ± 0.14	0.0 ± 0.00
CAL (mm; mean ± *SD*)	4.7 ± 1.22	1.3 ± 0.52
No. sites with CAL ≥ 4 mm	104.1 ± 28.40	5.3 ± 4.69
% sites with CAL ≥ 4 mm	67.0 ± 0.18	3.2 ± 0.03
No. sites with CAL ≥ 6 mm	49.0 ± 30.75	0.0 ± 0.16
% sites with CAL ≥ 6 mm	31.5 ± 0.20	0.0 ± 0.00

BoP, bleeding on probing; PD, probing depth; CAL, clinical attachment loss.
[a]Dichotomous variable (0 = absence and 1 = presence).

3.2 | Pulse wave velocity and uniformity in wave propagation

Median values and interquartile ranges for PWV and R^2 values in periodontitis patients and controls for the 33 matched pairs are illustrated in Figure 3. No significant differences were observed between the groups for PWV (median PWV 2.81 m/s *versus* 3.35 m/s in periodontitis and periodontally healthy controls, respectively, Wilcoxon signed rank test p = .31), but statistically significantly lower median R^2 values were observed in periodontitis patients than in controls (.43 *versus* .52, respectively, p = .01).

Univariate linear regression analysis based on values from 78 patients (i.e. all participants with obtainable PWI data) (Table 3) showed a significant, negative association between the uniformity in wave propagation (R^2) and presence of periodontitis (p = .004), current smoking (p = .02) as well as continuous measures of periodontitis including percentage of sites with BoP (p = .02), percentage of sites with dental plaque (p = .03), percentage of sites with PD ≥ 4 mm (p = .008), PD ≥ 6 mm (p = .02), CAL ≥ 4 mm (p = .003) and CAL ≥ 6 mm (p = .002). On the other hand, PWV did not show statistically significant associations with any of the variables studied apart form % of sites with BoP (p = .04). In the multivariate linear regression analysis (Table 4), based again on all participants with obtainable PWI data, after adjusting for gender, age, ethnicity, BMI, smoking, blood pressure medication, and number of teeth present, the only association that remained statistically significantly, negatively associated with R^2 was the presence of periodontitis (p = .01).

4 | DISCUSSION

In this study, we detected differences in the elastic properties of the common carotid artery between a group of patients with periodontitis and gender- and age-matched periodontally healthy controls, none of whom presented with a history of established CVD or diabetes mellitus. In multivariate analyses controlling for demographic variables, BMI, smoking, and blood pressure medication, it was shown that presence of periodontitis was associated with a less uniform propagation of the pulse wave along the carotid arteries, indicating arterial stiffness.

Our results are in line with a body of literature that has associated periodontitis with systemic inflammation, endothelial dysfunction and adverse cardiovascular outcomes. Arterial stiffness constitutes a strong, independent subclinical indicator of CVD and is a hallmark of atherosclerosis (Cecelja & Chowienczyk, 2012). Arterial stiffness and periodontitis have been associated using two major methodologies: those examining vessel anatomy—such as IMT (Beck & Offenbacher, 2001, 2005; Southerland et al., 2012)– or those assessing the vessel

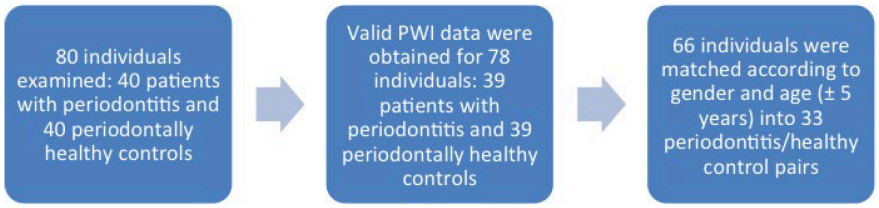

FIGURE 2 Flow chart indicating the analytical strategy (enrolled sample, availability of valid Pulse Wave Imaging data, and matching according to gender and age)

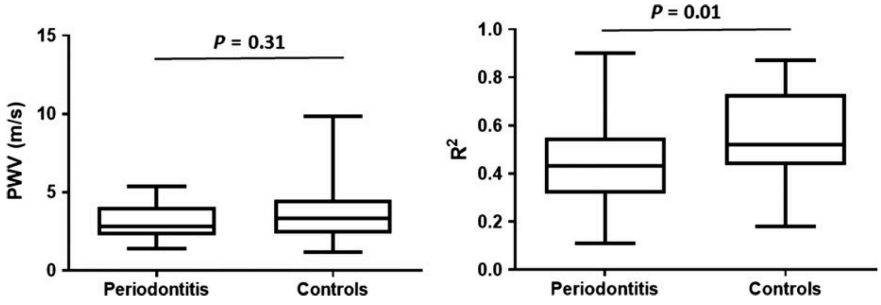

FIGURE 3 Graphic representation of pulse wave velocity (PWV) and R^2 in periodontitis cases and periodontally healthy controls, based on the 33 matched pairs. Horizontal lines describe medians and interquartile range; whiskers describe maximum and minimum values

TABLE 3 Univariate linear regression analysis of the association between explanatory variables and the main outcomes (PWV and R^2) in all participants with obtainable Pulse Wave Imaging data (n = 78)

	Outcome variable PWV		Outcome variable R^2	
Variable	Coefficient (95% CI)	*p*-value	Coefficient (95% CI)	*p*-value
Periodontitis[a]	−0.37 (−1.03, 0.30)	.27	−0.12 (−0.20, −0.04)	.004
Gender	−0.27 (−0.92, 0.38)	.41	−0.0005 (−0.09, 0.8)	.99
Age	−0.01 (−0.04, 0.02)	.49	−0.004 (−0.004, 0.003)	.80
Ethnicity	0.53 (−0.15, 1.21)	.13	−0.008 (−0.10, 0.08)	.83
BMI	−0.02 (−0.10, 0.06)	.57	0.009 (−0.0008, 0.02)	.07
Smoking[a]	0.16 (−0.92, 1.23)	.77	−0.16 (−0.29, −0.02)	.02
BP medication[a]	0.18 (−0.62, 0.98)	.66	−0.06 (−0.16, 0.4)	.23
BP systolic	0.001 (−0.01, 0.02)	.91	0.003 (−0.002, 0.003)	.76
BP diastolic	0.001 (−0.31, 0.03)	.94	0.0007 (−0.003, 0.004)	.73
Number of teeth	0.008 (−0.10,0.12)	.89	0.007 (−0.007, 0.02)	.36
% sites PD ≥ 4 mm	−0.92 (−2.08, 0.25)	.12	−0.20 (−0.35, −0.05)	.008
% sites CAL ≥ 4 mm	−0.79 (−1.74, 0.17)	.11	−0.18 (−0.30, −0.06)	.003
% sites PD ≥ 6 mm	−1.62 (−3.98, 0.74)	.18	−0.34 (−0.65, −0.5)	.02
% sites CAL ≥ 6 mm	−0.92 (−2.46, 0.62)	.24	−0.30 (−0.50, −0.11)	.002
% sites with plaque[a]	−0.72 (−1.67, 0.22)	.13	−0.14 (−0.26, −0.02)	.03
% sites with BoP[a]	−1.14 (−2.20, −0.9)	.04	−0.16 (−0.30, −0.3)	.02

Gender: 0 male, 1 female; Ethnicity: 0 Hispanic, 1 non-Hispanic.
BoP, bleeding on probing; BMI, body mass index; BP, blood pressure; PD, probing depth; CAL, clinical attachment loss; PWV, Pulse wave velocity.
[a]Dichotomous variable (0 = absence; 1 = presence).

TABLE 4 Multivariate linear regression analysis of the association between explanatory variables and the main outcomes (PWV and R^2) in all participants with obtainable Pulse Wave Imaging data (n = 78)

	Outcome variable PWV		Outcome variable R^2	
Variable	Coefficient (95% CI)	*p*-value	Coefficient (95% CI)	*p*-value
Periodontitis[a]	−0.57 (−1.34, 0.19)	.14	−0.12 (−0.21, −0.03)	.01
Gender	0.03 (−0.78, 0.86)	.92	−0.03 (−0.13, 0.06)	.48
Age	−0.01 (−0.04, 0.02)	.54	0.0009 (−0.003, 0.005)	.68
Ethnicity	0.50 (−0.31, 1.33)	.22	−0.01 (−0.11, 0.08)	.77
BMI	0.006 (−0.07, 0.09)	.87	0.009 (−0.001, 0.01)	.08
Smoking[a]	0.38 (−0.89, 1.66)	.55	−0.04 (−0.20, 0.11)	.57
BP medication[a]	0.26 (−0.60, 1.12)	.54	−0.03 (−0.14, 0.06)	.45
Number of teeth	−0.04 (−0.18, 0.09)	.51	−0.0004 (−0.01, 0.01)	.95

Gender: 0 male, 1 female; Ethnicity: 0 Hispanic, 1 non-Hispanic, BP medication: 0 no, 1 yes.
BoP, bleeding on probing; BMI, body mass index; BP, blood pressure; PD, probing depth; CAL, clinical attachment loss; PWV, Pulse wave velocity.
[a]Dichotomous variable (0 = absence; 1 = presence).

functionality—such as FMD (Amar et al., 2003; Blum et al., 2007; Elter et al., 2006; Higashi et al., 2008; Mercanoglu et al., 2004; Seinost et al., 2005; Tonetti et al., 2007) and foot-to-foot PWV (Franek et al., 2012; Hayashida et al., 2013; Kapellas et al., 2014; Miyaki et al., 2006; Vieira et al., 2011).

Intima-media thickness has been shown in large epidemiological studies to be positively associated with periodontitis (Beck et al., 2001; Desvarieux et al., 2005), although its actual association with arterial stiffness remains unclear (Cecelja & Chowienczyk, 2012). Importantly, IMT physiologically increases with age (O'Leary et al., 1999), which may confound its association with periodontitis.

Commonly, FMD is compared to nitroglycerin-mediated dilation (NMD), in order to differentiate it from endothelium-independent vasodilation, and is assessed through two-dimensional ultrasound imaging of the brachial artery after occlusion-induced reactive hyperemia. Case–control and cross-sectional observations (Amar et al., 2003; Blum et al., 2007; Elter et al., 2006; Higashi et al., 2008; Mercanoglu et al., 2004; Seinost et al., 2005; Tonetti et al., 2007), single-arm

intervention studies (Blum et al., 2007; Elter et al., 2006; Higashi et al., 2008; Mercanoglu et al., 2004; Seinost et al., 2005) and a single randomized controlled trial (Tonetti et al., 2007) have all demonstrated an impaired flow mediated dilatation in periodontitis, a temporary deterioration in FMD immediately after periodontal treatment due to the massive bacterial inoculation that occurs in conjunction with soft tissue instrumentation, but substantial improvement 6 months after completed periodontal therapy. A recent systematic review and meta-analysis concluded that individuals with periodontitis presented with an average FMD that was 5.1% lower (95% CI 2.08–8.11) than that of periodontally healthy controls, while periodontal treatment was estimated to result in approximately 6.6% improvement in FMD (95% CI 2.83–10.44), $p < .0001$). Nevertheless, the assessment of endothelial function with FMD is impacted by methodological, physiological and technical factors all of which can influence the validity, reproducibility and interpretation of results in clinical research (Orlandi et al., 2014).

Carotid-femoral PWV is considered the "gold-standard" measurement of arterial stiffness (Laurent et al., 2006). Although a significant association has been observed between periodontitis and higher values of PWV (Hayashida et al., 2013; Houcken et al., 2016; Jockel-Schneider et al., 2014; Kapellas et al., 2014; Miyaki et al., 2006; Shanker et al., 2013; Vieira et al., 2011), this association was attenuated or not detected in several studies after adjusting for age, systolic blood pressure, smoking and other common risk factors of CVD (Miyaki et al., 2006; Vieira et al., 2011). Interestingly, no differences in PWV between periodontitis patients and controls were detected in a number of studies (Franek et al., 2009, 2012; Hanaoka et al., 2013). A recent systematic review concluded that patients with periodontitis have increased arterial stiffness compared to controls (higher mean PWV by 0.85 m/s; 95% CI: 0.53–1.16; $p < .00001$) while the two available interventional studies showed inconsistent effects of periodontal treatment on PWV (Schmitt, Carra, Boutouyrie & Bouchard, 2015).

Pulse Wave Imaging is a novel methodology for the assessment of PWV that was developed in our institution. PWI holds several advantages compared to other means of assessing the elastic properties of the vessel and determining PWV. Traditional methods used to determine PWV are global rather than regional, i.e. the calculation of PWV is carried out at two distant sites of the arterial tree, such as carotid-femoral (Vidal, Cordovil, Figueredo & Fischer, 2013; Vieira et al., 2011), carotid-radial (Franek et al., 2009, 2012) and less commonly, carotid-dorsalis pedis (Kapellas et al., 2014) and brachial-ankle (Hanaoka et al., 2013; Miyaki et al., 2006; Schmitt et al., 2015) among others (Jockel-Schneider et al., 2014; Shanker et al., 2013). The involvement of two distant sites in the measurement of PWV entails several challenges including difficulties in determining small time shifts (as the wave travels fast), difficulties in obtaining an accurate measurement of the distance between the two sites, and dependence on the assumption that the waves travel along a straight and uniform pathway between the two loci. These issues are especially important in older cohorts and patients that have suffered CV events or present with other CV risk factors (Luo et al., 2012).

Pulse Wave Imaging provides quantitative (PWV) and qualitative (R^2) information with regard to the elastic properties of the arteries and the uniformity of wave transmission. In our study, we observed no statistically significant differences in PWV between periodontitis patients and controls, likely due to the fact that the recruited cohort did not have a history of established CVD, diabetes, obesity, or uncontrolled blood pressure, and the frequency of current smokers was low. Admittedly, our study was adequately powered to detect a difference of 1.4 m/s between periodontitis patients and periodontally healthy controls, while the actual observed difference in median PWV between the two groups amounted to only 0.46 m/s. On the other hand, our study identified a clearly inferior uniformity in the transmission of the pulse wave though the aortic wall in periodontitis, indicating a level of subclinical structural pathology compatible with arterial stiffness. Similar observations were made in a cohort of patients with history of abdominal aortic aneurysm and hypertension, in whom the shape of the waveform was substantially distorted in the most severe cases (Li et al., 2011). While the clinical impact of the observed 0.09 reduction in the determination coefficient between periodontitis and periodontal health remains speculative, recently published experimental evidence in a murine model demonstrated a less homogeneous propagation in the pulse wave (R^2) in unstable *versus* stable aortic aneurysms, despite similar PWV values between the groups (Nandlall & Konofagou, 2016).

It is difficult to compare the results of this and other published studies that also assessed PWV in periodontitis, due to several methodological aspects including the definition of cases and controls, differences in the CV status of the participants [presence of essential hypertension (Franek et al., 2009), type II diabetes (Franek et al., 2012), heterozygous familial hypercholesterolemia (Vieira et al., 2011), or differences in race/ethnicity and accompanying levels of susceptibility to CVD (Kapellas et al., 2014)]. There are notable differences in the findings from intervention studies that have assessed PWV before and after periodontal therapy, with some studies suggesting a reduction in PWV post treatment (Houcken et al., 2016; Vidal et al., 2013), while another study detected a reduction in IMT but not in PWV, suggesting treatment-mediated changes in the structure but not the function of the vessel wall (Kapellas et al., 2014).

5 | CONCLUSIONS

Our findings confirm an association between poor periodontal status and arterial stiffness, assessed through a novel pulse wave imaging technique. Specifically, our study demonstrated that the propagation of the pulse wave along the aortic wall occurs in a non-homogeneous fashion in periodontitis patients, suggesting an element of structural pathology that results in arterial stiffening and/or inhomogeneity. Our findings are based on patients seeking oral health care in a University clinic setting, and are readily generalizable for adult patients with periodontitis in an urban setting.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest.

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11. PUBLICATION 2: NONINVASIVE EVALUATION OF VARYING PULSE PRESSURES IN VIVO USING BRACHIAL SPHYMOMANOMETRY APPLANATION TONOMETRY, AND PULSE WAVE ULTRASOUND MANOMETRY

11.1. Reference: Li, R.X., Ip, A., Sanz-Miralles, E. and Konofagou, E.E., (2017) Noninvasive evaluation of varying pulse pressures in vivo using brachial sphymomanometry, applanation tonometry, and Pulse Wave Ultrasound Manometry. *Artery Research 18*, 22-28.

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11.1.1. Acceptance letter

From: John Cockcroft (Artery Research) <EviseSupport@elsevier.com>

Date: Tue, Feb 7, 2017 at 6:59 AM

Subject: Your manuscript ARTRES_2016_27 has been accepted

To: rxl2103@columbia.edu

Ref: ARTRES_2016_27 Title: Noninvasive Evaluation of Varying Pulse Pressures in vivo Using Brachial Sphymomanometry, Applanation Tonometry, and Pulse Wave Ultrasound Manometry Journal: Artery Research

Dear Dr. Li,

I am pleased to inform you that your paper has been accepted for publication. My own comments as well as any reviewer comments are appended to the end of this letter. Now that your manuscript has been accepted for publication it will proceed to copy-editing and production.

Thank you for submitting your work to Artery Research. We hope you consider us again for future submissions.

Kind regards,

John Cockcroft Editor-in-Chief Artery Research

The authors describe the application of an ultrasound technique, previously developed by the same group, to obtain central aortic pulse pressure. The study compares pulse pressure in the brachial artery obtained by sphygmomanometry and applanation tonometry respectively and abdominal aortic pressure by ultrasound. The findings are expected, where the largest pulse pressure is found in the hypertensive group. However, the positive correlation between peripheral and central pulse pressure found only in the hypertensive group is of interest, as this may indicate a higher sensitivity of pulse wave velocity to pulse pressure in this group. Indeed, a significant limitation of the study is the small number of subjects at different ages, but the work provides a proof-of-concept study that may be extended to age matched groups in different

categories of hypertension.

11.1.2. Publication

Artery Research (2017) 18, 22–28

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Noninvasive evaluation of varying pulse pressures *in vivo* using brachial sphymomanometry, applanation tonometry, and Pulse Wave Ultrasound Manometry

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Abstract The routine assessment and monitoring of hypertension may benefit from the evaluation of arterial pulse pressure (PP) at more central locations (e.g. the aorta) rather solely at the brachial artery. Pulse Wave Ultrasound Manometry (PWUM) was previously developed by our group to provide direct, noninvasive aortic PP measurements using ultrasound elasticity imaging. Using PWUM, radial applanation tonometry, and brachial sphygmomanometry, this study investigated the feasibility of noninvasively obtaining direct PP measurements at multiple arterial locations in normotensive, pre-hypertensive, and hypertensive human subjects. Two-way ANOVA indicated a significantly higher aortic PP in the hypertensive subjects, while radial and brachial PP were not significantly different among the subject groups. No strong correlation ($r^2 < 0.45$) was observed between aortic and radial/brachial PP in normal and pre-hypertensive subjects, suggesting that increases in PP throughout the arterial tree may not be uniform in relatively compliant arteries. However, there was a relatively strong positive correlation between aortic PP and both radial and brachial PP in hypertensive subjects ($r^2 = 0.68$ and 0.87, respectively). PWUM provides a low-cost, non-invasive, and direct means of measuring the pulse pressure in large central arteries such as the aorta. When used in

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conjunction with peripheral measurement devices, PWUM allows for the routine screening of hypertension and monitoring of BP-lowering drugs based on the PP from multiple arterial sites.

Introduction

Hypertension is a highly prevalent cardiovascular risk condition that affects nearly 1 billion people globally,[1] increasing the risk of heart disease and stroke by 3–4 times.[2] In current clinical practice, the diagnosis and monitoring of hypertension is based on peripheral blood pressure measurements taken from the brachial artery using a sphygmomanometer. However, the central blood pressure (CBP) near the heart (i.e. in large arteries such as the aorta) has been recognized to play a key role in the pathogenesis of cardiovascular disease.[3–5] The distending pressure in the large elastic arteries is a key determinant of the degenerative changes that characterize accelerated aging and hypertension.[6] Furthermore, it has been demonstrated that different anti-hypertensive pharmacological treatments may have different effects on CBP reduction while maintaining similar brachial BP reduction.[6,7] These findings support the need to account for CBP during hypertension treatment and monitoring.

Pulse pressure (PP), defined as the difference between the systolic and diastolic blood pressures (i.e. the pressure increase required to generate a pulse), has been recognized by several studies as a significant predictor of all-cause cardiovascular mortality and morbidity.[3,8,9] PP arises from the interaction of cardiac ejection (stroke volume) and the properties of the arterial circulation. An increased stiffness of the large arteries leads to an increase in PP due to a reduction in arterial compliance and increased speed of wave reflections.[10] The PP in peripheral arteries is commonly assessed using cuff sphygmomanometry at the brachial site and applanation tonometry at the radial site.[11] However, the PP in large arteries remains challenging to measure clinically, as the only method to obtain a direct measurement of central PP in the clinic is by way of a highly invasive arterial catheter. Many longitudinal clinical research studies[3,12–16] have employed radial applanation tonometry with a generalized transfer function to derive central PP in large populations of patients. However, an indirect method may not be used for evaluation on an individual case-by-case basis.

Pulse Wave Ultrasound Manometry (PWUM) was previously developed by our group[17] as a noninvasive, easy-to-use central PP measurement technique based on the regional pulse wave propagation characteristics obtained using ultrasound elasticity imaging. Initial feasibility studies have demonstrated the reproducibility (~11% average intra-subject variability) of the method and its high correlation ($0.94 < r^2 < 0.98$) with the aortic PP waveforms obtained using radial applanation tonometry and a generalized transfer function in healthy, normotensive subjects. A block diagram of the PWUM technique on a normal human aorta is shown in Fig. 1. Aortic wall displacements and pulse wave velocity (PWV) are estimated using our established Pulse Wave Imaging (PWI) technique,[18–25] and the incremental distension curve is obtained by subtracting the posterior wall displacements from anterior wall displacements at the central scan line to avoid angle artifacts. The theoretical basis for PWUM is formed by combining the Laplace Law[26] and the Modified Moens–Korteweg Equation,[27–29] thus relating an incremental change in fluid pressure (dP) to the PWV, incremental distension (dR), fluid density (ρ), Poisson's ratio (ν), and lumen radius (R).

While PWUM has been tested in healthy, normotensive subjects,[17] it has not yet been used to evaluate patients with elevated blood pressure. This study aims to evaluate the feasibility of a more complete assessment of PP variation throughout the arterial tree by performing direct measurement of PP at three arterial sites using three different instruments — a sphygmomanometer cuff for the left brachial artery, an applanation tonometer for the left radial artery, and PWUM for the infrarenal abdominal aorta.

Methods & study design

Study design

This study was approved by the Institutional Review Board (IRB) of Columbia University. Outpatients visiting the Dental Clinic at Columbia University Medical Center for routine dental exams were recruited. Patients who provided informed consent to participate in the study were instructed to lie in the supine position for the duration of the exam. Three brachial blood pressure measurements were performed on the left arm over a 15-minute period using a clinically recommended[30] automatic digital blood pressure monitor (HEM-705CP, Omron Corp., Kyoto, Japan). The first measurement was excluded, and the average of the latter two was used to classify each subject as pre-hypertensive (systolic blood pressure between 120 mmHg and 139 mmHg) or hypertensive (systolic blood pressure >140 mmHg) based on the American Heart Association (AHA) recommendation for blood pressure categorization.[31]

Brachial PP was calculated as the difference between the systolic and diastolic pressures. Because blood pressure is known to fluctuate throughout the day,[32] it was important to perform all measurements as concurrently as possible. In between each brachial cuff measurement, PWUM and radial applanation tonometry were performed to obtain the pulse pressure waveform in the aorta and left radial artery, respectively. Only the subjects who exhibited

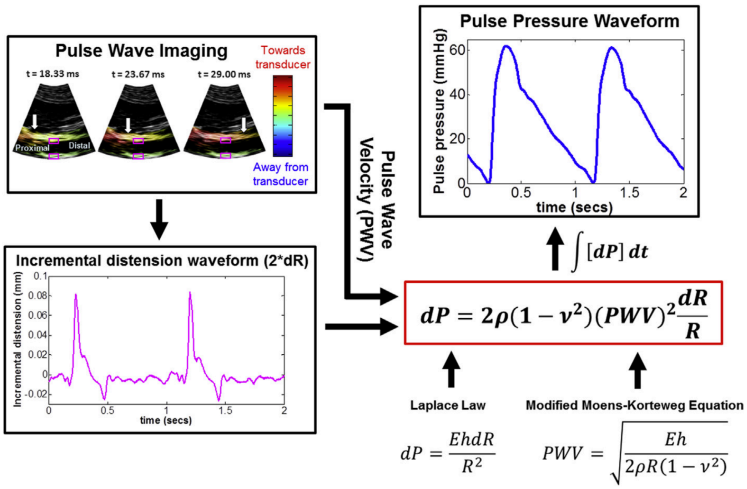

Figure 1 Block diagram of the Pulse Wave Ultrasound Manometry (PWUM) method on a normal human aorta *in vivo*. A previously developed technique, Pulse Wave Imaging (PWI), provides local PWV measurements by tracking the estimated aortic wall displacement waveform, shown here in color overlaid onto consecutive B-Mode frames. The incremental distension waveform (pink) obtained at the central scan line (pink squares) was used along with the PWV to derive the pulse pressure waveform based on the Laplace law and the Modified Moens—Korteweg equation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

a clear, unobstructed acoustic window of an infrarenal abdominal aortic segment were included in this study. This resulted in N = 9 pre-hypertensive (5 M, 4 F, mean age 37.8 ± 8.4 y.o.) and N = 5 hypertensive (3 M, 2 F, mean age 54.2 ± 3.1 y.o.) subjects. N = 5 normal subjects (3 M, 2 F, mean age 27.2 ± 3.2 y.o.) were also recruited for comparison, yielding a total subject population of N = 19.

PWUM

The infrarenal abdominal aorta of each subject was scanned in the longitudinal (i.e. long-axis) view using a SonixTOUCH system (Analogic Corp., Peabody, MA, USA) and a 3.3 MHz curvilinear array transducer, as shown in Fig. 2. Because the pulse wave travels at a high velocity (~4—12 m/s in human abdominal aortas[19,20]), a high frame rate was warranted to adequately track its propagation.[33] The imaging depth was adjusted to the minimum depth so as to visualize both the anterior and posterior aortic walls, and the lateral resolution was reduced to between 19 and 25 scan lines over the field of view. This resulted in imaging depths of 7—12 cm and frame rates of 222—351 Hz, which corresponded to maximum measurable PWVs[33] of ~14.9—23.3 m/s. RF frames were acquired over 2-second intervals during which the subject was required to perform breath holding in order to minimize rigid motion. One additional RF frame was acquired at a high line density (180) to provide a reference frame for accurate manual segmentation of the aortic walls. All RF signals were digitized at a sampling frequency of 40 MHz, and 5—7 acquisitions were performed for each subject in order to average the measurements over multiple cardiac cycles.

The incremental (i.e. inter-frame) axial displacements were estimated offline using a 1-D normalized cross correlation-based motion estimation method[34] on the RF signals with a 1.5 mm window size and 95% overlap. Due to its close proximity to the spine, the posterior aortic wall exhibited minimal motion *in vivo*. Manual segmentation of the anterior wall was performed on the high line density reference frame, generating a wall trace that was mapped onto the first frame of the RF sequence and automatically updated based on the inter-frame displacements to track the wall throughout the sequence.[35] The displacements at each point along the dynamic trace were mapped over time, generating a 2-D image depicting the spatio-temporal variation of the pulse wave propagation. Waveform tracking was performed by automatically identifying the 50% upstroke[33] of each displacement waveform on the

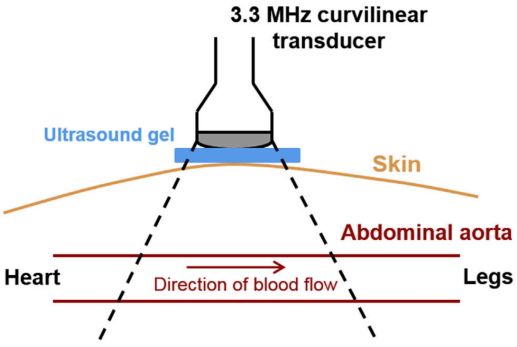

Figure 2 Schematic for *in vivo* data acquisition using PWUM. In order to minimize rigid motion, each subject was asked for perform breath-holding during each 2.5-second acquisition.

spatio-temporal map, and PWV was estimated via linear regression of the 50% upstroke markers. The incremental distension curve was obtained at the central scan line and inserted along with the PWV into the PWUM framework (Fig. 1) to arrive at the aortic PP waveform.

Radial applanation tonometry

An applanation tonometry system was assembled and calibrated for the purpose of this study by connecting a SPT-301 noninvasive pulse tonometer to the input channel of a PCU-2000 pressure control unit (Millar Instruments, Houston, TX, USA). The output channel of the control unit was connected to a USB digital I/O device (NI USB-6501, National Instruments Corp, Austin, TX, USA) for data acquisition. The I/O device was controlled by a MATLAB GUI (MathWorks, Natick, MA, USA) that displayed and saved the tonometer signal in real-time on the SonixTouch scanner. Once the pulse in the left radial artery was located by palpation, the tonometer was placed on the top of the artery to record 20 s of radial pressure waveforms.

For each subject, radial and aortic pulse pressures were calculated from the respective waveforms as the amplitude difference between the peak and the beginning of the upstroke (i.e. foot), and averaged over 5—10 cardiac cycles.

Statistical analysis

Due to unequal sample sizes, two-way ANOVA was performed using the Bonferroni method to evaluate statistical significance among the three subject groups.

Results

PWI yielded aortic PWV measurements of 5.00 $\pm$ 0.59 m/s, 7.37 $\pm$ 1.50 m/s, and 11.69 $\pm$ 2.72 m/s in the normotensive, pre-hypertensive, and hypertensive subject groups, respectively. Since PWV is a regional functional index of arterial stiffness over a certain arterial length,[36] the significantly higher PWV of the hypertensive group ($p < 0.01$) suggests that the aortas of the hypertensive subjects were stiffer than those of the normotensive and pre-hypertensive subjects.

Figure 3 shows the radial and aortic pulse pressure waveforms over one full cardiac cycle for (a) a normal subject (F, 23 y.o., brachial BP 113/70) in which radial PP $>$ aortic PP, (b) a pre-hypertensive subject (M, 51 y.o., brachial BP 136/87) in which radial PP $\approx$ aortic PP, and (c) a hypertensive subject (M, 60 y.o., brachial BP 153/91) in which radial PP $\approx$ aortic PP. Note the difference in the amplitude scale of the waveforms, which were manually aligned by the foot (i.e. beginning of the upstroke). In each case, the times corresponding to the peak of the forward wave, reflected wave, and dicrotic notch in the radial PP waveform are indicated by blue lines and labels.

In the normal and pre-hypertensive cases, the peak of the radial forward wave correlates with an inflection point in the aortic waveform representing the beginning of the augmentation pressure, which is commonly observed in the aorta when the forward and reflected waves merge during late systole.[37] By contrast, the greater separation between the forward and reflected peaks at the radial site indicates that the reflected wave merges with the forward wave during diastole. Also, the amplitude of the radial reflected wave is greater in the pre-hypertensive case compared to the normal case.

The radial waveform in the hypertensive case closely resembles the aortic waveform in the normal and pre-hypertensive cases — the peak of the forward wave appears as an inflection point rather than a relative maximum, suggesting that the higher velocity of the radial reflected wave in the hypertensive case has caused it to merge with the forward wave in late systole.[38] In the aortic waveform, the inflection point corresponding to the radial forward peak is followed by a decrease in pressure rather than the increase seen in the normal and pre-hypertensive cases. This suggests that the reflected wave in the aorta of the hypertensive case may be merging with the forward wave in early diastole rather than late systole.[37]

The bar graphs in Fig. 4 show the blood pressure measurements (brachial systolic, brachial diastolic, brachial PP, aortic PP measured by PWUM, and radial PP measured by applanation tonometry) averaged across each of the three

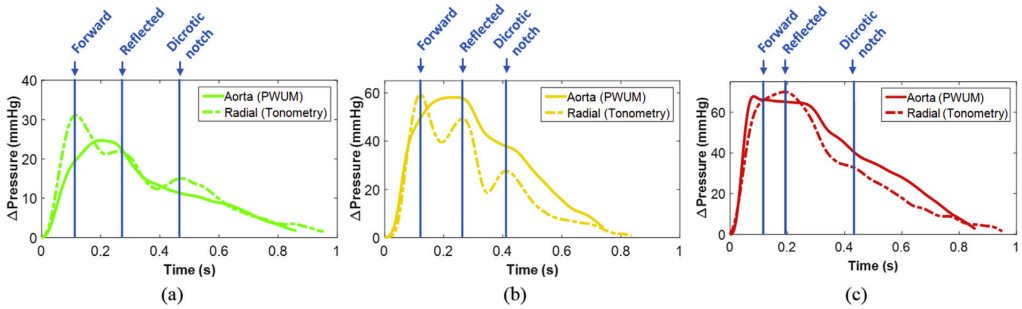

Figure 3 Representative radial and aortic pulse pressure waveforms, manually aligned by the foot of the wave, over a full cardiac cycle from (a) one normal subject (brachial BP 113/70), (b) one pre-hypertensive subject (brachial BP 136/87), and (c) one hypertensive subject (brachial BP 153/91). In each case, the times corresponding to the peak of the forward wave, reflected wave, and dicrotic notch in the radial PP waveform are indicated by blue lines and labels. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

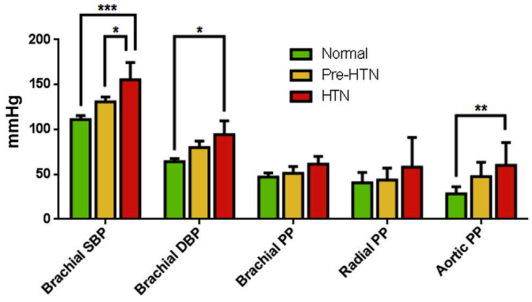

Figure 4 Summary of the average blood pressure measurements for each subject group (Pre-HTN = pre-hypertensive, HTN = hypertensive). For each of the five pressure measurements, statistical significances amongst the subject groups were determined using two-way ANOVA (*** denotes $p < 0.001$, ** denotes $0.001 < p < 0.01$, and * denotes $0.01 < p < 0.05$).

subject groups. For each of the five pressure measurements, statistical significance among the subject groups was determined using two-way ANOVA (*** denotes $p < 0.001$, ** denotes $0.001 < p < 0.01$, and * denotes $0.01 < p < 0.05$). Bonferroni's multiple comparisons test revealed that a significantly higher PP in the hypertensive group was detected only at the aortic site by PWUM, whereas the radial and brachial PPs were not significantly different among the three subject groups.

The aortic PP vs. (a) radial and (b) brachial PP for all subjects is shown in Fig. 5. While no strong correlation was observed in normal and pre-hypertensive subjects, there was a relatively strong positive correlation between aortic PP and both radial and brachial PP in hypertensive subjects ($r^2 = 0.68$ and 0.86, respectively).

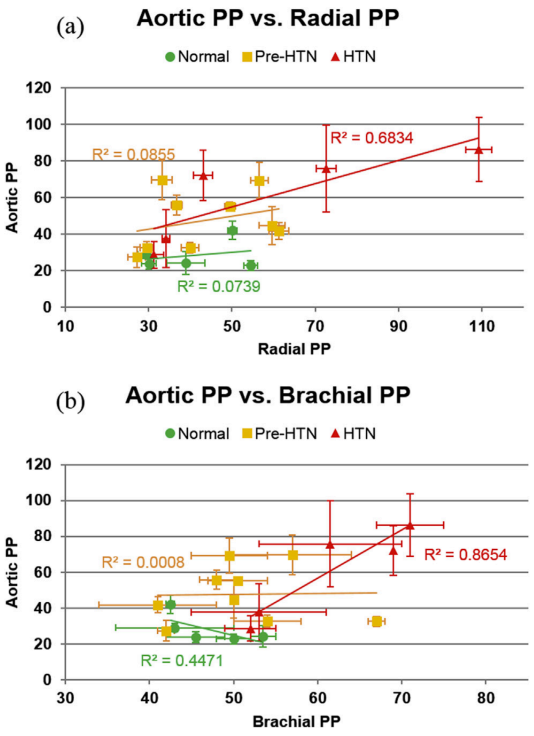

Figure 5 Aortic PP vs. (a) radial and (b) brachial PP for all subjects. In both plots, linear regression was performed on the data within each subject group. The greatest correlation between aortic PP and radial/brachial PP was observed in the hypertensive group.

Discussion

In this study, the aortic PP measured by PWUM was compared to the peripheral PPs measured using radial applanation tonometry and brachial cuff sphygmomanometry. It is important to note that due to the small sample sizes present in this study, the objective was not to establish definitive relationships in PP among different subject groups, but rather to evaluate the feasibility of noninvasively obtaining direct measurements of PP at different arterial locations. The authors acknowledge that larger sample sizes may affect the results of this study.

Due to the lack of ground-truth PPs, the measurements were compared relative to each other using correlations. Significantly higher ($p < 0.01$) PP in hypertensive subjects was detected at the aorta by PWUM but not in the case of the brachial and radial arteries (Fig. 4). This suggests that changes in overall blood pressure may occur in the central arteries before they are apparent at peripheral sites,[7,39] further stressing the importance of routine CBP evaluation.

While the subjects represented three distinct blood pressure levels, they also represented three distinct age groups. The pre-hypertensives were significantly older ($p < 0.05$) than the normals, and the hypertensives were significantly older ($p < 0.01$) than the pre-hypertensives. Thus, the effects of aging are also reflected in the PP variations at the three arterial sites. The metabolic and biochemical factors behind age-dependent arterial stiffening are well established.[40] Stiffening of the central arteries will result in changes in both the wave reflection patterns and arrival times of the reflected waves at the measurement site.[41] This can be either burdensome, if the reflected wave adds to the main systolic pressure peak, or beneficial, if the reflected wave arrives at late systole or early diastole.[42] If the reflected wave does not augment the main systolic pressure peak, an increase in PP will not be observed, which tends to be the case in younger, more elastic arteries exhibiting lower PWV and hence lower reflection amplitudes.[41] This phenomenon is evident from the results within the pre-hypertensive group in this study — the four subjects who exhibited aortic PP > 50 mmHg were significantly older (42.0 ± 5.7 y.o.) than the five subjects with aortic PP < 50 mmHg (33.4 ± 6.9 y.o.).

Figure 3 depicts the non-uniformity of the PP waveform at different arterial sites. The arrival of the reflected wave caused an amplification of the PP in the normal and pre-hypertensive aortas (Fig. 3a and b, respectively) as well as in the hypertensive radial artery (Fig. 3c), but not in the hypertensive aorta. This appears to contradict the notion that in older, hypertensive individuals, the reflected wave tends to

arrive at the ascending aorta during early systole, resulting in an even greater increase in PP.[37,43] However, the aforementioned studies have investigated central PP using either a generalized transfer function to derive the PP at the ascending aorta,[44,45] or cardiac catheterization at the aortic root.[46] Since this study represents the first non-invasive, direct measurements of PP in the abdominal aortas of pre-hypertensive and hypertensive subjects, it is possible that in some hypertensive cases (such as the one shown in Fig. 3c), the arrival of the reflected wave at the abdominal aorta may not always cause pressure amplification.

In the normal and pre-hypertensive groups, no strong correlation ($r^2 < 0.45$) was observed between the aortic and peripheral PPs, while in the hypertensive group, a relatively strong correlation was observed between aortic PP and both peripheral PPs ($r^2 = 0.68$ for the radial, $r^2 = 0.87$ for the brachial). A combination of prolonged peripheral hypertension and age-related arterial stiffening may have resulted in the positive relationship between radial, brachial, and aortic PP in the hypertensive group. However, it is worth noting that two of the hypertensive subjects exhibited PPs within the normal and pre-hypertensive range at all three arterial sites despite exhibiting systolic brachial pressures >140 mmHg. This is attributed to the increased diastolic brachial pressure in these subjects, which can serve as its own cardiovascular risk factor.[15] The lack of correlation between aortic and peripheral PPs in the normal and pre-hypertensive groups indicates that PP elevation throughout the arterial tree may not be uniform in relatively compliant arteries.

From a physiological perspective, the mechanical integrity of the aortic wall is mainly determined by its matrix constituents, namely elastin, collagen, and smooth muscle.[26,27] Elastin is highly distensible and load-bearing at low pressures, while collagen is 1000 times stiffer and load-bearing at high pressures.[26] As the PP rises, the increased intraluminal pressure exerts a greater force on the wall, engaging more collagen fibers and causing a reduction in arterial compliance. However, the arterial compliance is also dependent on the vessel size, which may explain the non-uniformity of PP measurements at the three different sites investigated in this study.

PWUM relies on fundamental physical assumptions that are inherent to the validity of the Laplace law and the Moens—Korteweg equation used in this study. These assumptions include a cylindrical geometry and the linear elasticity of the arterial wall. The linear elasticity assumption denotes that the distension and pressure waveforms have temporal variations that are in-phase. This is a commonly accepted hypothesis[47] relying on the fact that the nonlinear behavior of the arterial wall *in vitro* starts to prevail at higher deformation than the physiological one.[48] However, it is unclear how well this translates to human arteries *in vivo*, as the nonlinear behavior of the arterial wall may infer to a more complex relationship between distension and pressure.[49,50]

Conclusion

PWUM is a technique that can be used to measure the PP in any artery accessible by ultrasound (e.g. carotid, brachial, etc.). We have selected the abdominal aorta for all PWUM studies to date due to its clinical significance and the fact that it is impossible to directly access with other noninvasive methods such as brachial sphymomanometry or applanation tonometry. Future studies are aimed at applying the PWUM method at other imaging sites such as the carotid and brachial arteries.

Conflict of interest statement

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest in the subject matter or materials discussed in this manuscript.

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