Aus dem Institut für Biostatistik und Informatik in Medizin und Alternsforschung

Entwicklung und Anwendung von statistischen Auswertungsmethoden für Kohortenstudien am Beispiel der Studien getABI und BILANZ

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Verzeichnis der Abkürzungen und Tabellen

BILANZ BIsphosphonat-LANgZeittherapie

BMI Body-Mass-Index

CRP c-reaktives Protein

CVD kardiovaskuläre Erkrankung

getABI German epidemiological trial on Ankle-Brachial Index

GFR glomeruläre Filtrationsrate

GGT Gamma-Glutamyltransferase

ISCED International standard classification of education

PAVK periphere arterielle Verschlusskrankheit

MOF bedeutende osteoporotische Fraktur (major osteoporotic fracture)

1 Einführung in das Thema Entwicklung und Anwendung von Auswertungsmethoden für Kohortenstudien

1.1 Kohortenstudien

Es gibt vier Haupttypen von klinischen Studien: Querschnittstudien, Fall-Kontroll-Studien, Kohortenstudien und experimentelle Studien. Diese unterscheiden sich in der Anzahl der Beobachtungszeitpunkte und Gruppen, in der Art der Zuteilung auf die Studiengruppen und im zeitlichen Verlauf der Erhebung von Einflussgrößen bzw. Exposition und Zielgrößen bzw. Outcomes.

Bei Kohortenstudien werden in einer für die Fragestellung geeigneten Stichprobe zuerst die Einflussgrößen erhoben und nach einiger Zeit das Outcome, z.B. das Vorliegen einer Erkrankung.

Der Hauptvorteil ist die klare zeitliche Reihenfolge von Einflussgröße und Zielgröße, was im Idealfall eine Ursache-Wirkung-Beziehung belegt. Die Aussagekraft von Kohortenstudien ist eine Stufe niedriger als die von randomisierten kontrollierten (experimentellen) Studien, je nach Definition der Evidenzgrade (OCEBM Levels of Evidence Working Group 2011). Nachteilig ist ein unter Umständen hoher Aufwand an Kosten und Zeit, außerdem sind bei seltenen Erkrankungen bzw. Ereignissen hohe Fallzahlen notwendig.

Zwei typische Fragestellungen werden vorwiegend mit Kohortenstudien beantwortet. Ätiologische Kohortenstudien betrachten Ursachen von Erkrankungen an gesunden Personen durch Vergleiche von Gruppen mit und ohne bestimmte Risikofaktoren. Prognostische Kohortenstudien untersuchen das Auftreten von Ereignissen bei bereits erkrankten Personen mit und ohne Vorliegen eines bestimmten Faktors.

Ausgewertet werden diese mit Risikomaßen (relatives Risiko, Odds-Ratio, Hazard-Ratio) und dazugehörigen Konfidenzintervallen oder statistischen Tests, wie etwa Chi-Quadrat-Test oder Log-Rank-Test (Kaplan-Meier-Plot), letztere, falls die Zeit bis zum Ereignis mit einbezogen wird.

In vielen Fällen wird in statistischen Auswertungen mit solchen klassischen Verfahren gearbeitet. Ob das ausreicht, sollte hinterfragt werden, was aber in der Vergangenheit nicht immer der Fall war (Groenwold et al. 2008). Für einen fairen Vergleich zwischen den Gruppen sind jedoch univariable Auswertungen oder einfache Regressionsanalysen wie etwa Kaplan-Meier-Plots oft nicht ausreichend, da es

Störgrößen (Confounder), systematische Verzerrungen (Bias), oder eine Modifizierung von Assoziationen zwischen Exposition und Outcome (Interaktion) aus den verschiedensten Gründen geben kann. In der statistischen Auswertung wird dann zur Adjustierung der Risikomaße mit multivariablen Regressionsmodellen gearbeitet, z.B. logistische Regression oder Cox Proportional Hazards Regression (kurz: Cox-regression). Aber auch die Adjustierung stößt manchmal an Grenzen, weil diese mit der Annahme eines homogenen Einflusses für etwaige Confounder einhergeht (Sainani 2011).

Bei der Wahl der statistischen Verfahren für die Auswertung von Beobachtungsstudien müssen einige wichtige Aspekte berücksichtigt werden. Trotz einiger Sorgfalt fallen Publikationen von Beobachtungsstudien auch in hochgerankten Journals durch verschiedene Probleme auf. Es kommt gar nicht selten vor, dass Effekte überschätzt oder gar falsch hinsichtlich der Richtung geschätzt werden, wie spätere Studien mit besserem Setting herausfanden (Ioannidis 2005). Jedenfalls haben Beobachtungsstudien gegenüber randomisierten kontrollierten Studien (RCT) Mehrbedarf bei der Auswertung und es ist oft notwendig nach Lösungen für spezielle Probleme zu suchen.

Der Autor dieser Schrift ist im Rahmen seiner Tätigkeit als Wissenschaftler in der medizinischen Biometrie mit vielen solchen Situationen vertraut. Bekannte und innovative fortgeschrittene statistische Auswertungsmethoden kamen dabei in mehreren Analysen von zwei großen deutschen Kohortenstudien zum Einsatz und werden in dieser kumulativen Habilitationsschrift anhand von sieben Originalarbeiten präsentiert.

1.2 Die Studien getABI und BILANZ

Diese beiden an der Abteilung für medizinische Informatik, Biometrie und Epidemiologie der Ruhr-Universität Bochum mit öffentlichen Fördermitteln durchgeführten großen Kohortenstudien werden im Folgenden kurz vorgestellt.

Die periphere arterielle Verschlusskrankheit (PAVK) betraf im Jahre 2010 weltweit etwa 200 Millionen Menschen bei steigender Tendenz (Fowkes et al. 2013). Die PAVK ist im Anfangsstadium (Stadium I nach Fontaine) asymptomatisch. Zur (nichtinvasiven) Diagnose der asymptomatischen PAVK wird der arterielle Blutdruck an den Armen gemessen - eine Standarduntersuchung in der Hausarztpraxis - und mit dem Blutdruck der Knöchelarterien verglichen. Ist der Druck am Knöchel beim liegenden Patienten

deutlich niedriger als der Druck der Armarterien (Knöchel-Arm-Index <0,9, engl.: ankle-brachial index, ABI), gilt dies als Hinweis für eine Einengung der Beinarterien durch Atherosklerose. Die getABI-Studie wurde ab 2001 mit 6.880 Patienten über 65 Jahren in 344 Hausarztpraxen deutschlandweit durchgeführt und beobachtete in 7 Jahren Follow-Up primär die möglichen Ursachen und Folgen der peripheren arteriellen Verschlusskrankheit (PAVK) (Diehm et al. 2004).

Der Einsatz von Bisphosphonaten (BPs) zur Reduzierung des osteoporotischen Frakturrisikos geht bei Langzeitbehandlung einher mit einer Zunahme von seltenen, aber schwerwiegenden unerwünschten Wirkungen, wie atypischen Femurfrakturen und Osteonekrose des Kiefers. Da BPs auch nach dem Absetzen Auswirkungen auf den Knochenstoffwechsel haben, wurde vorgeschlagen, dass bei Patienten mit Osteoporose eine Unterbrechung der BP-Therapie mit regelmäßiger Neubewertung in Betracht gezogen werden kann, um unerwünschte Ereignisse zu minimieren und dabei möglicherweise einige Vorteile für das Skelett zu erhalten. Um die begrenzte Evidenz bezüglich der Risiken und Vorteile von BP-Pausen zu erweitern, wurden in der BILANZ-Studie prospektiv Frakturen und die Mortalität bei Patienten mit längerer BP-Vortherapie zwischen BP-Pausen und BP-Weiterbehandlung verglichen und das Frakturrisiko in Abhängigkeit von der BP-Pausendauer analysiert (Pfeilschifter et al. 2020).

1.3 Fortgeschrittene Auswertungsmethoden

In dieser Arbeit gibt es aus der statistischen Methodik für klinische Studien vier Schwerpunkte. Dazu gehören multivariable Überlebenszeitanalysen, Interaktionen bzw. Effektmodifikationen, Regressionsmethoden zur Bestimmung des Eintritts der peripheren arteriellen Verschlusskrankheit, und ein gleitender Durchschnitt zur Modellierung der zeitabhängigen, residualen Therapiewirkung von Bisphosphonaten nach Beginn einer Pause.

Multivariable Cox-Regression (Cox Proportional Hazards Regression): Für die Analyse von Ereigniszeiten ist die Cox Proportional Hazards Regression (kurz, Cox-Regression) das Standardverfahren. Die Hauptvoraussetzung ist hierbei, dass der zeitliche Verlauf der Ereignisse proportional in den Gruppen ist. Hierbei wird die Information verwendet, wann ein Ereignis eingetreten ist, ein Vorteil gegenüber der logistischen Regression, welche „nur“ den Anteil an Ereignissen in einem bestimmten Zeitraum verwertet. Dieses Mehr an Information wird durch zwei abhängige Variablen

transportiert, Zeit unter Risiko und Status. Der Status gibt zu jedem Zeitpunkt an, ob das Ereignis eingetreten ist oder nicht. Bei Beobachtungsende ohne Ereignis spricht man von Zensierung. In Beobachtungsstudien ist die Berücksichtigung von Störgrößen (Confoundern) notwendig, diese müssen vor Studienbeginn identifiziert und erfasst werden, um in der statistischen Auswertung berücksichtigt werden zu können. Man spricht bei der Hinzunahme solcher Faktoren in ein multivariables Modell von einer Adjustierung des eigentlich interessierenden Zusammenhangs von Einflussgröße und Outcome.

Interaktionen und Effektmodifikationen: Wenn der Effekt einer Exposition auf das Outcome durch einen sogenannten Modifikator bedeutsam beeinflusst wird, spricht man von einem Interaktionseffekt. Bei einer quantitativen Interaktion hat man auf den (i.a. zwei) Leveln des Modifikators verschieden große gleichgerichtete Effekte, während bei einer qualitativen Interaktion ein positiver und ein negativer Zusammenhang vorliegt. Im jeweiligen Modell befindet sich hierbei ein Interaktionsterm. Bei Subgruppenanalysen werden Effekte in Teilpopulationen, welche sich durch die Levels des Modifikators ergeben, geschätzt. Hierbei sollte es guter biometrischer Praxis nach zuvor einen Interaktionstest (Pocock et al. 2002) geben. Ansonsten kann es Probleme u.a. mit dem Signifikanzniveau (alpha-Fehler Kumulierung) oder Ungenauigkeiten bei der Schätzung durch zu kleine Teilkollektive geben. Das Problem zu weniger Ereignisse für die Zahl an Merkmalen tritt bei Subgruppenanalysen von Beobachtungsstudien mit multivariablen Modellen zur Adjustierung von Zusammenhängen eher auf als bei randomisierten kontrollierten Studien.

Regressionsmethode für den Zeitpunkt des Eintritts einer PAVK: Die Regressionsmethode zur Beschreibung des ABI-Verlaufs wurde entwickelt, um Ungenauigkeiten bei der Bestimmung des ABI auszugleichen. Zwar wird der ABI in Ruhe und liegend bestimmt, aber durch die Messung und Bildung des Quotienten des Blutdrucks an Beinen und Armen sind die Werte des ABI anfällig für zufällige Abweichungen. Somit können, wie bei anderer Diagnostik auch, falsch positive und falsch negative Ergebnisse entstehen. Bei Bestimmung des ABI zu mehreren Zeitpunkten kann eine Regressionsgerade berechnet werden. Dabei ist der Standardfehler der Vorhersage kleiner als der der einzelnen Bestimmung.

Gleitender Durchschnitt als zeitabhängige Therapievariable: Bei Vergleichen von Therapiegruppen in nicht-randomisierten Studien entstehen spezielle Situationen, insbesondere bei Betrachtung des zeitlichen Verlaufs. In Überlebenszeitanalysen können Einflussgrößen im Laufe der Beobachtung ihren Wert ändern. Eine Möglichkeit solche zeitabhängigen Kovariablen zu berücksichtigen ist für jeden Wechsel der Kovariable eine neue Beobachtung anzulegen, z.B. als Zeile in der Datenmatrix, bei Anpassung der Start- und Stoppzeiten. Um die residuale Therapiewirkung der Bisphosphonate zu untersuchen, wurde das Frakturrisiko in Abhängigkeit der Pausenlänge modelliert, unter der Annahme einer gleichmäßigen Abnahme der Wirkung seit Pausenbeginn. Dafür wurde ein gleitender Durchschnitt der Therapie der zurückliegenden 12 Monate verwendet, und in anschließenden Paarvergleichen wurden die Verzerrungen durch Selektions-Bias minimiert.

2 Zielsetzung der eigenen Arbeiten

2.1 Risikofaktoren für kardiovaskuläre Morbidität und Mortalität

In diesem Artikel wurden Risikofaktoren für Mortalität und kardiovaskuläre Erkrankungen von älteren Erwachsenen in der hausärztlichen Praxis im Längsschnitt der getABI-Studie analysiert. Mit den Daten bis zur vorletzten Nachuntersuchung (nach 5 Jahren) konnte 2009 gezeigt werden, dass eine periphere arterielle Verschlusskrankheit (PAVK) zum Beginn der Studie ein erhöhtes Risiko für den gemeinsamen Endpunkt von Sterblichkeit und Herz-/Kreislauferkrankungen anzeigte (Diehm et al. 2009). Mit den 7-Jahres-Daten konnten aufgrund der zahlreicheren Events komplexere Auswertungen vorgenommen werden. Das Ziel dieser Arbeit war, bisher nicht berücksichtigte potentielle Risikofaktoren hinzuzunehmen.

2.2 Prognose von kardiovaskulärer Mortalität mit NT-proBNP

Neben ihrer Rolle bei der Diagnose von Herzinsuffizienz bei symptomatischen Patienten mit Dyspnoe, wurden natriuretische Peptide zur Verbesserung der Risikovorhersage von kardialen Ereignissen und der Sterblichkeit in asymptomatischen Kohorten eingesetzt. Das Ziel war es, den prognostischen Wert von NT-proBNP (N terminales, pro B-Typ natriuretisches Protein) für kardiovaskuläre und Gesamtmortalität über die traditionellen Risikofaktoren hinaus in einer prospektiven Studie mit Patienten der hausärztlichen Primärversorgung zu untersuchen.

2.3 Interaktionen klassischer Risikofaktoren mit CRP

Bei Erwachsenen ist ein erhöhtes CRP ein etablierter Risikofaktor sowohl für kardiovaskulärer Morbidität als auch der Mortalität. Jedoch ist diese Assoziation bei verschiedenen Untergruppen von Individuen nicht homogen. Zum Beispiel variierten die Stärke des Einflusses bei verschiedenen Altersgruppen (Hamer et al. 2009). Daher war das Ziel, verschiedene potentielle Modifikatoren für den Zusammenhang zwischen erhöhtem CRP und Anstieg des Risikos zu versterben zu untersuchen. Diese waren Alter, Geschlecht, systolischer Blutdruck, Body-Mass-Index, und Diabetes mellitus.

2.4 Sozio-ökonomische Faktoren der PAVK

Es ist bekannt, dass der Wohnort einen Einfluss auf Gesundheit hat, insbesondere auf Herz-Kreislauf-Erkrankungen und das Charakteristika von Wohnvierteln mit der Häufigkeit von koronaren Herzkrankheiten zusammenhängen (Diez Roux et al. 2001). Außerdem sagt der Grad der Deprivation in der Nachbarschaft das Risiko eines ischämischen Schlaganfalls voraus, selbst nach Adjustierung der etablierten Risikofaktoren. Bezüglich der Assoziation von sozioökonomischen Faktoren und dem Risiko einer peripheren Arterienerkrankung (PAVK), einer weiteren Manifestation der atherosklerotischen Arterienerkrankung, ist die Evidenz spärlich. Da Untersuchungen zum Zusammenhang mit der Inzidenz der PAVK insbesondere in Europa bisher nicht bekannt wurden, sollten solche Zusammenhänge in dieser Arbeit aufgeklärt und außerdem nach potentiellen Modifikatoren gesucht werden.

2.5 Inzidenz der PAVK und Risikofaktoren bei Älteren

Für die Auswirkungen der Atherosklerose ist die Analyse von Symptomen der PAVK und von schwerwiegenden Konsequenzen wie der kardiovaskulären Morbidität und Mortalität sehr wichtig. Denkt man jedoch an die Prävention lohnt sich die Betrachtung der asymptomatischen PAVK. Daher wurden in dieser Arbeit die Inzidenz und die Risikofaktoren für neu aufgetretene PAVK untersucht. Das Eintreten der PAVK wurde definiert als Zeitpunkt des Auftretens eines peripheren Symptoms (Claudicatio Intermittens, Nekrose/Gangrän, periphere Revaskularisation, Amputation) oder durch den Beginn der asymptomatischen PAVK (nur das erste Ereignis wurde berücksichtigt). Dieser Beginn wurde mittels linearer Regression bestimmt; hierzu wurden die Messpunkte des gesamten Studienverlauf herangezogen; der Zeitpunkt, an dem die Regressionsgerade den Wert von 0,9 schneidet, wurde als Beginn der asymptomatischen PAVK definiert.

2.6 Vergleich verschiedener Definitionen der asymptomatischen PAVK

Die übliche Definition der asymptomatischen peripheren arteriellen Verschlusskrankheit (PAVK) durch eine einzelne Bestimmung des ABI (für Werte unterhalb des Schwellenwerts von 0,9) weist aufgrund von Messfehlern eine gewisse Unsicherheit auf. Dies kann sich auf die Schätzungen der PAVK-Inzidenz und die Bewertung der PAVK-Risikofaktoren auswirken. Um dieses Problem zu untersuchen, wurden drei Methoden zur Definition der asymptomatischen PAVK eingesetzt und

jeweils die Inzidenzrate und Risikofaktoren bestimmt und anschließend miteinander verglichen.

2.7 Das Frakturrisiko bei BP-Weiterbehandlung und Therapiepause

Die Wirksamkeit von Bisphosphonaten (BPs) zur Reduzierung des osteoporotischen Frakturrisikos wurde in großen randomisierten kontrollierten Studien nachgewiesen (Sanderson et al. 2016). Aufgrund von seltenen, aber schwerwiegenden unerwünschten Wirkungen einerseits und einer Wirkung von BPs auch nach dem Absetzen wurde vorgeschlagen, dass eine Unterbrechung der BP-Therapie mit regelmäßiger Neubewertung in Betracht gezogen werden kann.

Um das Wissen über BP-Pausen zu erweitern, wurde prospektiv das Frakturrisiko bei Patienten mit vorangegangener BP-Therapie zwischen BP-Pausen und BP-Weiterbehandlung verglichen und das Frakturrisiko in Abhängigkeit von der BP-Pausendauer analysiert. Da BPs mit einer reduzierten Mortalität assoziiert sind, wurde nach dem gleichen Schema auch die Mortalität untersucht.

Das Hauptproblem war dabei die Entwicklung eines Modells für die abklingende Wirkung der Bisphosphonate nach Pausenbeginn. Weiterhin interessierte, ob sich das Frakturrisiko in der Studienpopulation im Verlauf heterogen entwickelt.

3 Zusammenfassung und Diskussion der eigenen Arbeiten

3.1 Multivariable Modelle

Der Ankle-Brachial-Index (ABI) ist wesentlicher Faktor in dieser Arbeit. Die ersten Ergebnisse der getABI Studie (2001-2007) zu Prävalenz der PAVK anhand der Baseline-Daten und vom 5-Jahres Follow-Up gingen später in die S3-Leitlinie Diagnostik und Therapie der PAVK ein (Deutsche Gesellschaft für Angiologie 2015) (Lawall et al. 2011). In Abhängigkeit der Stadien der PAVK ergeben sich unterschiedliche Risiken für schwerwiegende Ereignisse. Die Ein-Jahres-Mortalität bei schwerer symptomatischer PAVK (critical limb ischemia, Stadium III nach Fontaine) lag in einer französischen Registerstudie bei 23% (Cambou et al. 2010). Aber auch die asymptomatische PAVK hat starke Auswirkungen.

Für den statistischen Beleg eines neuen Risikofaktors wird im Regelfall ein etabliertes Modell, welches sich für das Outcome eignet, für die Fragestellung ausgebaut, in dem der interessierende Faktor hinzugefügt wird. Auch bei Biomarkern, wie etwa Laborparametern im Blut, ist der inkrementelle Nutzen für die Prognose in der Auswertung aufzuzeigen. In beiden Fällen ist ein multivariables Regressionsmodell das Standardverfahren (Moons et al. 2009).

Anhand der Beurteilung der prognostischen Bedeutung des ABI sei dieses Verfahren exemplarisch dargestellt. Der ABI ist mit fatalen kardio- oder zerebrovaskulären Ereignissen assoziiert. Je schwerer die Durchblutungsstörung (je niedriger der ABI) desto höher ist die Wahrscheinlichkeit für Tod aus kardio- oder zerebrovaskulärer Ursache.

Merkmal		**Hazard-ratio (95%-KI)**	**Chi-Quadrat (Wald)**	**p-Wert**
Alter (pro Jahr)		1,10 (1,08-1,12)	109,25	<0.0001
männliches Geschlecht		1,89 (1,50-2,40)	28,15	<0.0001
Rauchstatus	ehem. R.	1,16 (0,90-1,48)	1,31	0.253
	aktiver R.	1,59 (1,12-2,25)	6,61	0,010

Hypertonie	1,40 (1,09-1,79)	6,93	0,009
Dyslipidämie	1,06 (0,86-1,31)	0,30	0,585
Diabetes Mellitus	2,11 (1,71-2,60)	48,16	<0.0001
ABI (pro 0,1)	0,83 (0,79-0,87)	50,62	<0.0001

Tabelle 1: Der ABI als unabhängiger Risikofaktor für Tod aus kardio- oder zerebrovaskulärer Ursache.

Ein bekanntes Modell für diese fatalen Ereignisse bei Älteren ist der europäische Risiko-Score SCORE-OP (Cooney et al. 2016). Bei Adjustierung für die enthaltenen prognostischen Faktoren Alter, Geschlecht, Rauchstatus, Bluthochdruck, Fettstoffwechselstörung und Diabetes zeigte sich in der getABI-Kohorte eine Erhöhung der Mortalität um 17% pro 0,1 Punkte Abnahme im ABI (Modellierung als stetiges Merkmal, Tabelle 1). Dieser sogenannte Zusatznutzen (engl.: added value) für die Prognose ist sehr deutlich, wie man auch an den Relationen der erklärten Variabilität des Outcomes (Werte der Wald-Chi-Quadrat Statistik) erkennen kann.

3.1.1 Risikofaktoren für kardiovaskuläre Morbidität und Mortalität

In dieser Arbeit zum 7-Jahres Follow-Up von getABI sollte mit Hilfe der Daten von 6880 Patienten bei einer Beobachtungszeit von sieben Jahren zunächst die Mortalität und die kardiovaskuläre Morbidität bestimmt werden. Dann wurden Modelle unter Berücksichtigung von klassischen (Rauchen, Diabetes mellitus, arterielle Hypertonie, Hypercholesterinämie) und neuen (u.a. Vitamin D, C-reaktives Protein, Nierenfunktion, Homocystein) Risikofaktoren sowie der prävalenten AVK zu Beginn der Beobachtung mit den Zielparametern Mortalität bzw. kardiovaskuläre Morbidität gebildet. Die neuen Faktoren ergaben sich aus der aktuellen (nach Diehm et al., 2009) Literatur, aus Nachberechnung (z.B. eGFR) oder Nachbestimmung (z.B. Vitamin D).

In dieser Publikation wurden die Ergebnisse berichtet und Gamma-Glutamyl-Transferase (GGT) als neuer Risikofaktor herausgestellt. In der Primärversorgung wird die Aktivität der GGT zur Beurteilung der hepatobiliären Dysfunktion verwendet, ist aber auch bekannt dafür, dass sie mit dem Risiko kardiovaskulärer Ereignisse sowie der Gesamtmortalität assoziiert ist. Da dieses Wissen hauptsächlich auf Kohorten mit Teilnehmern mittleren Alters basiert, wurden diese Zusammenhänge hier bei älteren Patienten in der Primärversorgung untersucht.

	All patients		≤3rd GGT quartile		>3rd GGT quartile	
	N	%	N	%	N	%

Total	6.882	100,0	5146	100,0	1676	100,0
Age >median	3.411	50,0	2652	51,5	759	45,3
Male sex	2.863	42,0	2174	42,2	689	41,1
Currently smoking	634	9,3	441	8,6	193	11,5
Arterial Hypertension	4738	69,5	3471	67,5	1267	75,6
Diabetes	1745	25,6	1166	22,7	579	34,5
LDL ≥130 mg/dL	2916	42,7	2224	43,2	692	41,3
Lipid lowering medication	1607	23,6	1160	22,5	447	26,7
BMI ≥30 kg/m²	1576	23,1	1133	22,0	443	26,4
Cardiovascular disease	1091	16,0	807	15,7	284	16,9
PAD	1434	21,0	1030	20,0	404	24,1
ISCED 0-2 (low)	1698	24,9	1285	25,0	413	24,6
Homocysteine >medina	3410	50,0	2573	50,0	837	49,9
sCRP >3 mg/dL	2642	38,7	1810	35,2	832	49,6
eGFR <60 mL/min/1,73m²	1337	19,6	1036	20,1	301	18,0
Lipoproteine(a) >50 mg/dL	1434	21,0	1102	21,4	332	19,8
Vitamin D <50 nmol/L	4741	69,5	3509	68,2	1232	73,5
Sodium <136 | >144 mmol/L	867	12,7	669	13,0	198	11,8

Tabelle 2: getABI - Baseline Charakteristika (Kreutzer et al. 2019).

Das Niveau der GGT-Werte in der Studie und die Normbereiche sind bei Männern höher als bei Frauen. Daher wurden zunächst je Geschlecht das 3.Quartil bestimmt, und dann dichotomisiert um hohe Werte als Risikofaktor zu untersuchen. In den Basisdaten waren bereits Unterschiede zu erkennen, so kamen in der exponierten Gruppen (GGT >3.Quartil) z.B. PAVK, positiver Raucherstatus, Hypertonie, erhöhtes CRP überproportional häufig vor (Tabelle 2). Im Vergleich der Inzidenzraten der Endpunkte zeigten sich bei der Gruppe mit GGT-Exposition jeweils numerisch höhere Ereignisraten.

Risk factor	**Overall death**		**Cerebrovascular event**	
	HR [95% CI]	p	HR [95% CI]	p
GGT >3rd quartile women >18, men >26 [U/L]	1,38 [1,22-1,56]	<0,001	1,39 [1,08-1,79]	0,010
Age >median	1,90 [1,67-2,17]	<0,001	1,61 [1,25-2,08]	51,5

Male sex	1,87 [1,64-2,12]	<0,001	1,66 [1,27-2,15]	42,2
Currently smoking	1,87 [1,59-2,19]	<0,001	1,07 [0,72-1,60]	0,738
Arterial Hypertension	1,21 [1,05-1,39]	0,008	1,02 [0,77-1,34]	0,901
Diabetes	1,43 [1,27-1,62]	<0,001	1,60 [1,25-2,05]	<0,001
LDL ≥130 mg/dL	0,75 [0,67-0,84]	<0,001	0,85 [0,66-1,08]	0,175
Lipid lowering medication	0,67 [0,58-0,77]	<0,001	0,89 [0,68-1,18]	0,430
BMI ≥30 kg/m²	1,02 [0,89-1,18]	0,739	1,04 [0,79-1,38]	0,778
Cardiovascular disease	1,45 [1,26-1,66]	<0,001	1,43 [1,07-1,91]	0,016
PAD	1,47 [1,30-1,67]	<0,001	1,45 [1,12-1,88]	0,006
ISCED 0-2 (low)	1,27 [1,11-1,46]	<0,001	1,19 [0,90-1,59]	0,229
Homocysteine >medina	1,27 [1,13-1,44]	<0,001	1,04 [0,82-1,32]	0,734
sCRP >3 mg/dL	1,30 [1,16-1,46]	<0,001	1,08 [0,85-1,37]	0,524
eGFR <60 mL/min/1,73m²	1,75 [1,53-1,99]	<0,001	1,17 [0,87-1,57]	0,291
Lipoproteine(a) >50 mg/dL	1,05 [0,92-1,20]	0,463	1,37 [1,06-1,78]	0,017
Vitamin D <50 nmol/L	1,39 [1,21-1,60]	<0,001	1,22 [0,93-1,60]	0,148
Sodium <136 | >144 mmol/L	1,14 [0,97-1,34]	0,120	1,39 [1,02-1,89]	0,038

Tabelle 3: Assoziation zwischen Risikofaktoren zu Baseline und Mortalität oder zerebrovaskulären Ereignissen (multivariable Auswertung) (Kreutzer et al. 2019).

Ohne weitere Adjustierung waren die Ereignisraten in der exponierten Gruppe ca. 40% höher (Ergebnisse nicht gezeigt). Erhöhte Werte beim GGT blieben auch nach Adjustierung für Standardrisikofaktoren und weitere Faktoren stabil (HR=1,38 für Tod jeglicher Ursache und HR=1,39 für Tod aus zerebrovaskulärer Ursache). Erwähnenswert ist weiterhin, dass Werte außerhalb des Normbereiches bei Natrium und erhöhte beim Lipoprotein(a) mit einem höheren Risiko von zerebrovaskulären Ereignissen assoziiert waren (Tabelle 3).

Der etablierten Vorgehensweise in Prognosestudien folgend, wurde von einem Modell für den Tod mit den etablierten Risikofaktoren ausgegangen und das Prognosemodell dann gezielt um die interessierenden Einflussgrößen, welche sich wie oben beschrieben, ergeben hatten, ergänzt. Aufgrund der Zahl der Ereignisse im Beobachtungszeitraum von 7,25 Jahren stellt die relativ hohe Zahl (18) der Einflussgrößen im Modell kein Problem dar, auch wenn man die Maßstäbe strenger anlegt als die Daumenregel mit 10 Ereignissen pro Prädiktor, wie etwa in Harrell et al. (1996) diskutiert.

3.1.2 Prognose von kardiovaskulärer Mortalität mit NT-proBNP

Diese Studie sollte an einem Kollektiv der repräsentativen Primärversorgung die Bedeutung von NT-proBNP (N terminales pro B-Typ natriuretisches Protein) für die Prognose von kardiovaskulärer und Gesamtmortalität über die traditionellen Risikofaktoren hinaus zeigen. Bisher war dieser Marker überwiegend in der Kardiologie im Einsatz, zur Diagnose von Herzinsuffizienz oder bei der Risikovorhersage von kardialen Ereignissen.

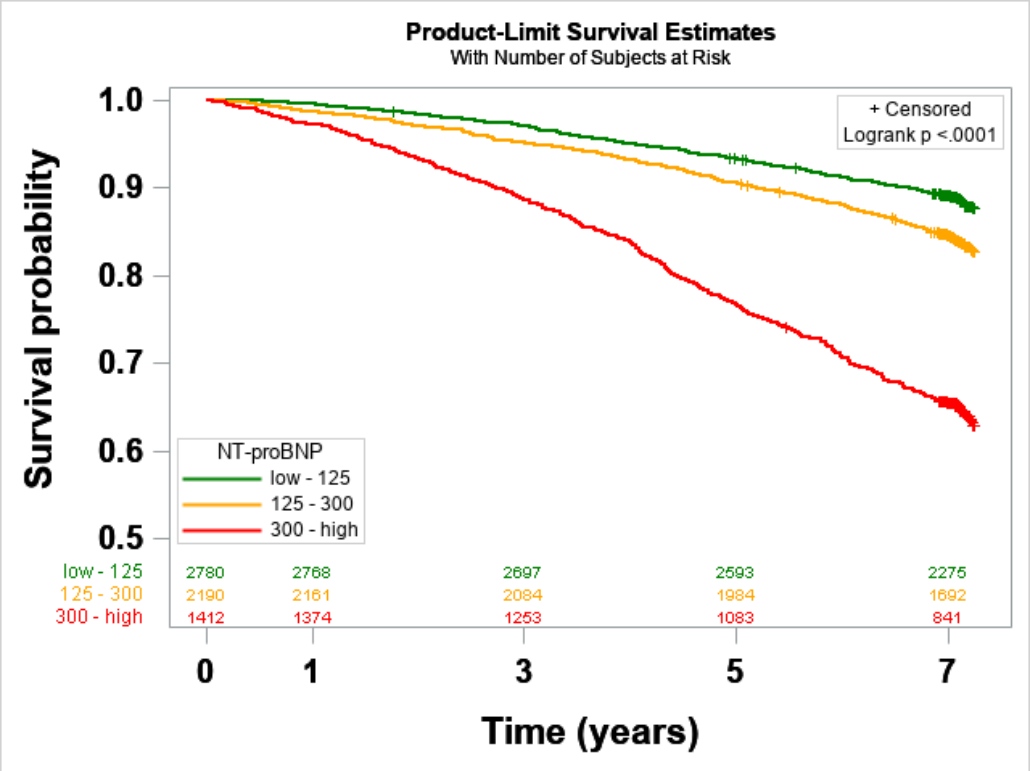

Abbildung 1: Kaplan-Meier-Plot für Mortalität nach den drei Kategorien von NT-proBNP

Der Kaplan-Meier-Schätzer zeigte Unterschiede zwischen den drei Gruppen (Abbildung 1). Weiterhin konnte auch der Zusatznutzen für die Prognose in der multivariablen Cox-Regression gezeigt werden (Tabelle 4).

NT-proBNP / **Model**	Hazard-Ratio (95% CI)		
	Continuous Log2-transf.	**Categorial in reference to the ≤125 group [pg/mL]**	
		125-300	>300
Unadjusted	1,83 (1,71 to 1,95)	1,62 (1,21 to 2,29)	6,38 (4,83 to 8,41
Model 1	1,70	1,64	5,22

(Age and sex)	(1,60 to 1,82)	(1,19 to 2,25)	(3,93 to 6,94)
Model 2 (Model 1 + cardiac medication)	1,60 (1,49 to 1,72)	1,58 (1,15 to 2,18)	4,31 (3,19 to 5,81
Model 3 (Framingham 10-year-risk)	1,70 (1,59 to 1,81)	1,61 (1,17 to 2,22)	5,06 (3,81 to 6,71)
Model 4 (Model 2 + Cardiac diseases)	1,58 (1,46 to 1,69)	1,51 (1,10 to 2,09)	3,93 (2,90 to 5,32)
Model 5 (Fully adjusted model)	1,51 (1,39 to 1,64)	1,48 (1,07 to 2,04)	3,41 (2,51 to 4,63)

Tabelle 4: Cox-Regression für Assoziation von NT-proBNP mit kardiovaskulärer Mortalität (Rudolf et al. 2020).

NT-proBNP ist somit als prognostischer Marker auch bei älteren Patienten im hausärztlichen Setting von Bedeutung. Für prädiktive Marker ist darüber hinaus Bedingung, dass je nach Klassifizierung (Schwellenwert oder Bereich) eine (andere) wirkungsvolle Therapieoption existiert. Dafür müsste in interventionellen Studien eine Interaktionen zwischen Marker und Therapie gezeigt werden.

In dieser Arbeit fehlte ein wichtiger Risikofaktor für die betrachteten kardiovaskulären Todesfälle: die Herzinsuffizienz. In getABI wurde erst nach 5 Jahren die Herzinsuffizienz abgefragt, klassiert nach den NYHA-Stadien (Criteria Committee of the New York Heart Association 1973). Da der zeitliche Zusammenhang nicht stringent ist und der Status bei zuvor Verstorbenen unbekannt ist, wurde stattdessen wurde die Medikamentenanamnese zu Beginn der Studie herangezogen. Es erfolgte eine Adjustierung für herzwirksame Medikamente wie etwa Beta-Blocker, Diuretika, und Digitalis. Dabei wurden für die Einnahme von Digitalis und Diuretika Hazard-Ratios ermittelt, welche mit ihrem Konfidenzintervall vollständig über der Eins lagen. Solche negativen Effekte für Medikamente erscheinen nur auf den ersten Blick widersprüchlich. In Beobachtungsstudien tritt dies häufiger auf, da Merkmale zur Medikamenteneinnahme ein Stellvertreter sind für die Schwere der Erkrankung, welche mit schlechterem Outcome assoziiert ist.

3.2 Interaktionen und Effektmodifikationen

In multivariablen Modellen sind die Haupteffekte im Allgemeinen additiv modelliert. Wenn bei der Modellierung für das gemeinsame Auftreten zweier (oder mehrerer) Effekte nicht nur additive Effekte angenommen werden, werden vorzugsweise

Interaktionen eingebaut. Dabei gibt es quantitative und qualitative Interaktionen. Arbeiten, die sich mit der Güte statistischer Auswertungen von klinischen Studien befassen, bemängeln u.a., dass Interaktionen zu selten berücksichtigt werden, obwohl die Annahme homogener Effekte sicher meist nicht zutrifft (Kraemer et al. 2006). In den nachfolgend gezeigten Arbeiten geht es um Modifikatoren der Effekte des CRP auf die Mortalität und um Interaktionen des Merkmals Geschlecht mit sozioökonomischen Faktoren.

3.2.1 Interaktionen klassischer Risikofaktoren mit CRP

Bei Erwachsenen ist ein erhöhtes CRP ein etablierter Risikofaktor sowohl für kardiovaskulärer Morbidität als auch der Mortalität. Jedoch ist diese Assoziation bei verschiedenen Untergruppen von Individuen nicht heterogen. Zum Beispiel variierten die Stärke des Einflusses bei verschiedenen Altersgruppen (Hamer et al. 2009) im primary care setting

Solche Effektmodifikationen wurden in dieser Arbeit (Rudolf et al. 2017) untersucht bezüglich der Assoziation vom Inflammationsmarker CRP und der Mortalität.

	Hazard Ratio* (95% CI)	**P value**
CRP x age	0,75 (0,60 to 0,94)	0,011
CRP x sex	1,38 (1,11 to 1,72)	0,004
CRP x diabetes	1,04 (0,83 to 1,31)	0,727
CRP x body mass index	0,88 (0,68 to 1,16)	0,367
CRP x syst blood pressure	0,64 (0,51 to 0,81)	<0,001

Tabelle 5: Interaktionen zwischen CRP und anderen Variablen (Alter, Geschlecht, Diabetes, BMI, systolischer Blutdruck) (Rudolf et al. 2017).

Dabei fanden sich Interaktionen von CRP mit Alter, Geschlecht und Blutdruck (Tabelle 5). Die geschätzten Hazard-Ratios für die Interaktionsterme bilden dabei den Teil des Risikos ab, welches noch über die zusammengesetzten Effekte der beiden beteiligten Merkmale hinausgeht. Eine solche ausführliche Darstellung der Effekte bei der alleinigen Exposition mit einem Faktor und des gemeinsamen Effekts gegenüber einer Referenzkategorie (Tabelle 6: Einfluss von CRP und Geschlecht auf Tod jeglicher Ursache (Rudolf et al. 2017).), folgt dabei den STROBE-Guidelines (Vandenbroucke et al. 2007). An dieser Stelle sei ferner erwähnt, dass die Interaktionen modellbedingt (logistische oder Cox-Regression) multiplikativ als relative Risiken interpretierbar sind,

während der Zusammenhang bei den Koeffizienten (β) additiv bleibt - durch den Faktor exp(Xβ) zwischen der Basis-Hazard und der Hazard bei Exposition.

	Events n	Person-years	Adjusted* Hazard-Ratio* (95% CI)	P value
Total (n=6817)	1.288	45.336	-	-
CRP ≤3 mg/dL female sex (n=2353)	327	16.121	Reference	Reference
CRP >3 mg/dL female sex (n=1592)	267	10.732	1,13 (0,96-1,33)	0,146
CRP ≤3 mg/dL male sex (n=1819)	348	11.999	1,44 (1,23-1,68)	<0,001
CRP >3 mg/dL male sex (n=1053)	346	6.484	2,25 (1,92-2,63)	<0,001

*Tabelle 6: Einfluss von CRP und Geschlecht auf Tod jeglicher Ursache (Rudolf et al. 2017).*adjustiert für Alter, Bildung, PAVK, kardiovaskuläre Komorbidität, Rauchstatus, Diabetes, systolischen Blutdruck, blutdrucksenkende Medikation, BMI, Gesamt-Cholesterin, lipidsenkende Medikation.*

Im Detail ergibt sich für die Interaktion aus CRP und Geschlecht bei Betrachtung der paarweisen Vergleiche zwischen den vier möglichen Kombination aus CRP und Geschlecht mit der Referenz CRP normal und Geschlecht weiblich folgendes: Das Hazard-Ratio von erhöhtem CRP und männlichem Geschlecht (HR=2,25) ist gleich dem Produkt der HRs von erhöhtem CRP und weiblichem Geschlecht (HR=1,13), normalem CRP und männlichem Geschlecht (HR=1,44) und dem für die Interaktion CRP und Geschlecht (HR=1,38). Das bedeutet einen um 38% höheren Anstieg der Mortalität beim gemeinsamen Auftreten der Faktoren erhöhtes CRP und männliches Geschlecht, als durch das Zusammenziehen der Effekte beider einzelner Merkmale zu erwarten gewesen wäre.

3.2.2 Sozio-ökonomische Faktoren der PAVK

Neben den herkömmlichen kardiovaskulären Risikofaktoren wurden sozioökonomische Ungleichheiten (niedriges Einkommen und niedriger Bildungsstand) mit der Prävalenz der PAVK bei Erwachsenen in den USA in Verbindung gebracht (Pande und Creager 2014). Aufgrund geringer Evidenz für eine Assoziation von sozio-ökonomischen Faktoren und dem Risiko einer PAVK, war diese Studie aufgesetzt worden. Hierbei wurde herausgefunden, dass Bildung sowie sozio-

ökonomische Indikatoren auf Gebietsebene wie Bevölkerungsdichte, Art der Gemeinde und lokale Arbeitslosenquote unabhängig assoziiert mit neu aufgetretenen Fällen von PAVK bei älteren Erwachsenen im Verlauf einer Nachbeobachtung von 7 Jahren waren (Tabelle 7).

Risk factor	**HR (95% CI)**	**P value**
Unemployment rate (per 1%)	1,04 (1,00 to 1,07)	0,032
Population density (per 500 Inhab./km²)	0,93 (0,89 to 0,98)	0,002
Education, ISCED 0-2	1,29 (1,14 to 1,46)	<0,001
Type of municipality		
Large city	Reference	Reference
Medium sized city	0,74 (0,60 to 0,92)	0,006
Small city	0,71 (0,53 to 0,96)	0,027
Village community	0,77 (0,51 to 1,15)	0,200
Age ≥71,9 years	1,33 (1,19 to 1,49)	<0,001
Male sex	0,83 (0,73 to 0,95)	0,007
Arterial hypertension	1,46 (1,29 to 1,66)	<0,001
Smoking status		
Never smoker	Reference	
Former smoker	1,23 (1,08 to 1,40)	0,002
Current smoker	1,96 (1,64 to 2.35)	<0,001
Diabetes mellitus	1,23 (1,09 to 1,39)	0,001
LDL cholesterol ≥130 mg/dL	1,22 (1,10 to 1,36)	<0,001
Lipid lowering therapy	1,15 (1,01 to 1,30)	0,040
sCRP >3 mg/L	1,19 (1,07 to 1,33)	0,002
eGFR <60 mL/min	1,03 (0,89 to 1,19)	0,696
Homocysteine ≥median	1,16 (1,04 to 1,29)	0,010
Vitamin D <50 nmol/L	1,11 (0,98 to 1,26)	0,088
History of cardiovascular events	1,41 (1,21 to 1,64)	<0,001

Tabelle 7: Multivariable Cox-Regression für neu aufgetretene PAVK im Beobachtungszeitraum von 7,25 Jahren (Rudolf et al. 2021).

Interessant ist der Befund, dass männliches Geschlecht mit einem niedrigeren Risiko verbunden war. Die Generation in dieser Studie ist sicherlich speziell, da geboren in den 30er Jahren des 20. Jahrhunderts die Bildung (50er und 60er Jahre) und Rauchgewohnheiten zwischen den Geschlechtern noch stark differierten. Daher ist die

um 17% niedrigere Rate bei Männern mit Vorsicht zu interpretieren. Eine Analyse mit Interaktionen zwischen Geschlecht und den genannte Faktoren konnte dies teilweise aufklären. Es lag nämlich eine Interaktion zwischen Geschlecht und Bildung vor, d.h. der Risikofaktor für neu aufgetretene PAVK, die niedrige Bildung (ISCED: 0-2 vs. 3-6), war bei Frauen (HR=1,22; 95%-KI 1,07-1,40) kleiner als bei Männern (HR=2,11; 59%-KI 1,64-2,72). Man muss dabei aber auch berücksichtigen, dass für Frauen der Zugang zu höherer Bildung seinerzeit nicht leicht war, und damit die Gruppe ISCED 0-2 bei Frauen vermutlich eine „bunte Mischung“ mit weit weniger Trennschärfe als bei Männern war.

3.3 Regressionsmethode für den Zeitpunkt des Eintritts einer PAVK

Länger ist bekannt, dass die Reliabilität des ABI zwar gut ist, aber aufgrund der Messungen des Blutdrucks Schwankungen unterworfen ist. Der mittlere Fehler von 8-9% sowohl innerhalb als auch zwischen Beobachtern bei wiederholten Messungen des ABI (Holland-Letz et al. 2007) führt auch zu einer Ungenauigkeit bei der Diagnose asymptomatische PAVK. Weiterhin trägt die Bildung von Quotienten nicht dazu bei, dass der ABI robust gegenüber Messfehlern ist. Daher wurde eine Methode entwickelt, die mehrere Zeitpunkte einbezieht.

3.3.1 Inzidenz der PAVK und Risikofaktoren bei Älteren

Für die Untersuchung von Risikofaktoren eignen sich auch bei der PAVK besonders neu Erkrankte. In prospektiven Studien ist dann auch der zeitliche Zusammenhang zwischen Exposition und Ereignis eindeutig. Für die Festlegung des Ereignisses PAVK zählte jedes periphere Symptom (nur das erste Ereignis wurde berücksichtigt) oder die asymptomatische PAVK, je nachdem, was zuerst auftrat. Anstatt die Diagnostik der asymptomatischen PAVK anhand eines einzelnen ABI-Wertes <0,9 vorzunehmen, wurde das Auftreten einer PAVK folgendermaßen festgelegt: Messpunkte über den Studienverlauf wurden für eine lineare Regression herangezogen und der Zeitpunkt, an dem die Regressionsgeraden den Wert von 0,9 schneidet, wurde als Beginn der PAVK definiert. Theoretisch ist diese Bestimmung genauer als bei der Einmalmessung. Der Standardfehler bei der Prognose mittels Regression hängt von der residualen Streuung und der Stelle der Prognose auf der x-Achse ab. Wenn diese zentral in den x-Werten liegt, ergibt sich die optimale Verringerung der Standardfehler des ABI um den Faktor Wurzel n, wobei n die Anzahl der ABI-Bestimmungen ist. Die

Risikofaktoren im multivariablen logistischen Regressionsmodell für einen Beobachtungszeitraum von 7 Jahren, zeigt bekannte Risikofaktoren (Tabelle 87).

Risk factor	**OR (95% CI)**	**P value**
Male	1,01 (0,85 to 1,10)	0,914
Age >75 years	1,49 (1,23 to 1,80)	<0,001
Education, ISCED 0-3	1,09 (0,86 to 1,39)	0,463
Current smoker	2,65 (2,08 to 3,37)	<0,001
BMI ≥30 kg/m²	1,10 (0,90 to 1,33)	0,347
Diabetes mellitus	1,35 (1,13 to 1,58)	0,001
Antihypertensive medication	1,34 (1,13 to 1,62)	0,001
Systolic BP ≥140 mmHg	1,13 (0,96 to 1,34)	0,154
Statin use	0,99 (0,80 to 1,22)	0,922
LDL ≥3,4 mmol/L	1,26 (1,07 to 1,48)	0,006
CVD comorbidity	1,62 (1,30 to 2.02)	<0,001
Vitamin D <50 nmol/L	1,15 (0,96 to 1,38)	0,137
sCRP >3 mg/L	1,23 (1,05 to 1,45)	0,013
Homocysteine ≥median	1,19 (1,01 to 1,41)	0,034
GFR <60 mL/min	1,27 (1,03 to 1,56)	0,029

Tabelle 8: Risikofaktoren zu Studienbeginn für das Auftreten einer PAVK innerhalb von 7 Jahren (Krause et al. 2016).

Hervorzuheben sind die neuen Faktoren erhöhtes CRP, erhöhtes Homocystein und Beeinträchtigung der Nierenfunktion.

3.3.2 Vergleich verschiedener Definitionen der asymptomatischen PAVK

Die oben erwähnte Ungenauigkeit im Zeitpunkt des Eintritts der PAVK spielt insbesondere in Überlebenszeitanalysen mit der Zielgröße Zeit bis zum Ereignis eine Rolle. Daher wurde für den Vergleich der Auswirkungen verschiedener Definitionen der asymptomatischen PAVK von der logistischen Regression auf die Cox-Regression gewechselt. Neben der ABI-Einzelbestimmung und der oben genannten Regressionsmethode, wurde eine dritte Definition der asymptomatischen PAVK erprobt, die Regressionsmethode mit Extrapolation. Diese hat den Vorteil, dass bei einem Lost-to-Follow-up ein möglicher Trend weiter betrachtet werden konnte. Da fehlende Werte oft bei prognostisch schlechten Patienten auftreten (Altman und Bland 2007), wirkt man so einer Unterschätzung der Inzidenz entgegen, die eintreten kann, wenn zum Zeitpunkt der letzten Information einfach zensiert wird.

Die Unterschiede in den Inzidenzraten der pAVK zwischen der ersten Definition der pAVK, bei der ein einziger ABI-Wert unter 0,9 zur Definition des Auftretens einer asymptomatischen pAVK verwendet wurde und denjenigen, die Regressionsmethoden verwendeten, war groß. Die 1. PAVK-Definition wies die höchste PAVK-Inzidenzrate auf (41,2 Ereignisse pro 1000 Personenjahre). Jahre). Die zweite PAVK-Definition (Regression A) wies die niedrigste Inzidenzrate auf (25,0). Dies könnte eine Unterschätzung sein, da ABI-Werte nicht zufällig fehlen und fehlende Werte einen schlechteren Zustand dieser Teilnehmer widerspiegeln können. Auch nach der Erweiterung der einzelnen Regressionsgeraden über den Zeitpunkt der letzten ABI-Bewertung hinaus (Regression B) blieben die Inzidenzraten deutlich niedriger: 33,9 (95% CI 31,91 bis 35,97) im Vergleich zur 1. PAVK-Definition mit der einzelnen ABI-Methode.

Im Vergleich der Risikofaktoren zwischen den drei Definitionen wurde einigen eine unterschiedlich große Bedeutung beigemessen (Tabelle 99).

	HR (95% CI)		
Risk factor	**1st Definition Single ABI**	**2nd Definition Regression A**	**3rd Definition Regression B**
Age ≥75 years	1,28 (1,12-1,46)	1,66 (1,42-1,95)	1,72 (1,50-1,97)
Male sex	0,89 (0,78-1,01)	1,10 (0,94-1,29)	1,06 (0,92-1,21)
Current smoker	1,79 (1,50-2.15)	2.36 (1,92-2.90)	2.08 (1,72-2.51)
Arterial hypertension	1,37 (1,21-1,56)	1,38 (1,17-1,63)	1,23 (1,07-1,41)
Diabetes mellitus	1,20 (1,06-1,37)	1,33 (1,13-1,56)	1,33 (1,16-1,53)
LDL ≥130 mg/dL	1,23 (1,10-1,38)	1,25 (1,08-1,44)	1,15 (1,02-1,30)
Lipid lowering therapy	1,12 (0,98-1,28)	1,04 (0,88-1,23)	0,95 (0,82-1,11)
BMI ≥30 kg/m²	1,17 (1,03-1,34)	1,19 (1,01-1,40)	1,19 (1,03-1,37)
CVD comorbidity	1,45 (1,24-1,70)	1,79 (1,48-2.15)	1,64 (1,39-1,93)
Low ISCED 0-2	1,27 (1,12-1,45)	1,36 (1,15-1,60)	1,36 (1,18-1,56)
HCY >14,1 µmol/L	1,18 (1,05-1,32)	1,15 (1,00-1,33)	1,10 (0,97-1,24)
CRP >3 mg/L	1,12 (1,00-1,26)	1,19 (1,03-1,38)	1,22 (1,08-1,38)
GGT highest quartile	1,05 (0,92-1,19)	1,13 (0,96-1,32)	1,08 (0,94-1,25)
eGFR <60 mL/min	1,04 (0,89-1,22)	1,06 (0,88-1,28)	1,18 (1,00-1,38)
Vitamin D <50 nmol/L	1,10 (0,97-1,26)	1,07 (0,91-1,26)	1,16 (1,01-1,33)

Tabelle 9 Multivariable Analyse der Assoziation zwischen Risikofaktoren und Inzidenz bei den drei Methoden zur Definition des Eintritts der PAVK (Lupilov et al. 2021).

So waren beispielsweise die Faktoren eGFR und Vitamin D nur bei Definition 3 bedeutsam. Auch bei Alter, Rauchen, Diabetes, CRP und kardiovaskulärer Komorbidität wurden bei Anwendung der Regressionsmethoden teilweise bis zu doppelt so große Risiken (Hazard-Ratio) bei Vorliegen dieser Expositionen geschätzt.

Wahrscheinlich kombiniert die dritte Definition die Vorteile von geringeren (Mess-) Fehlern und der Einbeziehung von Patienten mit schlechterem Gesundheitszustand, und könnte daher aus theoretischen Gründen vorzuziehen sein. Dies müsste in weiteren Studien überprüft werden, z.B. durch den Vergleich der Diskriminationsfähigkeiten der Definitionen für harte Endpunkte wie den Tod.

3.4 Gleitender Durchschnitt als zeitabhängige Therapievariable

Problematisch bei Therapiestudien ohne Randomisierung ist der faire Vergleich zwischen den Therapiearmen (Grimes und Schulz 2002). Oft bringt die Indikation zur Therapie bereits eine Selektion von Patienten „bei denen sich diese lohnt“ mit sich. Die besseren Aussichten auf ein gutes Outcome sind dann beim Vergleich der Therapiearme nicht gut zu berücksichtigen.

Dieses Problem ist nicht neu und von mehreren Seiten methodisch angegangen worden, eine Patentlösung gibt es aber nicht. Einige Verfahren sind etabliert, die Diskussionen darüber aber kontrovers. Das betrifft sowohl Matching-Strategien oder Propensity-Scores (Austin 2008) (Freemantle et al. 2013) oder auch die naheliegende Adjustierung für die bekannten Prädiktoren des Outcomes. Dieses sogenannte „Confounding by Indication“ ist eine Quelle von Verzerrungen in den Ergebnissen, und fällt unter die Kategorie Selektions-Bias. Das ist in vielen Fachgebieten der Fall, so auch im Beispiel der BILANZ-Studie.

3.4.1 Modellierung der residualen Therapiewirkung seit Pausenbeginn

Da die BILANZ-Studie entgegen der ursprünglichen Planung aufgrund von Rekrutierungsproblemen nicht als RCT durchgeführt wurde, sondern als Beobachtungsstudie, ergaben sich spezielle Herausforderungen in der Auswertung. „Eine generelle Begrenzung der Therapiedauer von Bisphosphonaten auf drei bis fünf Jahre sollte daraus nicht abgeleitet werden; pragmatisch erscheint eine Risiko-Nutzen-Bewertung nach jeweils 3-5 Jahren Therapie, um über die Fortführung der spezifischen Therapie zu entscheiden. Dabei sind insbesondere das aktuelle Frakturrisiko, die zu erwartenden positiven und negativen Therapieeffekte und

Patientenpräferenzen zu berücksichtigen.“ Eine Selektion von Patienten mit guter Prognose in die Therapiepause ergibt sich somit bereits aus der S3-Leitlinie zur Osteoporose (Dachverband Osteologie 2017). Dies führte vermutlich zum Beispiel bei kruder Zweigruppen-Auswertung entgegen bisheriger Evidenz bei der Bisphosphonat-Therapie zu einer erhöhten Mortalität in der Weitertherapiegruppe (Ergebnisse nicht gezeigt). Auch bei der primären Fragestellung des Vergleichs des osteoporotischen Frakturrisikos zwischen den Gruppen Therapiepause und BP-Weiterbehandlung ergab sich ein Hazard-Ratio unter Eins, also zu Gunsten der Pausengruppe. In beiden Fällen ist aber von einer Selektion von Patienten mit guter Prognose in die Pause auszugehen. Auch durch eine geeignete Adjustierung des Zusammenhangs von osteoporotischen Frakturen und der Therapie (Pause vs. BP-Weiterbehandlung) zur Zeit des ersten Interviews können Selektionseffekte nicht herausgerechnet werden. In einem Modell mit zeitabhängiger Therapievariable (aktuelle Therapie) waren ähnliche Auswirkungen festzustellen.

Fracture type	Adjusted HR (95% CI) for fractures		
	Without interaction	Interaction between BP-SMA and PVF	
		PVF = yes	PVF = no
Major osteoporotic fracture (MOF)			
0% vs. >0%-<50%	2,28 (1,07-4,86)	3,53 (1,19-10,51)	1,44 (0,49-4,22)
0% vs. ≥50%	1,54 (0,94-2,52)	2,42 (1,30-4,51)	0,93 (0,46-1,88)
>0%-<50% vs. ≥50%	0,67 (0,34-1.35)	0,69 (0,25-1,90)	0,65 (0,25-1,65)
Any clinical osteoporotic fracture			
0% vs. >0%-<50%	1,17 (0,68-2,01)	1,98 (0,85-4,63)	0,77 (0,37-1,58)
0% vs. ≥50%	1,20 (0,78-1,84)	1,60 (0,91-2,83)	0,91 (0,51-1,64)
>0%-<50% vs. ≥50%	1,02 (0,66-1,60)	0,81 (0,39-1,67)	1,19 (0,68-2,08)
Clinical vertebral fractures			
0% vs. >0%-<50%	1,93 (0,71-5,25)	1,65 (0,49-5,54)	3,30 (0,41-26,82)
0% vs. ≥50%	1,60 (0,78-3,29)	2,01 (0,78-5,17)	1,15 (0,43-3,09)
>0%-<50% vs. ≥50%	0,83 (0,34-2,02)	1,22 (0,44-3,40)	0,35 (0,05-2,54)

Tabelle 10: Adjustierte Hazard-Ratios für Frakturen, paarweise Vergleiche zwischen BP-SMA Stufen und Modifikation durch prävalente Wirbelkörperfraktur (Pfeilschifter et al. 2020).

Das Problem lag dabei auch in der geeigneten Modellierung der residualen Therapiewirkung seit Pausenbeginn im Verlauf der Beobachtung. Meine Idee war es, durch die Betrachtung von verschiedenen Zeitabschnitten seit Beginn einer Pause Selektionseffekte zu minimieren. Annähernd frei von Selektionseffekten sind hingegen die Vergleiche von Pausenabschnitten untereinander. Da das Outcome die Zeit bis zum Ereignis ist, braucht es dafür je Zeitpunkt eine Information, wie lange ein möglicher Pausenbeginn zurückliegt. Die zeitabhängige Betrachtung des Therapiestatus des aktuell zurückliegenden Jahres als gleitender Durchschnitt leistet genau dies. Die dabei auftretenden Werte zwischen Eins (durchgehende Behandlung) und Null (bereits mindestens ein Jahr Pause) wurden weiter in 3 Level kategorisiert. Das erste Level entspricht einer Weitertherapie oder kürzeren Zeitabschnitten seit Beginn einer Pause von bis zu einem halben Jahr. Für den Kontrast zwischen dem Zweiten, korrespondierend zu Zeitabschnitten von einem halben bis zu einem Jahr, und Dritten (mehr als ein Jahr) untereinander sind so nur geringe Verzerrungen aufgrund von Selektionseffekten zu erwarten. Für Zeitabschnitte einer Pause, die bis zu einem halben Jahr nach deren Beginn liegen, konnten wir keine erhöhte Wahrscheinlichkeit für osteoporotische Frakturen finden. Aber es zeigte sich, dass Zeitabschnitte von >12 Monate seit Pausenbeginn gegenüber denen von 6-12 Monaten mit einem höheren Risiko für MOFs (major osteoporotic fractures) assoziiert waren (HR=2,28 (1,07-4,86), Tabelle 10). Bei Betrachtung der Modifikation durch prävalente Wirbelkörperbrüche (ein Kriterium für Hochrisikopatienten) fand sich eine signifikante Interaktion, d.h. bei Hochrisikopatienten ist in längeren Pausenabschnitten vermutlich eine stärkere Zunahme des Frakturrisikos zu erwarten.

3.5 Schlussfolgerungen und Ausblick

Die Ergebnisse der großen, in Hausarztpraxen Deutschlands durchgeführten Kohortenstudie getABI zeigen die Bedeutung der PAVK als prognostischer Faktor für schwere Verläufe infolge von Atherosklerose und auch die Relevanz der PAVK als Outcome für eine Prävention oder Behandlung von speziellen Risikofaktoren einer neu aufgetretenen PAVK.

Neben der PAVK sind andere bedeutende Risikofaktoren für schwerwiegende Endpunkte hervorgetreten. Neben Alter und Geschlecht sowie den klassischen Faktoren einer erhöhten Morbidität und Mortalität wie etwa Rauchen, Diabetes, Lipidstoffwechselstörung, Bluthochdruck sind weitere teils bekannte (CRP, NTpro-

BNP) und neue (GGT) als unabhängige Einflussfaktoren in multivariablen Überlebenszeitmodellen analysiert worden. Im Fall von erhöhtem CRP sind Interaktionen mit Geschlecht, Alter und Bluthochdruck erkannt worden, womit sich Modifikationen des Einflusses von Inflammation auf die Mortalität zeigten und damit der Nutzen einer umfassenden Betrachtung des individuellen Risikoprofils auch aus statistischen Gesichtspunkten belegt ist.

Für die PAVK als Endpunkt hat sich in den vorgestellten Analysen der sozio-ökonomische Status neu als bedeutend herausgestellt. Neben individuellen Charakteristika wie Bildung standen Faktoren der Umgebung des Wohnortes (Arbeitslosenquote, Bevölkerungsdichte, Stadt-Gemeinde-Typ) im Fokus.

Methodisch war hierbei die Definition des Zeitpunkts des Eintritts von Interesse. Die angewendete Regressionsmethode bietet die Möglichkeit einer genaueren und weniger von Messungenauigkeiten beim Blutdruck gestörten Analyse der Zeit bis zum Ereignis als bei der einmaligen Bestimmung des ABI.

Beim Vergleich der Verfahren zur Bestimmung der inzidenten PAVK zeigten sich Unterschiede in der Inzidenzrate (wobei von einer Überschätzung im Falle der Einmalmessung ausgegangen werden kann) und auch in der Größe der geschätzten prognostischen Faktoren. Dabei sind BMI, Diabetes und GFR hervorzuheben. Es liegt nahe, dass das Risiko des Übersehens bestimmter Risikofaktoren in epidemiologischen Studien mit Mehrfachbestimmung des ABI geringer ist.

Ein Vergleich von 6 verschiedenen Definitionen mit PAVK als zeitabhängigem Risikofaktor für kardiovaskuläre Morbidität ist als nächstes vorgesehen. Die Publikation von Analysen der Studie als Ganzes mit ihren Endpunkten in einem Mehrstadien-Modell ist in Vorbereitung. Hierbei erfolgte die gemeinsame Betrachtung der Endpunkte in Abhängigkeit des PAVK-Status unter Einbeziehung von Missings (not at random) und einem Schwerpunkt auf die Langzeitwirkung von Umweltschadstoffen am Wohnort.

In der BILANZ Studie konnte für Zeitabschnitte einer Pause, die bis zu einem halben Jahr nach deren Beginn liegen, keine erhöhte Wahrscheinlichkeit für osteoporotische Frakturen gefunden werden. Für den Endpunkt Tod ergaben sich tendenziell sogar Vorteile für Patienten, die zur Zeit des ersten Interviews in einer Pause waren. In beiden Fällen ist aber von einer Selektion von Patienten mit guter Prognose in die

Pause auszugehen. Annähernd frei von Selektionseffekten sind hingegen die Vergleiche von Pausenabschnitten untereinander, der Zeitabschnitte >12 Monate gegenüber 6-12 Monate seit Beginn der Pause. Da das Outcome die Zeit bis zum Ereignis ist, brauchte es dafür je Zeitpunkt eine Information, wie lange ein möglicher Pausenbeginn zurückliegt. Die zeitabhängige Betrachtung des Therapiestatus des aktuell zurückliegenden Jahres als gleitender Durchschnitt leistet genau dies. Die dabei auftretenden Werte zwischen Eins (durchgehende Behandlung) und Null (bereits mindestens ein Jahr Pause) wurden weiter in 3 Level kategorisiert. Das erste Level entspricht einer Weitertherapie oder kürzeren Zeitabschnitten seit Beginn einer Pause von bis zu einem halben Jahr. Für den Kontrast zwischen dem Zweiten, korrespondierend zu Zeitabschnitten von einem halben bis zu einem Jahr, und Dritten (mehr als ein Jahr) untereinander sind so nur geringe Verzerrungen aufgrund von Selektionseffekten zu erwarten. Im Ergebnis waren spätere gegenüber früheren Abschnitten einer Therapiepause mit einem erhöhten Risiko für bedeutende osteoporotische Frakturen assoziiert. Darüber hinaus zeigte sich eine Interaktion zwischen diesem Kontrast und prävalenten Wirbelkörperbrüchen. Die Zunahme des Frakturrisikos bei längeren Pausen war größer bei Patienten mit vertebraler Fraktur zu Baseline. Das bedeutet für die Praxis, dass das Pausenmanagement bei Hochrisikopatienten anspruchsvoller ist und engmaschig das individuelle Frakturrisiko berücksichtigen sollte.

Die Einbeziehung fachlicher, biometrischer beziehungsweise statistischer Expertise in Publikationen klinischer Studien ist mittlerweile die Regel und in vielen Journals werden methodenaffine Reviewer hinzugezogen. Weiterhin gibt es zahlreiche Arbeiten die typische Fehler erfassen, auswerten, und Alternativen aufzeigen (Strasak 2007; Real et al. 2016). Am anderen Ende seinen als Check für vernünftige Auswertungen noch die Guidelines für Berichte von Studienergebnissen (Initiativen der CONSORT-Gruppe) oder die STROBE-Guidelines (Noah 2008) speziell für Beobachtungsstudien genannt. Letztlich bleibt es in Beobachtungsstudien aus Sicht des Autors aber oft eine Einzelfallentscheidung.

Das war auch ein Gedanke beim Schreiben dieser Arbeit, die den Leser in vier teils speziellen Situationen durch diesen Prozess führte.

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5 Reprints der in dieser Schrift zusammengefassten Publikationen

(in der Reihenfolge der Nennung auf Seite 7)

Original communication

Gamma-glutamyl transferase as a risk factor for mortality and cardiovascular events in older adults – results from a prospective cohort study in a primary care setting (getABI)

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Summary: *Background:* In primary care, the gamma-glutamyl transferase (GGT) activity is used for assessing hepatobiliary dysfunction, but is also known to be associated with the risk of cardiovascular events as well as overall mortality. As this knowledge is mainly based on cohorts with middle-aged participants, we aim to assess these associations in elderly patients in a primary care setting. *Patients and methods:* 6,880 unselected primary care patients, aged 65 years or older, were enrolled by 344 general practitioners all over Germany (getABI study). During seven years of follow-up, coronary heart disease (CHD) events (myocardial infarction or coronary revascularization), cerebrovascular events (stroke or carotid revascularization) and deaths were recorded. Event rates were calculated and Cox regression analysis with adjustment for age, gender, GGT, classical and other risk factors (e.g. education, homocysteine, C-reactive protein, vitamin D) was performed. *Results:* 1,243 patients died. 27.8 deaths per 1,000 patient years (0.95 confidence interval [0.95 CI]: 26.2–29.3) occurred in the whole cohort. 605 participants had a CHD event, i.e. 16.1 per 1,000 patient years (0.95 CI: 14.8–17.4). 296 cerebrovascular events were observed, i.e. 7.7 per 1,000 patient years (0.95 CI: 6.9–8.6). Cox regression analysis with adjustment for the above-mentioned risk factors showed a significant impact of baseline elevation of GGT above the 3rd quartile (women > 18 U/L, men > 26 U/L) compared to the rest on mortality (hazard ratio [HR] 1.38, 95% CI 1.22–1.56, $p < 0.001$) and cerebrovascular events (1.39, 95% CI: 1.08–1.79), $p = 0.010$), whereas the association with CHD events (HR: 1.16, 95% CI: 0.97–1.39, $p = 0.103$) showed no significance. *Conclusions:* In a primary care setting, GGT values have a significant association with overall mortality and cerebrovascular events, but not with CHD events in elderly patients.

Keywords: Gamma-glutamyl transferase, risk factor, mortality, cardiovascular events, older adults

Introduction

Gamma-glutamyl transferase (GGT) is found on the external surface of cell membranes. It cleaves extracellular glutathione, an antioxidant in several defense mechanisms in the body, thereby providing cells with the precursors of glutathione. GGT activity is elevated in case of a depletion of glutathione. Thus, it can be regarded as marker of oxidative stress [1].

In routine primary care, the GGT activity is determined in order to assess hepatobiliary dysfunction or alcohol abuse. Over the last twenty years, epidemiology studies have found an association of increased GGT activity and cardiovascular diseases (CVD) as well as overall mortality [2]. Moreover, as increased GGT activity was seen in atherosclerotic plaques and was associated with plaque instability, GGT seems to directly contribute to the pathophysiology of CVD [3]. Nonetheless, as increased GGT activity is correlated with a lot of CVD risk factors such as body mass index, alcohol use, smoking, total lipoprotein and HDL, serum cholesterol, uric acid, serum triglycerides, and systolic blood pressure [4], it remains unclear whether

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the determination of GGT provides independent information about long-term outcomes [3].

Epidemiology studies regarding the impact of GGT on CVD and mortality are mainly based on middle-aged participants [4–6]. As CVD risk increases with age, the prognostic value of GGT may be pronounced in older adults. There are only few studies focusing on the elderly population, e.g. population based [7, 8] or confined to elderly twins [9].

We aimed to assess the impact of increased GGT values on coronary heart disease (CHD) events and cerebrovascular events as well as overall mortality in unselected elderly men and women in a primary care setting. Therefore, we performed this post-hoc analysis of data from a primary care cohort of more than 6800 participants with a follow-up of seven years (the getABI study) [10].

Patients and methods

In October 2001, the German epidemiological study on ankle brachial index (getABI) was set up as a prospective cohort study. 344 general practitioners (GPs) enrolled 6,880 unselected attendees [11]. Medical history was taken followed by a physical examination (including determination of the ankle brachial index [ABI]) and blood sampling. After one year, three, five, and seven years, there were follow-up examinations.

Definition of risk factors

GGT values at baseline were dichotomized by the 0.75 quantile; this was 18 U/L for women and 26 U/L for men. This dichotomization was chosen because subjects in the highest GGT quarter showed the highest increase in the risk of CVD and mortality in other studies [12]. Besides GGT we took 17 potentially relevant cardiovascular risk factors (all assessed at baseline) into account: age and gender, four classical risk factors (smoking status, arterial hypertension, diabetes, LDL cholesterol), and eleven other factors (lipid lowering treatment, body mass index, history of cardiovascular events, peripheral artery disease (PAD), education, homocysteine, C-reactive protein (CRP), estimated glomerular filtration rate (eGFR), lipoprotein (a), vitamin D, and sodium). For the analysis we defined these covariates as follows:

- *age:* dichotomized by the median (71.9 years),
- *gender:* status at baseline,
- *smoking status:* positive in case of current smoking,
- *arterial hypertension:* diagnosis made by the treating physician or in case of medication with angiotensin-1 receptor antagonists, angiotensin converting enzyme (ACE) inhibitors, or diuretics at baseline,
- *diabetes:* diagnosis at baseline made by the treating physician or in case of treatment with insulin or other antidiabetics or of an HbA1c $\geq$ 6,5%,
- *LDL-cholesterol:* dichotomized by a value of 130 mg/dL,
- *lipid lowering therapy:* use of statins or fibrates,
- *body mass index (BMI):* ratio of body weight (kg) and the square of the body height (m^2); dichotomized by the value of 30 kg/m^2,
- *history of cardiovascular disease:* established diagnosis of coronary heart disease or stroke or a history of coronary or carotid revascularization,
- *PAD:* history of claudication, peripheral revascularization, necrosis/gangrene, and/or amputation due to PAD for the diagnosis of symptomatic PAD or an ABI value < 0.9 for the diagnosis of asymptomatic PAD, respectively; participants with symptomatic PAD or asymptomatic PAD were considered as one PAD group,
- *education:* scaled by the International Standard Classification of Education (ISCED) score; an ISCED score of 0 to 2 coined as low and compared to a score of 3 to 6 [13],
- *homocysteine:* dichotomized by its median 14.1 µmol/L,
- *CRP*: dichotomized by 3 mg/L,
- *eGFR:* dichotomized by 60 mL/min/1.73 m^2, calculated by the Cockcroft Gault formula with the Dubois correction [14],
- *lipoprotein (a):* dichotomized by 50 mg/dL,
- *vitamin D:* dichotomized by 50 nmol/L
- *sodium:* dichotomized by 136 to 144 mmol/L versus < 136 or > 144 mmol/L.

Primary outcome measures

Primary outcome measures were death from any cause, CHD event (myocardial infarction or coronary revascularization) and cerebrovascular event (stroke or carotid revascularization). Death was determined by information provided by the general practitioners and by demand at the residents' registration office. CHD events and cerebrovascular events were recorded according to notifications by the general practitioners or patient information obtained personally or by telephone interviews.

Statistical methods

First, we made an estimation of incidence rates by the ratio of observed events and the number of patient years under risk. Then, multivariable Cox proportional hazards regression was done for the three endpoints using the above mentioned 18 risk factors. These factors comprised the already used selection of risk factors from a recent published work on this cohort [10] and the newly added variables lipoprotein(a), sodium and GGT. Thus, with adjustment of 17 risk factors, the hypothesis of an independent effect of GGT on the endpoints was investigated. Missing values were substituted by randomly chosen values from the cohort. This was done in 0.00 to 1.95% of the cases, respectively.

We used two-sided p-values and labeled p-value < 0.05 as significant. Analyses were performed using SAS, version 9.4 (2013, SAS Institute Inc., Cary, NC, USA).

The institutional review board of the University of Heidelberg had approved the getABI trial. Each patient had provided written informed consent. The getABI trial followed the recommendations of Good Epidemiological Practice and was supported by unrestricted grants from Sanofi-Aventis GmbH, Berlin, Germany, and the German Federal Ministry of Education and Research. The protocol of this post-hoc analysis was reviewed and approved by the ethics committee of the Ruhr University Bochum (registration number: 16-5907). Trial registration: DRKS 00011624.

Results

6,880 patients had participated in the getABI trial. For this post-hoc analysis, 58 of them were excluded due to the diagnosis of mediasclerosis because their ABI (above 1.5) was not likely to provide a reliable estimation of a state of asymptomatic PAD, which was regarded as an important covariate.

Thus, 6,822 patients were included in this analysis. The median age was 71.9 years, 58% were female. Patients with a GGT-value above the 3rd quartile showed higher baseline percentages of e.g. currently smoking, arterial hypertension, diabetes, peripheral artery disease, and elevated sCRP compared to the other patients (Table I). 241 women (6.1%) and 133 men (4.6%) had values above the normal range of GGT for adults, considered as 40 U/L and 65 U/L, respectively.

1,243 participants died during the observation period of seven years. 27.8 deaths per 1,000 patient years (0.95 confidence interval [0.95 CI], 26.2–29.3) occurred in the whole cohort, compared to 35.9 (0.95 CI, 32.3–40.0) in the subgroup with GGT > 3rd quartile. 605 participants had a CHD event with an event rate of 16.1 per 1.000 patient years (0.95 CI, 14.8–17.4) while in the subgroup with GGT values > 3rd GGT the respective event rate was 19.0 (0.95 CI, 16.2–21.9). 296 cerebrovascular events were observed leading to an event rate of 7.7 per 1,000 patient years (0.95 CI, 6.9–8.6) compared to 10.1 (0.95 CI, 8.1–12.2) in the subgroup with GGT values > 3rd quartile (Table II).

Cox regression analysis with adjustment for age, gender, classical and the other above-mentioned risk factors showed a significant impact of baseline GGT values above the 3rd quartile (women > 18 U/L, men > 26 U/L) compared to the rest regarding overall death (hazard ratio [HR], 1.38; 0.95 CI, 1.22–1.56) and cerebrovascular events (HR, 1.39; 0.95 CI, 1.08–1.79). The HR for CHD events was less elevated (1.16) and its 0.95 CI showed no significance (0.95 CI, 0.97–1.39; p = 0.103). Many of the other risk factors used for adjustment had an impact on death as well as on CHD, or cerebrovascular events. Regarding the newly added risk factors, Lipoprotein(a) had an impact on CHD events (HR, 1.53; 0.95 CI, 1.28–1.82) and cerebrovascular events (HR, 1.37; 0.95 CI, 1.06–1.78). Sodium showed a significant association only with cerebrovascular events (HR, 1.39; 0.95 CI, 1.02–1.89). There were several risk factors that failed to confirm the expected and in other studies proven impact on the chosen endpoint, e.g. the

Table I. Baseline characteristics.

	All patients		≤ 3rd GGT quartile		> 3rd GGT quartile	
	N	%	N	%	N	%
Total	6,822	100.0	5,146	100.0	1,676	100.0
Age > median (71.9 years)	3,411	50.0	2,652	51.5	759	45.3
Male gender	2,863	42.0	2,174	42.2	689	41.1
Currently smoking	634	9.3	441	8.6	193	11.5
Arterial hypertension	4,738	69.5	3,471	67.5	1,267	75.6
Diabetes	1,745	25.6	1,166	22.7	579	34.5
LDL-cholesterol ≥ 130 mg/dL	2,916	42.7	2,224	43.2	692	41.3
Lipid lowering medication	1,607	23.6	1,160	22.5	447	26.7
BMI ≥ 30 kg/m^2	1,576	23.1	1,133	22.0	443	26.4
Cardiovascular disease	1,091	16.0	807	15.7	284	16.9
PAD	1,434	21.0	1,030	20.0	404	24.1
ISCED 0–2 (low)	1,698	24.9	1,285	25.0	413	24.6
Homocysteine > median (14.13 µmol/L)	3,410	50.0	2,573	50.0	837	49.9
sCRP > 3 mg/L	2,642	38.7	1,810	35.2	832	49.6
eGFR < 60 mL/min/1.73 m^2	1,337	19.6	1,036	20.1	301	18.0
Lipoprotein(a) > 50 mg/dL	1,434	21.0	1,102	21.4	332	19.8
Vitamin D < 50 nmol/L	4,741	69.5	3,509	68.2	1,232	73.5
Sodium < 136 or > 144 mmol/L	867	12.7	669	13.0	198	11.8

BMI: body mass index; eGFR: estimated glomerular filtration rate; GGT: gamma-glutamyl transferase; ISCED: International Standard Classification of Education; LDL: low density lipoprotein; PAD: peripheral artery disease; sCRP: sensitive C-reactive protein.

Table II. Number of events and event rates.

Event	Number of events	Number of patient years	Events per 1,000 patient years [95% CI]
Death from any cause	1,243	44,764	27.8 [26.2–29.3]
≤ 3rd GGT quartile	859	34,081	25.2 [23.5–26.9]
> 3rd GGT quartile	384	10,683	35.9 [32.3–40.0]
Coronary heart disease event	605	37,625	16.1 [14.8–17.4]
≤ 3rd GGT quartile	434	28,635	15.2 [13.7–16.6]
> 3rd GGT quartile	171	8,990	19.0 [16.2–21.9]
Cerebrovascular event	296	38,276	7.7 [6.9–8.6]
≤ 3rd GGT quartile	203	29,109	7.0 [6.0–7.9]
> 3rd GGT quartile	93	9,167	10.1 [8.1–12.2]

CI: confidence interval; GGT: gamma-glutamyl transferase.

Table III. Association between risk factors at baseline and overall mortality, coronary heart disease events or cerebrovascular events during seven years of follow-up (multivariable analysis).

Risk factor	Overall death		Coronary heart disease event		Cerebrovascular event	
	HR [0.95 CI]	p	HR [0.95 CI]	p	HR [0.95 CI]	p
GGT > 3rd quartile (women > 18 U/L, men > 26 U/L)	**1.38 [1.22–1.56]**	**< 0.001**	**1.16 [0.97–1.39]**	**0.103**	**1.39 [1.08–1.79]**	**0.010**
Age > median (71.9)	1.90 [1.67–2.17]	< 0.001	1.06 [0.89–1.26]	0.537	1.61 [1.25–2.08]	< 0.001
Male gender	1.87 [1.64–2.12]	< 0.001	2.56 [2.11–3.10]	< 0.001	1.66 [1.27–2.15]	< 0.001
Arterial hypertension	1.21 [1.05–1.39]	0.008	1.41 [1.15–1.74]	0.001	1.02 [0.77–1.34]	0.901
Currently smoking	1.87 [1.59–2.19]	< 0.001	1.53 [1.21–1.95]	0.001	1.07 [0.72–1.60]	0.738
Diabetes	1.43 [1.27–1.62]	< 0.001	1.18 [0.99–1.40]	0.074	1.60 [1.25–2.05]	< 0.001
LDL-cholesterol ≥ 130 mg/dL	0.75 [0.67–0.84]	< 0.001	1.15 [0.97–1.35]	0.113	0.85 [0.66–1.08]	0.175
Lipid lowering medication	0.67 [0.58–0.77]	< 0.001	1.19 [0.99–1.43]	0.064	0.89 [0.68–1.18]	0.430
BMI ≥ 30 kg/m^2	1.02 [0.89–1.18]	0.739	1.10 [0.90–1.34]	0.368	1.04 [0.79–1.38]	0.778
Cardiovascular disease	1.45 [1.26–1.66]	< 0.001	1.79 [1.48–2.16]	< 0.001	1.43 [1.07–1.91]	0.016
PAD	1.47 [1.30–1.67]	< 0.001	1.62 [1.36–1.94]	< 0.001	1.45 [1.12–1.88]	0.006
ISCED 0–2 (low)	1.27 [1.11–1.46]	< 0.001	0.95 [0.76–1.19]	0.648	1.19 [0.90–1.59]	0.229
Homocysteine > median (14.1 μmol/L)	1.27 [1.13–1.44]	< 0.001	0.98 [0.83–1.16]	0.828	1.04 [0.82–1.32]	0.734
sCRP > 3 mg/L	1.30 [1.16–1.46]	< 0.001	1.24 [1.05–1.46]	0.012	1.08 [0.85–1.37]	0.524
eGFR < 60 mL/min/1.73 m^2	1.75 [1.53–1.99]	< 0.001	1.54 [1.25–1.88]	< 0.001	1.17 [0.87–1.57]	0.291
Lipoprotein(a) > 50 mg/dL	1.05 [0.92–1.20]	0.463	1.53 [1.28–1.82]	< 0.001	1.37 [1.06–1.78]	0.017
Vitamin D < 50 nmol/L	1.39 [1.21–1.60]	< 0.001	1.07 [0.90–1.28]	0.442	1.22 [0.93–1.60]	0.148
Sodium < 136 or > 144 mmol/L	1.14 [0.97–1.34]	0.120	0.89 [0.69–1.16]	0.389	1.39 [1.02–1.89]	0.038

BMI: body mass index; CI: confidence interval; eGFR: estimated glomerular filtration rate; GGT: gamma-glutamyl transferase; HR, hazard ratio; ISCED: International Standard Classification of Education; LDL: low density lipoprotein; PAD: peripheral artery disease; sCRP: sensitive C-reactive protein.

effect of diabetes on CHD events was not significant. In general, these risk factors were mainly used for adjustment and are not in the focus of this study. (Table III).

Discussion

In this post-hoc analysis of a large-scale prospective cohort of older adults in a primary care setting with a seven-year follow-up, significant associations could be found between high GGT values on the one hand and increased overall mortality as well as cerebrovascular event rates on the other hand, respectively. Even after adjustment for 17 risk factors, the HRs of the subgroup with a baseline elevation of GGT above the 3rd quartile compared to the rest of the cohort were significantly elevated regarding overall death and cerebrovascular events, but not regarding CHD events. Of note, the 3rd quartile (18 U/L for women and 26 U/L for men) is in the lower half of the normal rage of GGT (upper limit of normal about 40 U/L for women and about 65 U/l for men). This means that values in the upper half of the normal range may already indicate increased risks for overall mortality and cerebrovascular events requiring the appropriate therapeutic measures.

Strength and limitations of this study

A strength of this study is the large sample size of 6,822 primary care patients with a follow-up of seven years. In order to increase generalizability of results, thirty-four vascular specialists in all parts of Germany had chosen about ten general practitioners in their vicinity for participation in this study. These 344 general practitioners had enrolled about twenty patients each.

The recruitment of unselected primary care patients took place in three prespecified weeks in order to reduce selection bias. During the seven-year follow-up, CHD and cerebrovascular events were not only recorded by the general practitioners, but also by telephone interviews in order to achieve results as comprehensive as possible. Furthermore, all reported cases of stroke were independently verified. Death was determined by information provided by the general practitioners and/or by demand at the residents' registration office.

Compared to population-based cohorts, the primary care setting may provide results that are more likely to be directly applicable to routine care. On the other hand, as population-based studies are going to comprise more and more participants, the sample size of our cohort will be surpassed by those of e.g. nationwide cohorts.

Of course, the inclusion criteria (age $\geq$ 65 years) may limit the applicability of results to this age group. Moreover, alcohol intake was not evaluated. However, as societies in Europe are becoming older, the results of our study may even gain more relevance in the future.

Comparison with other studies

The association between GGT values and overall survival is apparent in many other studies. In the British Regional Heart Study with 7,613 middle-aged men followed for 11.5 years, GGT levels were strongly associated with all-cause mortality, due to an increase in deaths in the top fifth of the GGT distribution ($\geq$ 24 U/L) with a relative risk vs. the rest of 1.22 (0.95 CI, 1.01–1.42) [4]. 3,451 participants were included in the Framingham Heart Study (mean age of 44 years, mean follow-up of 19 years); those with GGT-levels over the 3rd quartile yielded a HR of 1.83 (0.95 CI, 1.29–2.60) compared to the 1st quarter after adjustment for multiple risk factors [12]. This choice of the reference leads to higher HRs than in the analysis presented here. A cohort of 283,438 attendants of the Vienna General Hospital with a follow-up of 7.6 years showed that GGT values above the reference category were significantly ($P < 0.001$) associated with all-cause mortality [15]. In this cohort, an age dependency of the HR for all-cause mortality was apparent, with younger individuals having far worse outcomes than older individuals. Recently, a very large Korean nationwide cohort database of 16,624,006 Korean adults with a follow-up of 9.1 years exhibited a positive relationship among GGT and mortality in a multivariate adjusted model (HR, 1.46; 0.95 CI, 1.40–1.52) [16]. Nearly all these HRs for all-cause mortality from cohorts with middle-aged participants are in the same range as the HR in our cohort. The only exception was the Framingham Heart Study that compared the quarter of patients with the highest GGT values with that of the lowest GGT values, thereby yielding a higher HR than studies that compared the quarter with the highest GGT values with the rest of the cohort.

The association between GGT values and mortality could also be seen in elderly people, taking part in population-based cohorts or in a twin cohort. The Rancho Bernardo Study was a prospective cohort study including 2,364 community-dwelling participants (mean age 70 years) with a mean follow-up of 13.7 years. In a multivariable analysis, serum GGT elevation (> 51 U/L in men and > 33 U/L in women) was significantly associated with all-cause mortality (HR, 1.55; 0.95 CI, 1.21–1.98) [7]. In the Rotterdam Study, a large population-based cohort of persons aged 55 years or older, with a follow-up of up to 19.5 years, 2,997 of 5,186 (57.8%) participants died; serum liver enzyme elevations were associated with all-cause mortality (all $p < 0.001$) [8]. Data from the Longitudinal Study of Aging Danish Twins (686 twins, 73–94 years old) showed that an increase in 1 logarithmically transformed U/L of GGT was associated with a 15% increase in the HR for mortality (0.95 CI, 0.99–1.32) [9]. Thus, the results of these population-based cohorts and of the twin cohort provided result comparable to our primary care based cohort.

Also, the relationship between GGT and cerebrovascular events has been investigated by several prospective cohort studies. 14,874 Finnish men and women aged 25 to 64 years participated in a cardiovascular risk-factor survey. After adjustment for other risk factors, serum GGT concentration was associated with the risk of total and ischemic stroke in both genders. By the way, self-reported alcohol drinking was not associated with stroke [17]. In the EUROSTROKE project, a nested case-control analysis, an increase in GGT of one standard deviation was associated with an age and sex adjusted increase in risk of stroke of 26% (0.95 CI, 5%–53%) [18]. In a study with general practices in 24 British towns including 6,997 men aged 40–59 with a follow-up of 24 years, GGT values were significantly associated with an increased risk of major stroke events after adjustment for established CVD risk factors. The adjusted relative risk of the highest to the lowest category according to quartiles was 1.56 (0.95 CI, 1.20–2.04) for stroke [19]. A Japanese prospective cohort study enrolling 6,281 women and 3,471 men, aged 40 to 69 years, with 18 years of follow-up, found HRs of total stroke for the highest quarter of GGT compared with the lowest quarter of 1.56 (0.95 CI, 1.01–2.39) for women and 1.37 (0.95 CI, 0.89–2.11) for men [20]. Thus, all these studies had similar results to our study. In contrast, the Reasons for Geographic and Racial Differences in Stroke (REGARDS) cohort of 30,239 American adults found an inverse association between high GGT values and the risk of stroke in men (but not in women). Of course, these surprising data require further confirmation [21].

Concerning the association between GGT and CHD events, the results of other studies have been somewhat heterogeneous. In a cohort of 163,944 Austrian adults with a follow-up of 17 years, high GGT values were significantly ($P < 0.001$) associated with total mortality from CVD. In men, subgroup analyses showed that high GGT was positively associated with congestive heart failure ($P < 0.001$) as well as hemorrhagic ($P = 0.01$) and ischemic stroke ($P < 0.001$), but not with acute myocardial infarction ($P = 0.16$) [5]. A prospective study of 28,838 Finnish men and women (median follow-up time of 11.9 years) observed a HR for non-fatal myocardial infarction of 1.16 (0.95 CI, 0.88–1.53) among men and 1.53 (0.95 CI, 1.06–2.22) among women with GGT values between the 75th and 90th sex-specific percentiles compared to the quarter with the lowest GGT values, respectively [22]. A meta-analysis of twenty-nine cohort studies with aggregate data on over 1.23 million participants and 20,406 cardiovascular outcomes yielded relative risks CVD of 1.23 (0.95 CI, 1.16–1.29) per 1-standard deviation change in log baseline levels of GGT level [22]. Thus, there seems to be a modest association between GGT values and CHD events, though significance is lacking in our cohort due to the relatively small sample size.

In summary, our results that were acquired in a primary care setting confirm findings from other study designs: Higher GGT values are associated with elevated overall mortality and an increased risk for cerebrovascular events. Therefore, elevated GGT values should raise physician's awareness for the management of modifiable risk factors in elderly patients.

Unanswered questions and future research

The underlying pathogenetic mechanisms for the above discussed associations need to be fully elucidated, potentially leading to novel therapeutic options in the treatment of CVD. Furthermore, the recent data of an inverse correlation between elevated GGT values and the risk of stroke need to be confirmed.

Conclusions

In a primary care setting, GGT values show a significant association with overall mortality and cerebrovascular events in elderly patients.

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NT-proBNP for risk prediction of cardiovascular events and all-cause mortality: The getABI-study

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ABSTRACT

Background: Beside their role in the diagnosis of heart failure in symptomatic patients with dyspnea, natriuretic peptides have been suggested to improve risk prediction of cardiac events and mortality in asymptomatic cohorts. We aimed to evaluate the prognostic value of NT-proBNP for cardiovascular and all-cause mortality above traditional risk factors in a prospective cohort study of unselected elderly patients in a representative primary care setting.

Methods: We followed 6382 patients of the getABI-study for 7 years. Associations of NT-proBNP levels (≤125; 125–300; >300pg/ml for all) with all-cause and cardiovascular mortality were assessed using cox regression analysis.

Results: The incidence of all-cause and cardiovascular mortality was higher in subjects with higher levels of NT-proBNP (all-cause mortality/cardiovascular mortality: 35.4%/6% for NT-proBNP > 300 pg/ml; 16.2%/40% for NT-proBNP 125–300 pg/ml vs. 11.4%/4% for NT-proBNP ≤ 125 pg/ml. Participants with a NT-proBNP levels > 300pg/ml had increased incidence of hard endpoint (hazard ratio (HR) (95% confidence interval (CI)): 3.62 (3.15–4.17) for all-cause mortality, and 6.38 (4.84–8.41) for cardiovascular mortality). These associations remained after adjustment for traditional risk factors and cardiac medications and diseases (HR = 2.64 (2.26–3.08) for all-cause mortality, and HR = 3.93 (2.90–5.32) for cardiovascular mortality).

Conclusion: Our results show strong associations of higher NT-proBNP levels with cardiovascular and all-cause mortality in an unselected, large population of elderly patients in the primary care setting independent of traditional risk factors indicating that NT-proBNP can help identifying subjects at high risk for cardiac events.

1. Introduction

Cardiac risk stratification detecting silent cardiac damage remains a challenge in modern medicine. B-type natriuretic peptide (BNP) and N-terminal pro BNP (NT-proBNP) are secreted from cardiac myocytes in response to ventricular and atrial wall stress and serve as serum biomarkers for the diagnosis of heart failure [1].

Besides their established role in patients with heart failure, several studies have described the powerful prognostic role of natriuretic peptides for prediction of cardiovascular outcomes in asymptomatic cohort-studies [2–6].

Natriuretic peptides are increased in subjects with early stages of cardiac diseases such as diastolic dysfunction, left ventricular

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hypertrophy, and silent myocardial ischemia with plasma-levels markedly below the cut-off value used for the diagnosis of heart failure [4,7]. Thus, natriuretic peptides seem to reflect pancardiac functional heart disease also in early asymptomatic stages and thus may hereafter be useful in cardiac screening programs [4,7].

However, there is a lack of studies investigating the association of NT-proBNP levels with cardiac events in a large cohort from primary care setting.

Therefore, aim of this analysis was to determine the association of NT-proBNP with all-cause mortality and cardiovascular mortality above traditional risk factors in a prospective cohort study of unselected elderly patients in a representative primary care setting in Germany (getABI Study).

2. Methods

2.1. Participants

The German Epidemiological Trial on Ankle Brachial Index (getABI) is a prospective observational cohort study initiated in October 2001. Details about recruitment and study design have been previously published [8]. In brief, 34 vascular physicians trained and supervised 344 physicians throughout Germany. Patients were included in a prespecified week in October 2001 regardless of seeing the doctor. In total, 6880 elderly (>65 years) patients were recruited. Each practice included an average of 20 patients, the only exclusion criteria was life expectancy less than 6 month as judged by the doctor. At baseline, a medical history assessment including prescribed medications, physical examination, and blood sampling were performed. Follow-up included physician visits at one, three, five and seven years after baseline.

2.2. Measurement of NT-proBNP

Subjects blood samples were collected at baseline. NT-proBNP was determined using a chemoluminescent microparticle immunoassay (CMIA, Abbott Diagnostics, Chicago, Illinois, USA) on an Abbott Architect i200SR analyzer. Interassay CVs were 2.8% and 2.3% at mean values of 153 and 4849 pg/ml.

NT-proBNP was categorized into 3 levels as clinically established thresholds: minimum up to 125, 125 to 300, and>300 (unit pg/ml) [9]. GetABI participants with missing NT-proBNP values were excluded. Thus, 6382 patients comprised the analysis population for this work.

2.3. Cardiovascular diseases and endpoint definition

Cardiovascular diseases (CVDs) at baseline were defined as myocardial infarction, stroke, coronary revascularization, or revascularization of the carotid arteries. The endpoints death and death from cardiovascular cause were considered if declared by the physician (CRF) or via death certificate. If possible, they were verified by clinical reports. For lost to follow-up participants the registration office was asked for living status. Death from Cardiovascular cause comprised fatal myocardial infarction and cerebrovascular events.

2.4. Statistical analysis

On basis of the pre-determined NT-proBNP categories, characteristics of participants are presented descriptively with mean and standard deviations for continuous variables and counts and proportions for categorical variables. Survival analyses were done by means of Kaplan-Meier plots for the univariable analysis according to NT-proBNP categories, and by Cox proportional hazards regression for the adjusted models. Variables for adjustment of hazards were selected from previously published work of the getABI study group [10,11]. The Missing values for predictor variables were imputed by randomly selected values from the cohort. Results for predictors are given as hazard ratios (HR) and 95% confidence intervals (CI). Statistical analyses were performed using SAS, version 9.4 (SAS Institute, Cary, North Carolina, USA).

3. Results

3.1. Baseline characteristics

A total of 6382 patients were included (mean age 72.5 ± 5.3, 42.2% female) in this analysis. The median NT-proBNP level was 143.15 pg/ml (Q1: 83.6; Q3: 271.8). In average, NT-proBNP levels were higher in male patients compared to female (304,6 pg/ml vs. 271,7 pg/ml).

The baseline characteristics were depicted in Table 1 stratified for NT-proBNP using 125 and 300 pg/ml as threshold [9].

3.2. Association of NT-proBNP with all-cause mortality and cardiovascular mortality

After a follow-up time of 41,873 person-years 1204 participants died from any cause and 347 patients had a fatal cardiovascular event.

The incidence of all-cause and cardiovascular mortality was higher in subjects with higher levels of NT-proBNP (all-cause mortality/cardiovascular mortality: 35.4%/13.6% for NT-proBNP > 300 p g/ml; 16.2%/4.0% for NT-proBNP 125–300 pg/ml vs. 11.4%/2.4% for NT-proBNP $\leq$ 125 pg/ml.

Table 2a and 2b show the Cox-regression analysis for all-cause mortality and fatal cardiovascular events. Unadjusted, subjects with a NT-proBNP level > 300 pg/ml had more than three-fold risk for all-cause mortality and more than six-fold increased risk for cardiovascular mortality. Associations were attenuated but remained statistically significant after further adjustment for age, sex, traditional risk factors, cardiac diseases and cardiac medications using NT-proBNP as continuous variable as well as using the defined thresholds. After further adjustment (full model) for cardiac diseases and cardiac medications associations remained unchanged as depicted in Table 3. In particular, when modelling NT-proBNP as a continuous variable, a 2-fold increase of it was associated with a 50% increase in the risk of cardiovascular death in the model M5. The ability of the model M5 to distinguish between alive and death was good, since the area under the curve (AUC) was determined as 0.77.

Further, we investigated if the associations between continuous NT-proBNP and endpoints were modified by sex. Regarding all-cause mortality, in females a one-unit change in log2-transformed NT-proBNP resulted in elevation of the hazard by 38,9%, and for men by 26,7% (p-value for interaction 0.015 in fully adjusted model), whereas for cardiovascular mortality, although hazards were still numerically different, that interaction was not significant (p-value 0.39, results not shown).

Fig. 1 displays the survival probability according to NT-proBNP levels, showing a significant difference in mortality rate in patients with a NT-proBNP level > 300 pg/ml.

4. Discussion

In this large prospective cohort study we examined the association of NT-proBNP with all-cause and cardiovascular mortality above traditional risk factors.

Table 1
Baseline characteristics.

	Overall	NT-proBNP ≤ 125 pg/ml	NT-proBNP 125–300 pg/ml	NT-proBNP > 300 pg/ml
N	6382	2780	2190	1412
Age (years)	72.51 (5.27)	71.23 (4.76)	72.71 (5.17)	74.68 (5.64)
Males (%)	2693 (42.20%)	1316 (47.34%)	763 (34.84%)	614 (43.48%)
Education: ISCED < 3 (%)	1586 (24.85%)	634 (22.81%)	576 (26.30%)	376 (26.63%)
Current Smoker (%)	578 (9.06%)	259 (9.32%)	205 (9.36%)	114 (8.07%)
Systolic blood pressure (mmHg)	143.72 (19.40)	142.10 (18.39)	144.45 (19.51)	145.78 (20.85)
Diastolic blood pressure (mmHg)	81.37 (9.58)	81.56 (9.40)	81.06 (9.43)	81.50 (10.12)
Vitamin D < 50 ng/ml (%)	4401 (68.96%)	1839 (66.15%)	1520 (69.41%)	1042 (73.80%)
CRP > 3 mg/L (%)	2468 (38.67%)	1009 (36.29%)	823 (37.58%)	636 (45.04%)
GGT > 3rd quartile (%)	1560 (24.44%)	667 (23.99%)	482 (22.01%)	411 (29.11%)
HCY > 15 μmol/L (%)	3188 (49.95%)	1247 (44.86%)	1072 (48.95%)	869 (61.54%)
PAD (%)	1319 (20.67%)	414 (14.89%)	468 (21.37%)	437 (30.95%)
Diabetes (%)	1626 (25.48%)	716 (25.76%)	487 (22.24%)	423 (29.96%)
Arterial hypertension (%)	4136 (64.81%)	1624 (58.42%)	1458 (66.58%)	1054 (74.65%)
LDL-C ≥ 130 mg/dl (%)	2736 (42.87%)	1309 (47.09%)	928 (42.37%)	499 (35.34%)
GFR < 60 ml/min (%)	1257 (19.70%)	317 (11.40%)	416 (19.00%)	524 (37.11%)
Lipid-lowering medication (%)	1499 (23.49%)	563 (20.25%)	556 (25.39%)	380 (26.91%)
Beta blocker (%)	1935 (30.32%)	516 (18.56%)	743 (33.93%)	676 (47.88%)
Diuretics (%)	1786 (27.98%)	626 (22.52%)	584 (26.67%)	576 (40.79%)
Digitalis (%)	516 (8.09%)	113 (4.06%)	140 (6.39%)	263 (18.63%)
Antihypertensive medication (%)	3284 (51.46%)	1259 (45.29%)	1102 (50.32%)	923 (65.37%)
Total Cholesterol (mg/dl)	212.29 (37.84)	215.09 (36.67)	213.46 (37.82)	204.95 (39.21)
HDL Cholesterol (mg/dl)	52.73 (17.58)	52.08 (16.03)	54.53 (19.57)	51.20 (16.99)
LDL Cholesterol (mg/dl)	125.23 (31.22)	127.96 (30.30)	124.91 (31.49)	120.37 (31.91)

ISCED: International Standard Classification of Education; CRP: C-reactive protein; GGT: Gamma-Glutamyl Transferase; HCY: Homocystein; PAD: peripheral artery disease; LDL; Low density lipoprotein; GFR: Glomerular filtration rate; HDL: High density lipoprotein.

Table 2a
Cox regression for the association of NT-proBNP with all-cause mortality.

	NT-proBNP as continuous variable (Log2-transformed)	NT-proBNP as categorical variable (in reference to the ≤ 125 pg/ml group)	
		125–300 pg/ml	> 300 pg/ml
Model	Hazard Ratio (95%-CI)	Hazard Ratio (95%-CI)	Hazard Ratio (95%-CI)
Unadjusted	1.51 (1.46; 1.57)	1.46 (1.25; 1.70)	3.62 (3.15; 4.17)
Model 1 (Age and gender)	1.41 (1.35; 1.46)	1.41 (1.21; 1.64)	2.90 (2.51; 3.35)
Model 2 (Model 1 + Cardiac medication)	1.37 (1.32; 1.43)	1.43 (1.22; 1.67)	2.74 (2.35; 3.19)
Model 3 (FRV)	1.43 (1.38; 1.49)	1.43 (1.23; 1.67)	3.09 (2.67; 3.56)
Model 4 (model 2 + Cardiac diseases)	1.36 (1.31; 1.42)	1.40 (1.20; 1.64)	2.64 (2.26; 3.08)
Model 5 (Full adjusted model)	1.31 (1.26; 1.37)	1.36 (1.17; 1.59)	2.34 (1.99; 2.74)

Cardiac diseases: cardiovascular diseases including coronary heart diseases, cerebral diseases and peripheral artery diseases.
Cardiac medication including beta-blocker, diuretics and digitalis.

Table 2b
Cox regression for the association of NT-proBNP with Cardiovascular mortality.

	NT-proBNP as continuous variable (Log2-transformed)	NT-proBNP as categorical variable (in reference to the ≤ 125 pg/ml group)	
		125–300 pg/ml	> 300 pg/ml
Model	Hazard Ratio (95%-CI)	Hazard Ratio (95%-CI)	Hazard Ratio (95%-CI)
Unadjusted	1.83 (1.71; 1.95)	1.62 (1.21; 2.29)	6.38 (4.83; 8.41)
Model 1 (Age and gender)	1.70 (1.60; 1.82)	1.64 (1.19; 2.25)	5.22 (3.93; 6.94)
Model 2 (Model 1 + Cardiac medication)	1.60 (1.49; 1.72)	1.58 (1.15; 2.18)	4.31 (3.19; 5.81)
Model 3 (FRV)	1.70 (1.59;1.81)	1.61 (1.17, 2.22)	5.06 (3.81,6.71)
Model 4 (Model 2 + Cardiac diseases)	1.58 (1.46; 1.69)	1.51 (1.10; 2.09)	3.93 (2.90; 5.32)
Model 5 (Fully adjusted model)	1.51 (1.39; 1.64)	1.48 (1.07; 2.04)	3.41 (2.51; 4.63)

Cardiac diseases: cardiovascular diseases including coronary heart diseases, cerebral diseases and peripheral artery diseases;
Cardiac medication including beta-blocker, diuretics and digitalis.

Increased NT-proBNP levels were associated with excessive prevalence of all-cause and cardiovascular mortality, resulting in an overall mortality of 35.4% and cardiovascular mortality of 13.6% in 7 years for patients with a NT-proBNP level > 300 pg/m l. The associations remained statistically significant after adjustment for traditional risk factors, cardiac diseases and medications.

Our results show strong associations of higher NT-proBNP levels with mortality in an unselected, large population of elderly patients in the primary care setting independent of traditional risk factors. Our results suggest that the mechanism involved in event manifestation and that are reflected in elevated NT-proBNP levels, are different from those mediated trough traditional risk factors.

Natriuretic peptides are commonly used for the diagnosis of heart failure in symptomatic patients with dyspnoea. Moreover, it was showed, that natriuretic guided treatment for chronic heart failure improves mortality. [12]

However, there is growing evidence, that higher levels of natriuretic peptides predict cardiac events and mortality in the general population and therefore serve as markers of cardiovascular risk.

Table 3
Predictors of all-cause mortality in the fully adjusted model, multivariable analysis.

Risk factor	Hazard-Ratio	95%-CI
Age (per year)	1.062	1.05–1.08
Sex	1.983	1.74–2.26
NT proBNP 125–300 pg/ml	1.363	1.17–1.59
NT proBNP > 300 pg/ml	2.335	1.99–2.74
Hypertension	0.918	0.80–1.05
Diabetes mellitus	1.445	1.27–1.64
Smoker (current)	2.027	1.72–2.39
LDL (≥130 mg/dl)	0.806	0.71–0.91
Lipid-lowering medication	0.722	0.62–0.84
CVD	1.267	1.09–1.47
ISCED 0–2 vs. 3–6	1.279	1.11–1.48
CRP (>3 mg/l)	1.186	1.05–1.33
GGT (>Q3)	1.302	1.14–1.48
GFR (per 10 ml/min/1.73 m^2)	0.923	0.89–0.96
Homocystein (>15 μmol/l)	1.171	1.04–1.32
Vitamin D (<50 nmol/l)	1.398	1.21–1.61
PAD	1.389	1.22–1.58
Use of Diuretics	1.220	1.07–1.39
Use of Beta blocker	0.836	0.73–0.96
Use of Digitalis	1.360	1.15–1.61

LDL: Low density lipoprotein; CVD: cardiovascular diseases; ISCED: International Standard Classification of Education; CRP: C-reactive protein; GGT: Gamma-Glutamyl Transferase; PAD: peripheral artery disease; LDL; Low density lipoprotein; GFR: Glomerular filtration rate; HDL: High density lipoprotein.

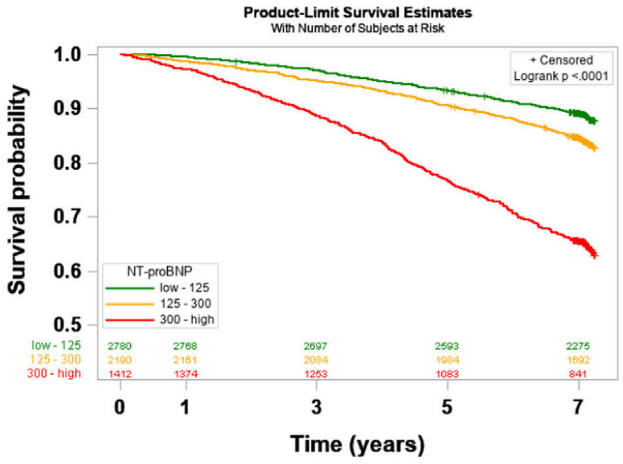

Fig. 1. Kaplan Meier Analysis for survival probability as stratified by a NT-pro-BNP-threshold of ≤ 125; 125–300; >300 pg/ml. Median follow up was 7.06 (Q1 7.00; Q3 7.14) years.

Wang et al. reported an association of BNP with all-cause mortality and cardiovascular events in a healthy population [3]. These results were confirmed in a *meta*-analysis with 87,747 subjects showing a 3-fold risk for cardiovascular events in subjects with a higher level of natriuretic peptides [13]. Another work of McKie et al including 2042 individuals showed that NT-proBNP independently predicted mortality and heart failure in the general population free of overt heart failure [14]. In another *meta*-analysis, recently published, on 25,715 subjects, elevated NT-proBNP levels appeared to be independently associated with increased risk for cardiovascular and all-cause mortality in the general population [15]. Participants in the highest NT-proBNP concentration had a significantly increased 3.77 fold cardiovascular mortality and 2.44 fold all-cause mortality, even after adjustment for traditional risk factors. In this setting, authors stated that higher NT-proBNP levels might reflect the degree of systemic atherosclerosis and/or an unknown initial cardiac overload.

Other studies demonstrated the prognostic value of natriuretic peptides in different settings:

Bibbings et al. showed, that elevated levels of NT-proBNP predict cardiovascular morbidity and mortality in patients with stable coronary heart disease independent of systolic and diastolic function assessed by echocardiography [16]. Paniagua et al. demonstrated the prognostic value of natriuretic peptide in dialysis patients. [17] and Tu et al. showed that NT-proBNP may be useful as independent prognostic markers in patients with ischemic stroke [18].

NT-proBNP is strongly associated with all-cause mortality also in hypertensive population. Paget et al. have demonstrated that plasma NT-proBNP levels ≥ 133 pg/mL were associated with a threefold increase of the risk of death in comparison with levels < 50.8 pg/ml, even after adjustment for confounders. Moreover, NT-proBNP resulted to be a stronger prognostic marker than ECG and its dosage has been proposed as first test for the cardiovascular stratification instead of ECG [19].

In our previously published work we demonstrated an association of BNP with coronary events and all-cause mortality, with BNP significantly improving prediction of risk in the general population above and complementary to coronary artery calcification and traditional risk factors [2]. For the risk prediction in the general population it was suggested that BNP reflects early stages of systolic and diastolic dysfunction; additionally it was hypothesized that BNP is linked with asymptomatic chronic cardiac ischemia. These suggestions were confirmed in a recently published study showing that BNP screening - in asymptomatic treated primary prevention patients - is able to identify existing left ventricular hypertrophy, systolic and diastolic dysfunction, left atrial enlargement, and ischemia [7].

As a conclusion, it has been suggested, that BNP may reflect "pancardiac" damage in early stages and that measurements of BNP may help to identify those who need closer examination and further risk stratification [20].

Our here presented data revealed that elevated NT-proBNP levels in the primary care setting are able to identify patients at high risk for a future cardiac event.

4.1. Clinical Implications

In our study we describe a significant association of NT-proBNP with fatal cardiac events and all-cause mortality, suggesting that NT-proBNP is the first biomarker which may improve risk prediction independent of and complementary to traditional cardiovascular risk factors. Together with previously published results, these data suggest that NT-proBNP may improve risk discrimination for cardiac events above and beyond traditional cardiovascular risk factors and therefore may help to identify subjects that may profit from aggressive risk modification. Furthermore, subjects with higher NT-proBNP levels may benefit from a cardiological workup for detections of early stages of a systolic or diastolic heart dysfunction even in the absence of symptoms.

We emphasize that assessment of NT-proBNP is feasible using a simple blood.

5. Conclusion

In our study we describe a significant association of NT-proBNP with fatal cardiovascular events and all-cause mortality, suggesting that NT-proBNP is the first biomarker which may improve risk prediction for cardiovascular events in the primary care setting independent of traditional cardiovascular risk factors.

6. Limitations

A limitation of our study is that we did not perform an echocardiography at baseline to get more information und reasons for

elevated levels of NT-proBNP as heart failure. We presented heart failure medications and we included heart failure medication into Cox proportional hazard analyses.

Furthermore, our study includes the measurement of NT-proBNP only, not of BNP.

CRediT authorship contribution statement

Henrik Rudolf: Conceptualization, Methodology, Software, Writing - original draft. **Andreas Mügge:** Writing - review & editing, Supervision. **Hans J. Trampisch:** Investigation, Writing - review & editing, Supervision. **Hubert Scharnagl:** Investigation, Supervision. **W. März:** Investigation, Supervision. **Kaffer Kara:** Supervision, Conceptualization, Methodology, Software, Writing - original draft.

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Declaration of Competing Interest

The authors report no relationships that could be construed as a conflict of interest.

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Original communication

Interactions between C-reactive protein and traditional risk factors in predicting mortality of older adults

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Summary: *Background:* Elevated levels of C-reactive protein (CRP) are known to be associated with cardiovascular (CV) morbidity and mortality in older adults, however, there seems to be heterogeneity of this association across subsets of individuals. We aim to assess the effects of interactions between CRP and one of the following traditional CV risk factors regarding all-cause mortality in unselected elderly men and women: age, sex, body mass index, diabetes, and hypertension. *Patients and methods:* Three hundred and forty-four general practitioners all over Germany enrolled 6,817 unselected participants, aged 65 years or older, and performed thorough examinations, including CRP measurement at baseline (getABI study). All-cause mortality was determined in the following seven years. Cox regression analyses were done using uni- and multivariable models. *Results:* At baseline 4,172 participants of this cohort had a CRP value of ≤ 3 mg/L (low level CRP group), 2,645 participants had a CRP value of > 3 mg/L (high level CRP group). The unadjusted hazard ratio for all-cause death of the high level CRP group compared to the low level CRP group was 1.49 (95 % confidence interval [95 %CI] 1.34 to 1.66). After adjustment for sex, age, education, peripheral artery disease/media sclerosis, other prior vascular events, smoking status, diabetes, systolic blood pressure, antihypertensive medication, body mass index, cholesterol, and statin use, the hazard ratio was 1.34 (95 %CI 1.20 to 1.50). Significant interactions with CRP were found for sex (adjusted hazard ratio 1.38, 95 %CI 1.11 to 1.72), age (0.75, 95 %CI 0.60 to 0.94), and baseline systolic blood pressure (0.64, 95 % CI 0.51 to 0.81). The interactions of CRP with body mass index and of CRP with diabetes were not significant. *Conclusions:* In older German adults, there seem to be effect modifications by age, sex, and arterial hypertension regarding the effect of CRP in the prediction of all-cause mortality.

Keywords: Risk factors, CRP, interaction, mortality, older adults

Introduction

Elevated levels of C-reactive protein (CRP) are known to be associated with cardiovascular morbidity [1, 2] and mortality [3] in older adults. This association is thought to be caused by systemic inflammation leading to the initiation and progression of atherosclerosis [4]. This was most clearly seen with CRP levels of >3 mg/L [5]. However, there seems to be heterogeneity of this association across subsets of individuals raising the suspicion of interactions [6] between the effects of elevated CRP levels and other variables, such as age, sex, metabolic syndrome, diabetes, and arterial hypertension.

The effects of elevated CRP levels on cardiovascular diseases varied across different age groups [7]. The ten-year follow-up results of the Cardiovascular Health Study showed a different impact of elevated CRP levels on the incidence of coronary heart disease for men and women [8]. CRP enhanced the prognostic information of the metabolic syndrome for coronary heart disease in the West of Scotland Coronary Prevention Study [9]. CRP was a significant predictor of cardiovascular diseases in participants without diabetes, but not in patients with diabetes in the Strong Heart Study [10]. This finding was confirmed in two other studies [11, 12]. Higher CRP levels were associated with pre-clinical atherosclerosis in participants with normal blood pressure but not in those with arterial hypertension in a large Korean cohort [13].

Because study findings are heterogeneous and are still under debate, we performed a retrospective analysis of the seven-year data of the German epidemiological study on ankle brachial index (getABI), a prospective cohort study set up in October 2001 [14]. We focused on the question of interactions of elevated CRP levels with the above mentioned traditional risk factors regarding all-cause mortality in the elderly.

This study aims for assessing the impact of interactions between CRP and one of the following traditional cardio-

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vascular risk factors regarding all-cause mortality in unselected elderly men and women: age, sex, body mass index (BMI), diabetes, and arterial hypertension.

Patients and methods

The getABI study is a prospective cohort study. Three hundred and forty-four general practitioners all over Germany enrolled 6,880 participants aged 65 years or older with a follow-up of seven years. A thorough physical examination including blood pressure measurement and blood sampling was performed at baseline and at the follow-up visits one, three, five, and seven years after baseline. High sensitive CRP measurements were made in a central laboratory. Details have been presented elsewhere [15].

Definition of risk factors

The diagnosis of diabetes was assumed if it was clinically diagnosed by the physician, if the HbA1c was ≥6.5% or if participants were receiving any type of oral anti-diabetic drug or insulin at baseline.

Systolic blood pressure measurements were usually obtained by a standardized Doppler ultrasonic device, preferably on the left arm. If ultrasound measurements were missing, results obtained by conventional blood pressure measurement were used. The intake of angiotensin-1 receptor antagonists, ACE inhibitors or diuretics at baseline was labelled as antihypertensive medication.

A pre-existing cardiovascular disease was assumed if there was a history of prior myocardial infarction, stroke, coronary revascularisation or revascularisation of the carotid arteries. Participants with an ankle brachial index (ABI) of >1.5 were labelled as having media sclerosis, those with an ABI of <0.9 as having peripheral artery disease (PAD). PAD was also assumed in participants with typical signs such as claudication, history of peripheral revascularisation, necrosis/gangrene, and/or limb amputation due to arterial occlusion.

A current smoker status was defined by currently smoking cigarettes with a history of one pack (20 cigarettes) per day for more than one year.

Statistical models

The characteristics of the participants with CRP values at baseline are presented descriptively.

Event rates are expressed per 1,000 person-years with corresponding 95% confidence interval (95%CI). Cox proportional hazards models are used to assess the association of risk factor interactions with all-cause mortality in the course of the seven-year follow-up. This was done for the interaction term of the CRP level (≤3 versus [vs] >3 mg/L) with one of the following risk factors: age (≤75 vs >75 years), sex, BMI (<30 vs ≥30 kg/m^2), diabetes, and arterial hypertension (defined as systolic blood pressure at baseline ≥140 mm Hg).

Univariable as well as multivariable Cox regression analyses were used. The multivariable models comprised 13 parameters as covariates:

- sex,
- age (≤75 vs >75 years),
- education as classified by the International Standard Classification of Education (ISCED) (0–3: pre-primary up to upper secondary education vs 4–6: post-secondary, non-tertiary education up to second stage of tertiary education),
- smoking status (never or past vs currently smoking),
- BMI (<30 vs ≥30 kg/m^2),
- diabetes,
- systolic blood pressure (<140 vs ≥140 mm Hg),
- antihypertensive medication,
- statin use,
- cholesterol (< 240 vs ≥240 mg/dl),
- PAD or media sclerosis (as defined above),
- pre-existing other cardiovascular conditions, and
- CRP (≤3 vs >3 mg/L).

All p-values are two-sided with a p-value of <0.05 labelled as statistically significant. Statistical analyses were performed using SAS, version 9.4 (SAS Institute, Cary, North Carolina, USA).

The getABI trial was approved by the institutional review board of the University of Heidelberg with an amendment for this post-hoc analysis by the ethics committee of the University of Bochum. The getABI trial was supported by an unrestricted educational grant from Sanofi-Aventis GmbH, Berlin, Germany (2001 to 2007) and the German Federal Ministry of Education and Research (2007 to 2010).

Results

Baseline characteristics

In the getABI study 6,880 participants were enrolled. Sixty-three of them had no (high sensitivity) CRP values determined at baseline. The remaining 6,817 participants made up the cohort for this post-hoc analysis. Of the cohort 4,172 participants (61.2%) had a CRP value of ≤3 mg/L, 2,645 participants (38.8%) had a CRP value of >3 mg/L. Baseline characteristics are shown in Table I.

CRP level and all-cause mortality

Before assessing the impact of the interactions of the CRP level (≤3 vs >3 mg/L) with other risk factors, we determined the association of CRP with overall mortality. The group of participants with a baseline CRP level of ≤3 mg/L showed 24 deaths in 1,000 patient-years compared to more than 35

deaths in the group with a CRP level of >3 mg/L (Table II). This corresponds with an unadjusted hazard ratio of elevated CRP levels for all-cause death of 1.49 (95%CI 1.34 to 1.66). After adjustment for sex, age, education (ISCED), PAD/media sclerosis, other prior vascular events, smoking status, diabetes, systolic blood pressure, antihypertensive medication, BMI, cholesterol, and statin use, the hazard ratio of elevated CRP levels (1.34, 95%CI 1.20 to 1.50) remained significant (p<0.001) (Table II).

Interactions of CRP level and traditional risk factors

Five possible CRP interactions were investigated. Significant interactions with CRP levels of >3 mg/L were found for sex, age, and baseline systolic blood pressure. The interaction term of CRP and diabetes as well as that of CRP and BMI showed no significant impact on all-cause mortality (Table III).

In the group with female participants with a baseline CRP level of ≤3 mg/L, there were 20 deaths in 1,000 patient-years compared to 25 deaths in the group with a CRP level of >3 mg/L (Table IV). By contrast, in the male participant group 29 deaths in 1,000 person-years occurred if the baseline CRP level was ≤3 mg/L compared to 53 deaths in 1,000 patient-years for those with a CRP level of >3 mg/L. This yielded an adjusted hazard ratio for the interaction term of 1.38 (95% CI 1.11 to 1.72) (Table III).

Participants younger than 75 years with a baseline CRP level of ≤3 mg/L had an event rate of 16 deaths in 1,000 patient-years compared to nearly 27 deaths in the group with a CRP level of >3 mg/L (Table V). Older participants with a CRP level of ≤3 mg/L showed a higher event rate of

Table I. Participant characteristics with CRP measures at baseline

	All participants (n=6,817)	CRP ≤3 mg/l (n=4,172)	CRP >3 mg/l (n=2645)
CRP, mean (SD)	4.5 (8.3)	1.6 (0.7)	9.2 (11.9)
Female sex, n (%)	3,945 (57.9)	2,353 (56.4)	1,592 (60.2)
Mean age, years (SD)	72.5 (5.3)	72.5 (5.3)	72.6 (5.3)
65–69 years, n (%)	2,348 (34.4)	1,433 (34.3)	915 (34.6)
70–74 years, n (%)	2,199 (32.3)	1,361 (34.3)	838 (31.7)
75+ years, n (%)	2,270 (33.3)	1,378 (33.0)	892 (33.7)
ISCED 0-3, n (%)	1,690 (24.8)	948 (22.8)	742 (28.0)
Peripheral artery diseases or mediasclerosis, n (%)	1,276 (18.7)	677 (16.2)	599 (22.6)
Other prior vascular event, n (%)	1,090 (16.0)	646 (15.5)	444 (16.8)
Current smoker, n (%)	631 (9.3)	317 (7.6)	314 (11.9)
Diabetes, n (%)	1,743 (25.6)	958 (23.0)	785 (29.7)
Systolic blood pressure ≥140 mm Hg (%)	4,374 (64.2)	2,603 (62.4)	1,771 (67.0)
Antihypertensive medication, n (%)	3,518 (51.6)	1,989 (47.7)	1,529 (57.8)
BMI ≥30 kg/m², n (%)	1,570 (23.0)	738 (17.7)	832 (31.5)
Cholesterol ≥240 mg/dl, n (%)	1,530 (22.4)	933 (22.4)	597 (22.6)
Statin use, n (%)	1,390 (20.4)	915 (21.9)	475 (18.0)

CRP: C-reactive protein; n: number; SD: standard deviation; ISCED: international standard classification of education (range 0-6); BMI: body mass index

Table II. Association between CRP and all-cause mortality

	Events n	Person-years n	Event rate per 1,000 person-years (95% CI)	Unadjusted hazard ratio (95% CI)	p-value	Adjusted* hazard ratio (95% CI)	p-value
Total	1,288	45,336	28.4 (26.9 to 30.0)	–	–	–	–
CRP ≤3 mg/l	675	28,120	24.0 (22.2 to 25.8)	Reference	–	Reference	–
CRP >3 mg/l	613	17,216	35.6 (32.8 to 38.4)	1.49 (1.34 to 1.66)	<0.001	1.34 (1.20 to 1.50)	<0.001

CRP: C-reactive protein; n: number; CI: confidence interval
* adjusted for sex, age, education (ISCED), peripheral artery disease/mediasclerosis, other prior vascular events, smoking status, diabetes, systolic blood pressure, antihypertensive medication, body mass index, cholesterol, statin use

42 deaths per 1,000 patient-years with only a slight increase of up to 54 deaths if the baseline CRP level was >3 mg/L. This corresponds to an adjusted hazard ratio of all-cause death for the interaction term of CRP and age of 0.75 (95% CI 0.60 to 0.94) (Table III). On the other hand, the absolute increase in event rates was almost the same in both age groups (11.3 vs 11.6 deaths in 1,000 person-years) (Table V).

In participants with a baseline systolic blood pressure of <140 mm Hg, a baseline CRP level of ≤3 mg/L was associated with a rate of nearly 21 deaths in 1,000 patient-years; the rate increased to more than 40 deaths in the group with a CRP level of >3 mg/L (Table VI). A baseline systolic blood pressure of ≥140 mm Hg was associated with a rate of 26 deaths per 1,000 patient-years if the CRP level was not above 3 mg/L and a relative small increase of up to 33 deaths per 1,000 patient-years if the baseline CRP level was >3 mg/L. This is in accordance with an adjusted hazard ratio of the interaction term of CRP and systolic blood pressure of 0.64 (95% CI 0.51 to 0.81) (Table III).

Table III. Interactions between CRP and one other variable (out of age, sex, diabetes, body mass index, systolic blood pressure) regarding all-cause mortality

	Adjusted* hazard ratio (95% CI)	p-value
CRP × age	0.75 (0.60 to 0.94)	0.011
CRP × sex	1.38 (1.11 to 1.72)	0.004
CRP × diabetes	1.04 (0.83 to 1.31)	0.727
CRP × body mass index	0.88 (0.68 to 1.16)	0.367
CRP × systolic blood pressure	0.64 (0.51 to 0.81)	<0.001

CRP: C-reactive protein; CI: confidence interval
* adjusted for sex, age, CRP, education (ISCED), peripheral artery disease/mediasclerosis, other prior vascular events, smoking status, diabetes, systolic blood pressure, antihypertensive medication, body mass index, cholesterol, statin use

Discussion

Over the past two decades, inflammation has proved to be a pivotal factor in the pathogenesis of atherosclerosis, especially in the initiation, growth, and complication of atherosclerotic plaques [5]. As CRP can be found in direct contact with activated complement fragments in atherosclerotic lesions and in vitro results point to a role of CRP in complement activation, CRP seems to be not only a marker of inflammation, but also an active component in human atherogenesis [16].

In this post-hoc analysis of a large German cohort with a follow-up of seven years, elevated CRP levels predicted increased all-cause mortality (adjusted hazard ratio 1.34, 95%CI 1.20 to 1.50) in older adults. Moreover, there is heterogeneity in the impact of elevated CRP levels on mortality, indicating interactions of CRP with age, sex, and elevated systolic blood pressure: elevated CRP levels had a relatively higher prognostic value in participants younger than 75 years, in males, and in participants without elevated systolic blood pressure at baseline. In general, there were effect modifications by the traditional risk factors sex, age, and systolic blood pressure. No significant heterogeneity was found for the impact of elevated CRP levels on mortality between participants with low or high BMI and between participants with or without diabetes.

The different prognostic impact of elevated CRP levels in various age groups was also reported in the Helsinki Ageing Study. In this study participants with a baseline CRP level lower or higher than 5 mg/L were compared. The prognostic value of the baseline CRP became weaker with increasing age and was only significant in the youngest group (75 years old) [3]. This result corresponds to our finding in which the relative increase in mortality rates in participants with elevated baseline CRP levels was higher in the younger subgroup. Nonetheless, the absolute increase was nearly identical in both age groups (11.3 vs 11.6 events in 1,000 person-years; Table V). This indicates that the age-related heterogeneity of the prognostic value of elevated baseline CRP is only due to the general increase of event rates in older groups and does not reflect a higher

Table IV. Impact of CRP level and gender on all-cause mortality

	Events n	Person-years n	Event rate per 1,000 person-years (95% CI)	Unadjusted hazard ratio (95% CI)	p-value	Adjusted* hazard ratio (95% CI)	p-value
Total (n = 6,817)	1,288	45,336	28.4 (26.9 to 30.0)	–	–	–	–
CRP ≤ 3 mg/dl and female sex (n = 2353)	327	16,121	20.3 (18.1 to 22.5)	Reference	–	Reference	–
CRP > 3 mg/dl and female sex (n = 1592)	267	10,732	24.9 (21.9 to 27.9)	1.23 (1.04 to 1.44)	0.013	1.13 (0.96 to 1.33)	0.146
CRP ≤ 3 mg/dl and male sex (n = 1819)	348	11,999	29.0 (26.0 to 32.0)	1.45 (1.25 to 1.69)	<0.001	1.44 (1.23 to 1.68)	<0.001
CRP > 3 mg/dl and male sex (n = 1053)	346	6,484	53.4 (47.7 to 59.0)	2.71 (2.33 to 3.16)	<0.001	2.25 (1.92 to 2.63)	<0.001

CRP: C-reactive protein; n: number; CI: confidence interval
* adjusted for age, education (ISCED), peripheral artery disease/mediasclerosis, other prior vascular events, smoking status, diabetes, systolic blood pressure, antihypertensive medication, body mass index, cholesterol, statin use

grade of the underlying inflammatory vascular process in younger persons. This is confirmed by results from the National Health and Nutrition Examination Survey in which no interaction between the CRP and leptin levels with age could be found [17].

Many studies have shown differences in men and women regarding the prognostic value of elevated CRP levels. In the National Health and Nutrition Examination Survey III, men with a CRP of >3.0 mg/L had increased all-cause mortality hazards compared to those with a CRP of ≤3.0 mg/L (hazard ratio 1.57, 95% CI 1.29–1.90). In women, elevated CRP values were not significantly associated with higher all-cause mortality hazards (HR 1.09, CI 0.93–1.29) [18]. Similar results were shown in the EPIC-Norfolk study in which the association between elevated CRP levels and all-cause mortality was apparent in all men, but only in those women with the highest levels of the CRP distribution [19]. Although CRP levels are lower in Japanese subjects compared with Western ones, CRP was an independent predictor of all-cause mortality in apparently healthy Japanese men but not women [20]. The reason for this gender difference needs to be elucidated.

It is well known that arterial hypertension is associated with elevated CRP levels [21]. In this cohort, a significant interaction between CRP and blood pressure values at baseline was found: an elevated CRP level nearly doubled the mortality rate in participants with normal blood pressure, whereas the mortality rate increased by less than one third in patients with high blood pressure. This result is comparable to the above mentioned finding in a large Korean cohort in which an association of a CRP level >2 mg/L with pre-clinical atherosclerosis was found in participants with normal blood pressure but not in those with arterial hypertension [13]. The pathophysiological background of this questionable association is not clearly understood.

Our results concerning the lack of an interaction between CRP levels and diabetes confirm the findings of a pooled analysis of 25,979 participants from four U.K. prospective cohort studies in which no significant interaction

Table V. Impact of CRP level and age on all-cause mortality

	Events n	Person-years n	Event rate per 1,000 person-years (95% CI)	Unadjusted hazard ratio (95% CI)	p-value	Adjusted* hazard ratio (95% CI)	p-value
Total (n = 6,817)	1,288	45,336	28.4 (26.9 to 30.0)	–	–	–	–
CRP ≤ 3 mg/dl and age < 75 years (n = 2,794)	298	19,230	15.5 (13.7 to 17.3)	Reference	–	Reference	–
CRP > 3 mg/dl and age < 75 years (n = 1,753)	313	11,663	26.8 (23.9 to 29.8)	1.74 (1.49 to 2.04)	<0.001	1.56 (1.33 to 1.84)	<0.001
CRP ≤ 3 mg/dl and age ≥ 75 years (n = 1,378)	377	8,890	42.4 (38.1 to 46.7)	2.77 (2.38 to 3.23)	<0.001	2.71 (2.32 to 3.16)	<0.001
CRP > 3 mg/dl and age ≥ 75 years (n = 892)	300	5,553	54.0 (47.9 to 60.1)	3.55 (3.03 to 4.17)	<0.001	3.18 (2.69 to 3.75)	<0.001

CRP: C-reactive protein; n: number; CI: confidence interval
* adjusted for sex, education (ISCED), peripheral artery disease/mediasclerosis, other prior vascular events, smoking status, diabetes, systolic blood pressure, antihypertensive medication, body mass index, cholesterol, statin use

Table VI. Impact of CRP level and systolic blood pressure on all-cause mortality

	Events n	Person-years n	Event rate per 1,000 person-years (95% CI)	Unadjusted hazard ratio (95% CI)	p-value	Adjusted* hazard ratio (95% CI)	p-value
Total (n = 6,817)	1,288	45,336	28.4 (26.9 to 30.0)	–	–	–	–
CRP ≤ 3 mg/dl and systolic blood pressure < 140 mm Hg (n = 1,569)	221	10,598	20.9 (18.1 to 23.6)	Reference	–	Reference	–
CRP > 3 mg/dl and systolic blood pressure < 140 mm Hg (n = 874)	226	5,592	40.4 (35.1 to 45.7)	1.96 (1.63 to 2.36)	<0.001	1.79 (1.49 to 2.16)	<0.001
CRP ≤ 3 mg/dl and systolic blood pressure ≥ 140 mm Hg (n = 2,603)	454	17,522	25.9 (23.5 to 28.3)	1.25 (1.06 to 1.46)	0.007	1.12 (0.96 to 1.32)	0157
CRP > 3 mg/dl and systolic blood pressure ≥ 140 mm Hg (n = 1,771)	387	11,624	33.3 (30.0 to 36.6)	1.60 (1.36 to 1.89)	<0.001	1.30 (1.10 to 1.54)	<0.001

CRP: C-reactive protein; n: number; CI: confidence interval
* adjusted for age, sex, education (ISCED), peripheral artery disease/mediasclerosis, other prior vascular events, smoking status, diabetes, antihypertensive medication, body mass index, cholesterol, statin use

between diabetes and CRP regarding all-cause mortality was found [22]. The same was deduced from data of the third National Health and Nutrition Examination Survey, where the interaction term for CRP and diabetes concerning all-cause mortality was not significant [17].

Finally, in this study as well as in the third National Health and Nutrition Examination Survey [17] interaction between BMI and CRP regarding all-cause mortality was not apparent. In our study the cut point was set at a BMI of 30 kg/m², whereas in the third National Health and Nutrition Examination Survey the cut point was 25 kg/m². Different results were seen in a Japanese study [23] that divided the cohort of 1,871 patients with coronary artery disease into three groups: BMI <24 kg/m², 24.0–27.9 kg/m² and ≥28 kg/m². Using the group with a BMI of 24.0–27.9 kg/m² and CRP <3 mg/L as reference, the hazard ratio was 1.16 for the group with the same BMI but with CRP values of ≥3 mg/L. The group with a BMI of <24 kg/m² had a hazard ratio of 0.93 if the CRP was <3 mg/L but 2.55 if the CRP was ≥3 mg/L. A similar difference was seen in patients with a BMI of ≥28 kg/m² who had a hazard ratio of 1.42 if the CRP was <3 mg/L compared to 3.41 if the CRP was ≥3 mg/L. Thus, a J-shaped heterogeneity could be seen that might have been missed by dichotomising the cohort as it was done in this study and in the third National Health and Nutrition Examination Survey.

The strengths of the getABI study lie in the large cohort in a primary care setting and in the effort to reduce selection bias. This was achieved by the setting the study over three pre-specified weeks, incorporating general practitioners from all parts of Germany, including about 20 primary care attendees. On the other hand, there are some limitations to the getABI study. Only persons of at least 65 years were recruited. Therefore, study results may only be applicable to this age group. The analyses of this study used single CRP measurements at baseline. Thus it is not possible to allow for regression dilution bias. However, CRP values have been shown to be fairly stable in individual patients [24] and are therefore considered suitable for long-term predictions.

Unanswered questions and future research

The most striking interaction in this analysis is seen between CRP and gender. The pathophysiological concept for this observation is still lacking. The interaction between CRP and blood pressure needs to be confirmed in further studies.

As vascular inflammation may cause plaques and promote their instability, the Cardiovascular Inflammation Reduction Trial (CIRT) investigates if low dose methotrexate, an anti-inflammatory agent which is widely used in rheumatic diseases, can reduce morbidity and mortality among patients with a coronary artery disease (NCT02576067) [25]. In case of positive results, the presence of CRP interactions might guide differential therapeutic decisions.

Conclusions

In elderly German adults, the effect of CRP in predicting all-cause mortality seems to be modified by age, gender, and arterial hypertension.

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Original communication

Socioeconomic factors and the onset of peripheral artery disease in older adults

Results from a prospective cohort study in a primary care setting (getABI)

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Summary: *Background:* As evidence concerning the impact of socioeconomic factors on the risk of peripheral artery disease (PAD) is sparse, we assessed the association of education and area-level factors (population density, type of municipality and local unemployment rate) on the onset of PAD in older adults. *Patients and methods:* The analysis used data of the getABI study, a prospective cohort study with seven years of follow-up. Onset of PAD was determined by ankle brachial index (<0.9) or PAD symptoms. Cox regression analysis was employed. *Results:* Out of 5,444 primary care attendees without PAD at baseline, there were 1,381 participants with PAD onset (cumulative observation time 31,739 years), yielding an event rate of 43.5 (0.95 confidence interval [0.95 CI] 41.2–45.8) per 1,000 person-years. Multivariable Cox regression analysis showed an association of PAD onset with low education (hazard ratio 1.29; 0.95 CI 1.14–1.46; P<0.001), high population density (0.93; 0.89–0.98; P=0.002), small cities (compared to large cities) (0.71; 0.53–0.96; P=0.027) and high local unemployment rate (1.04; 1.00–1.07; P=0.032). The impact of low education on PAD onset was higher for men (2.11; 1.64–2.72) than for women (1.22; 1.07–1.40) (interaction term P=0.013). *Conclusions:* Socioeconomic factors, education as well as area-level socioeconomic indicators, make independent contributions to PAD onset in older adults.

Keywords: Peripheral artery disease, socioeconomic factor, incidence, older adults

Introduction

The place of residence is known to have an impact on health, especially on cardiovascular diseases. Characteristics of neighborhoods were shown to be related to the incidence of coronary heart disease [1]. Moreover, levels of neighbourhood deprivation predict the risk of ischemic stroke, even after adjustment of established risk factors [2]. Concerning the association of socioeconomic factors and the risk of peripheral artery disease (PAD), another manifestation of atherosclerotic artery disease, evidence is sparse.

The number of PAD patients seems to be increasing with more than 200 million affected persons globally [3]. In Germany, more than 8% of men and more than 5% of women between 45 and 75 years of age show signs or symptoms of PAD [4]. Due to the systemic spreading of the underlying atherosclerotic artery disease, PAD not only causes local symptoms but also shows an association with coronary heart disease (CHD) and cerebrovascular events as well as with increased mortality [5].

Beside conventional cardiovascular risk factors, socioeconomic inequalities (low income and lower attained education level) are associated with PAD prevalence in US adults [6]. Low individual and area-level socioeconomic status are strong predictors of hospitalization with PAD [7] and socioeconomic deprivation leads to higher rates of major lower limb amputation secondary to peripheral arterial disease [8].

However, the relationship of socioeconomic factors with PAD incidence remains unclear. To shed light on this issue, we made a post-hoc analysis of data from a cohort of more than 6,800 participants in a general practice setting with a follow-up of seven years.

Material and methods

In October 2001, the German epidemiological study on ankle brachial index (getABI) was set up as a prospective cohort study. 344 general practitioners (GPs) trained by 34 vascular specialists all over Germany enrolled unselected participants aged 65 years or older (getABI study) [5]. Medical history was taken by the treating physician, followed by a physical examination including blood

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sampling. Bilateral Doppler ultrasound measurements were performed with determination of the ankle brachial index (ABI) [9]. After one year, three years, five years and seven years, there were follow-up examinations.

A history of claudication, peripheral revascularization, necrosis/gangrene and/or amputation due to PAD was sufficient for the diagnosis of symptomatic PAD. In patients without these symptoms, the diagnosis of asymptomatic PAD was based on an ABI value <0.9. Participants with an ABI >1.5 were not regarded as PAD patients unless they had PAD symptoms.

In this post-hoc analysis, participants with symptomatic PAD or asymptomatic PAD were considered as one group. Those with signs or symptoms of PAD at baseline were excluded from the analysis.

Definition of risk factors

Socioeconomic factors were chosen on the individual level (education) and on an area-based level (local unemployment rate, population density and type of municipality). Level of education was scaled by the International Standard Classification of Education (ISCED) score. An ISCED score of 0 to 2 was coined as low and compared to a score of 3 to 6 [10].

Population density data from the geographic information system ArcGIS (ESRI Inc., Environmental Systems Research Institute, Redlands, California, USA) were used. ArcGIS also provided local unemployment data. Information concerning type of municipality was gained from the Federal Institute for Building, Urban and Spatial Research in the Federal Office for Building and Regional Planning (Bundesinstitut für Bau-, Stadt- und Raumforschung im Bundesamt für Bauwesen und Raumordnung). These informations were linked with participants' addresses by 8-digit district municipality key numbers provided by the LOCAL® KGS system (Nexiga GmbH, Bonn, Germany). The types of municipality were defined as follows: large city, ≥500,000 inhabitants; medium sized city, 100,000 to 499,999 inhabitants; small city, 5,000 to 99,999 inhabitants; village community, <5,000 inhabitants.

Besides these four factors, we took 12 potentially relevant cardiovascular risk factors into account: age and gender, four classical risk factors (smoking status, arterial hypertension, diabetes, LDL cholesterol) and six other factors (lipid lowering treatment, C-reactive protein (CRP), estimated glomerular filtration rate (eGFR), homocysteine, vitamin D, history of cardiovascular events). These 12 risk factors were evaluated at baseline and defined for this analysis as follows:

- *age:* dichotomized by the median of the whole getABI cohort (71.9 years),
- *gender:* status at baseline,
- *smoking status:* never smoker, former smoker, current smoker,
- *arterial hypertension:* diagnosis by the treating physician or medication with angiotensin-1 receptor antagonists, angiotensin converting enzyme inhibitors, or diuretics at baseline,
- *diabetes:* diagnosis at baseline or treatment with insulin or other antidiabetics or in case of an HbA1c≥6.5%,
- *LDL cholesterol:* dichotomized by 130 mg/dl,
- *lipid lowering therapy:* use of statins or fibrates,
- *CRP*: dichotomized by 3 mg/l,
- *eGFR:* dichotomized by 60 ml/min/1.73 m^2, calculated by the Cockcroft Gault formula with the Dubois correction [11],
- *homocysteine:* dichotomized by its median 14.1 mol/l,
- *vitamin D:* dichotomized by 50 nmol/l,
- *history of cardiovascular events:* diagnosis of coronary heart disease or stroke or a history of coronary or carotid revascularization.

Dichotomization was used to provide conservative estimations whenever the number of missings seems to require this procedure.

Statistical methods

Primary outcome measure was PAD onset as defined above. We provided an estimation of the PAD incidence rate by the ratio of observed events and the number of person-years under observation. Missing values appeared in less than 1%. We categorized variables according to the above mentioned cutoffs into the levels "yes" and "no/unknown". Then, multivariable Cox-regression was done for the primary outcome measure taking the four socioeconomic factors as covariates with adjustment for the above mentioned 12 risk factors. Interaction analysis was performed for level of education and gender. We used two-sided p-values and labeled p-values <0.05 as significant. Analyses were performed using SAS, version 9.4 (2013, SAS Institute Inc., Cary, NC, USA).

The getABI trial was approved by the institutional review boards of the University of Heidelberg (reference number 202/2001). Before entering the trial, each participant provided written informed consent. The getABI trial followed the recommendations of Good Epidemiological Practice and was supported by unrestricted grants from Sanofi-Aventis GmbH, Berlin, Germany, and the German Federal Ministry of Education and Research. This post-hoc analysis was started after the approval of the ethics committee of the Ruhr University Bochum (registration number: 16-6003) and the registration with the German Clinical Trials Register (registration number: DRKS 00014099).

Results

6,880 patients participated in the getABI trial. Home addresses were missing for four participants. Of the remaining 6,876 participants, 1,432 (21%) had signs or symptoms of PAD at baseline and were excluded from further analysis. Thus 5,444 participants made up the baseline cohort of this post-hoc analysis (Figure 1).

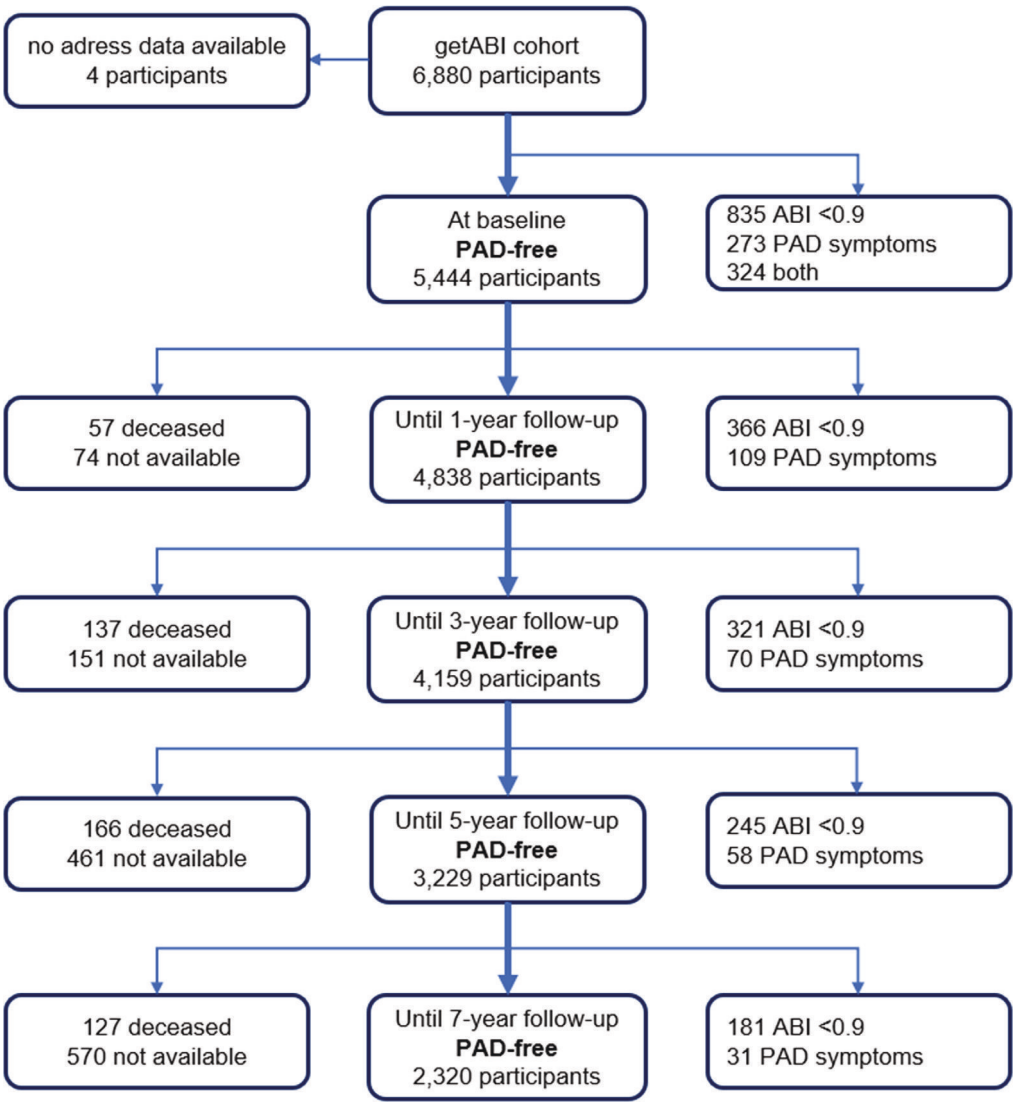

Figure 1. Flow diagram of the study population.

59% of these 5,444 participants were female; 76% had an ISCED level of 3 or higher. Only 4% lived in village communities, nearly one third of the others in small, medium sized or large cities, respectively. 66% had arterial hypertension, only 8% were currently smoking, 23% had diabetes and 67% a low vitamin D level (Table I). There were movements in 10% of the participants, but only 20% of them (2% of the cohort) moved to another city or region. Data from a subset of participants (n=2,120) shows that nearly 90% were born in Germany, nearly 10% in Eastern Europe, only 0.14% in Southern Europa and 0.3% outside Europe. Almost all risk factors were more pronounced among the participants with incidental PAD. Considering the entire study time, crude odds for participants with incidental PAD vs. PAD-free participants were particularly increased for smoking, age, diabetes, history of cardiovascular events and renal insufficiency.

In the course of seven-year follow-up with 31,739 person-years under risk, there were 1,381 participants with PAD onset. This yielded an incidence rate of 43.5 per 1,000 person-years (0.95 confidence interval [0.95 CI] 41.2 to 45.8). 1,113 patients had asymptomatic PAD and 268 had symptomatic PAD. 2,320 participants remained PAD-free until the 7-year follow-up (Figure 1).

Univariable Cox regression yielded significant results for the unemployment rate and low education as measured by ISCED. Multivariable Cox regression analysis with adjustment for classical and other well known risk factors showed a significant association of PAD onset with all chosen socioeconomic factors. Low education defined by an ISCED score of 0 to 2 had a significant impact on the PAD risk (hazard ratio [HR] 1.29; 0.95 CI 1.14 to 1.46; P<0.001). The same was true for a high local unemployment rate (HR 1.04; 0.95 CI 1.00 to 1.07; P=0.032). An increase of 500 inhabitants/km^2 in population density was inversely related to PAD onset (HR 0.93; 0.95 CI 0.89 to 0.98; P=0.002). In comparison to large cities, living in small cities (HR 0.71; 0.95 CI 0.53 to 0.96; P=0.027) or in medium sized cities (HR 0.74; 0.95 CI 0.60 to 0.92; P=0.005) was associated with lower PAD risk (Table II).

An interaction test of education and gender made it obvious that in case of low education levels the HR for PAD onset was higher in men (HR 2.11; 0.95 CI 1.64 to 2.72) than

Table I. Participant characteristics at baseline

	All participants (n=5,444)		Incidental PAD (n=1,381)		PAD-free (n=2,320)	
	n	%	n	%	n	%
ISCED						
0 to 2	1,311	24.08	411	29.76	460	19.83
3 to 6	4,133	75.92	970	70.24	1,860	80.17
Type of municipality						
Large city	1,644	30.20	429	31.06	714	30.78
Medium sized city	1,924	35.34	475	34.40	805	34.70
Small city	1,633	30.00	415	30.05	693	29.87
Village community	243	4.46	62	4.49	108	4.66
Age≥71.9 years	2,550	46.84	729	52.79	862	37.16
Female sex	3,200	58.78	869	62.93	1,327	57.20
Arterial hypertension	3,566	65.50	1,017	73.64	1,406	60.60
Smoking status						
Never smoker	3,110	57.13	740	53.58	1,382	59.57
Former smoker	1,909	35.07	492	35.63	809	34.87
Current smoker	425	7.81	149	10.79	129	5.56
Diabetes	1,246	22.89	366	26.50	449	19.35
LDL-cholesterol≥130 mg/dl	2,331	42.82	644	46.63	998	43.02
Lipid-lowering therapy	1,189	21.84	341	24.69	529	22.80
sCRP≥3 mg/l	1,993	36.61	577	41.78	749	36.61
eGFR<60 ml/min/1.73 m^2	924	16.97	261	18.90	265	11.42
Homocysteine>14.1 µmol/l	2,553	46.90	709	51.34	978	46.90
Vitamin D<50 nmol/l	3,666	67.34	991	71.76	1,475	63.58
History of CV-events	717	13.17	232	16.80	249	10.73

PAD: peripheral artery disease; ISCED: International Standard Classification of Education; LDL: low density lipoprotein; sCRP: sensitive C-reactive protein; eGFR: estimated glomerular filtration rate. *Definitions.* Large city: ≥500,000 inhabitants; medium sized city: 100,000 to 499,999 inhabitants; small city: 5,000 to 99,999 inhabitants; village community: <5,000 inhabitants.

in women (HR 1.22; 0.95 CI 1.07 to 1.40). The interaction term was significant (P<0.001) (Table III).

Discussion

In this large-scale prospective cohort study, 5,444 elderly participants without signs or symptoms of PAD at baseline were followed up to 7 years. 1,381 participants acquired PAD, yielding a cumulative incidence rate of 43.5 per 1,000 person-years at risk. After adjusting for classic and other well established PAD risk factors, we found significant associations of PAD onset with socioeconomic factors, such as education, population density, type of municipality and local unemployment rate.

Depending on the definition of PAD and the mean age of the study cohort, the reported incidence rates of overall PAD vary from 3.8 per 1,000 person-years at risk in a population based cohort (age between 35 and 79 years) [12] and 8.6 per 1,000 person-years in a Spanish cohort with participants older than 50 years [13] up to 22.9 for women in the age group ≥65 years in the Limburg PAOD study [14] that had defined PAD by an ABI of less than 0.95, measured twice at intervals of one week. In our analysis, one ABI-measurement of less than 0.9 was regarded sufficient for assigning to PAD, as recommended by the AHA/ACC Guideline [15]. However, single ABI measurement may lead to overestimation of PAD rates. The use of repeated measurements for diagnosis results in lower estimated rates. In a recent publication using a regression method with repeated measurements for defining PAD in getABI participants, the cumulative incidence rate was 20.3 per 1,000 person-years at risk [9], comparable with the result in the Limburg PAOD study.

Population density is often thought to be associated with increased mortality [16]. A recently published study confirmed this finding but stated that more walking and cycling may compensate part of this effect [17]. In our analysis, an increase in population density significantly lowered PAD incidence. This seems to be counterintuitive, as higher population densities are often thought to be associated with increased air pollution affecting arterial vessels and thereby promoting PAD. Because our multivariable model accounted for type of municipality, education and unemployment rate, population density on its own may reflect better infrastructural facilities, including medical care. As expected, the univariable analysis of population density was no longer significant.

Unemployment is known to predict, at least in men, death from cardiovascular disease and from all causes in the individual [18]. This link was also seen when examining the relationship of unemployment rates and mortality rates

Table II. Univariable and multivariable Cox regression for PAD onset in the course of 7-year follow-up

	Univariable model		Multivariable model	
Risk factor	Hazard-ratio (0.95 CI)	P value	Hazard-ratio (0.95 CI)	P value
Unemployment rate	1.04 (1.01–1.07)	0.007	1.04 (1.00–1.07)	0.032
Population density per 500	0.99 (0.97–1.01)	0.178	0.93 (0.89–0.98)	0.002
ISCED 0 to 2	1.39 (1.24–1.57)	<0.001	1.29 (1.14–1.46)	<0.001
Type of municipality				
Large city	Reference	Reference	Reference	Reference
Medium sized city	0.94 (0.83–1.08)	0.387	0.74 (0.60–0.92)	0.006
Small city	0.97 (0.85–1.12)	0.701	0.71 (0.53–0.96)	0.027
Village community	1.03 (0.79–1.35)	0.813	0.77 (0.51–1.15)	0.200
Age ≥71.9 years	1.37 (1.24–1.53)	<0.001	1.33 (1.19–1.49)	<0.001
Male sex	0.85 (0.76–0.95)	0.004	0.83 (0.73–0.95)	0.007
Arterial hypertension	1.60 (1.42–1.81)	<0.001	1.46 (1.29–1.66)	<0.001
Smoking status				
Never smoker	Reference	Reference	Reference	Reference
Former smoker	1.12 (0.99–1.26)	0.053	1.23 (1.08–1.40)	0.002
Current smokier	1.69 (1.42–2.02)	<0.001	1.96 (1.64–2.35)	<0.001
Diabetes	1.32 (1.17–1.49)	<0.001	1.23 (1.09–1.39)	0.001
LDL-cholesterol≥130mg/dl	1.17 (1.05–1.30)	0.004	1.22 (1.10–1.36)	<0.001
Lipid-lowering therapy	1.19 (1.05–1.35)	0.005	1.15 (1.01–1.30)	0.040
sCRP≥3 mg/l	1.32 (1.19–1.47)	<0.001	1.19 (1.07–1.33)	0.002
eGFR<60 ml/min/1.73 m^2	1.24 (1.08–1.42)	<0.001	1.03 (0.89–1.19)	0.696
Homocysteine>14.1 µmol/l	1.26 (1.14–1.40)	<0.001	1.16 (1.04–1.29)	0.010
Vitamin D<50 nmol/l	1.31 (1.17–1.48)	<0.001	1.11 (0.98–1.26)	0.088
History of cardiovascular events	1.49 (1.30–1.72)	<0.001	1.41 (1.21–1.64)	<0.001

ISCED: International Standard Classification of Education; LDL: low density lipoprotein; sCRP: sensitive C-reactive protein; eGFR: estimated glomerular filtration rate. *Definitions*. Large city: ≥500,000 inhabitants; medium sized city: 100,000 to 499,999 inhabitants; small city: 5,000 to 99,999 inhabitants; village community: <5,000 inhabitants.

Table III. Association of low ISCED levels (0 to 2) with PAD onset in women and men (univariable analysis)

Covariate	Hazard-Ratio (0.95 CI)	P-value for interaction
ISCED 0 to 2 in women	1.22 (1.07–1.40)	<0.001
ISCED 0 to 2 in men	2.11 (1.64–2.72)	

ISCED: International Standard Classification of Education.

of ischemic heart disease [19]. Moreover, a positive association between unemployment rate and hospital admissions due to acute myocardial infarction was observed [20]. In our cohort, a positive association could be found between unemployment rate and PAD incidence. The effect of unemployment was independent of education and other variables used for adjustment.

The disparity of cardiovascular risk factors in rural and urban communities seems to be homogeneous between European countries. In 2009, the rural population in northern Sweden was older, had less education, a higher BMI, a more sedentary lifestyle and higher cholesterol levels than the urban population [21]. The predicted 10-year risk of cardiovascular mortality in a 40 to 69-year old general population without cardiovascular diseases in Germany, measured by newly recalibrated SCORE (Systematic COronary Risk Evaluation) [22] Deutschland risk charts, was lower for inhabitants in larger cities. This was mostly striking for women living in communities with ≥ 100,000 inhabitants compared to <20,000 inhabitants, even after adjustment for the socioeconomic status including education and employment status (5.4% vs. 10.9%; odds ratio 0.49, 0.95 CI 0.27 to 0.85) [23]. The SCORE risk charts include gender, age, systolic blood pressure, total cholesterol and smoking status, leaving out other known risk factors for PAD onset. In our analysis with additional adjustment for population density, small and medium sized cities had a lower HR for PAD onset than large cities; the HR for village communities was in the range of small or medium sized cities. The link between area-level factors such as high employment rate or living in a large city and PAD onset seems to be complex, including air and noise pollution [24].

It is well known that low education status is associated with PAD prevalence when compared to high education [25]. A similar association was found for an index of multiple deprivation including education [26] and PAD onset in a large cohort from the United Kingdom [27]. This is in accordance with our findings that yielded a HR of 1.28 for low education regarding PAD onset. The pathway from a low education level to increased cardiovascular event-rates may be determined by health behaviors such as unhealthy diets, sedentary lifestyle and less physical activity [24].

A focus of our analyses lay on gender inequality concerning the impact of low education. As our participants had their education period mostly during or shortly after the World War II, we assumed disadvantages in the availability of higher education for female participants. This may weaken the link between education and socioeconomic situation in general, leading to a smaller impact of low education on PAD onset in women compared to men. Indeed, this assumption was supported by our findings; the interaction term for education and gender was significant, indicating a higher PAD risk for men with low education compared to women.

The getABI trial involved one of the largest PAD cohorts in a primary care setting. This setting may provide a more direct applicability of results to routine care than those of population-based cohorts. With participation of primary care physicians from all parts of Germany, the generalizability of results may additionally increase.

Limitations

Although removals of participants were recorded (about 10%), we did not include that into the analysis, because only approximately 2% of removals changed the area of municipality. Further, we had no information, how long the participants had lived at their place before the beginning of the study. This might disturb estimations on long-term effects of address; on the other hand, it can be assumed that people born in the 1930s or earlier moved less often than is usual today.

Conclusions

Education as well as area-level socioeconomic indicators such as population density, type of municipality and local unemployment rate are independently associated with PAD onset in older adults in the course of seven years of follow-up. Primary care attendees from a deprived place of residency and men with a low educational level should undergo peripheral vascular assessment. Studying socioeconomic risk factors may contribute to the identification of patients at risk of PAD.

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Original communication

The risk of peripheral artery disease in older adults – seven-year results of the getABI study

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Summary: *Background:* To assess the risk of peripheral artery disease (PAD) in older adults and the contribution of traditional and novel risk factors to the incidence of PAD. *Patients and methods:* 344 general practitioners (GPs), trained by vascular specialists all over Germany, enrolled 6,880 unselected participants aged 65 years or older (getABI study). The onset of PAD was determined by a regression method in the course of repeated measurements of the ankle brachial index (ABI) over seven years. PAD onset was defined by the declining linear regression ABI line reaching 0.9 or by PAD symptoms. *Results:* The cumulative PAD incidence over seven years was 12.9%, corresponding to an incidence rate of 20.3 per 1000 person years (95% confidence interval [95%CI] 18.8 to 21.7). Logistic regression analysis showed that traditional risk factors contributed significantly to the risk of PAD: current smoker status (odds ratio 2.65, 95%CI 2.08 to 3.37), diabetes (1.35, 95%CI 1.13 to 1.62), and low-density lipoprotein >130 mg/dl (1.26, 95%CI 1.07 to 1.48). Three novel risk factor candidates showed significant impact on PAD incidence: elevated sensitive C-reactive protein level (1.23, 95%CI 1.05 to 1.45), impaired estimated glomerular filtration rate (1.27, 95%CI 1.03 to 1.56), and elevated homocysteine level (1.19, 95%CI 1.01 to 1.41). *Conclusions:* Older adults in Germany have a PAD risk of 12.9% per seven years. Potentially modifiable traditional PAD risk factors yield high impact on PAD incidence. Novel risk factor candidates may contribute to the risk of PAD

Key words: Peripheral artery disease, risk factors, incidence, ankle brachial index, getABI study

Introduction

Peripheral artery disease (PAD) is a widespread manifestation of systemic atherosclerotic artery disease, affecting 202 million people globally, with figures increasing during the preceding decade [1]. Stenosis or occlusion of the lower-extremity arteries may result in symptoms of claudication, critical limb ischemia, and the need for revascularization or limb amputation. In Germany, PAD affects 8.2% of all men and 5.5% of all women between the ages of 45 and 75 [2]. Even asymptomatic PAD, as determined by the ankle brachial index (ABI), is associated with premature mortality and cardiovascular or cerebrovascular events [3].

Although prevalence of PAD in primary care practice is high and PAD is associated with cardiovascular morbidity and mortality, physician awareness is low. Underdiagnosed PAD leads to less intensive treatment of preventable or treatable risk factors such as lipid disorders and hypertension and to less effective secondary prevention of cardiovascular or cerebrovascular events in general [4].

Despite the high prevalence, the number of studies on the incidence of PAD is sparse. Recently, a large prospective study in the United States examined the associations between conventional cardiovascular risk factors and the risk of PAD in men. Smoking, hypertension, hypercholesterolemia, and type 2 diabetes were the main risk factors for symptomatic PAD [5]. Recent epidemiological and clinical studies have indicated novel treatable risk factors for PAD such as vitamin D deficiency [6].

To estimate the incidence rates of symptomatic and overall PAD in unselected elderly men and women as well as to assess the impact of conventional and novel risk factors on PAD incidence, we studied a cohort of more than 6800 participants in a general practice setting with a follow-up of seven years [3].

Patients and methods

The German epidemiological study on ankle brachial index (getABI)

The German epidemiological study on ankle brachial index (getABI) is a prospective cohort study set up in October 2001. Thirty-four vascular specialists in Germany chose about ten general practitioners (GPs) in their vicinity; these 344 GPs and their staff were trained in clinical assessments and the measurement of the ankle brachial index (ABI) under standardized conditions. Unselected primary care attendees were assessed for enrollment within three pre-specified weeks in October 2001 [7]. In total, the GPs enrolled 6880 participants and followed them up over seven years.

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At baseline, a medical history assessment, physical examination, and blood sampling were performed [3]. Examinations comprised bilateral Doppler ultrasound measurements and determination of the ABI, laboratory evaluations at baseline, and physical examinations. For ABI measurements, a standardized Doppler ultrasonic device was used (Kranzbühler 8 MHz, Solingen, Germany). Blood pressure measurements and ABI calculations were performed according to the recommendations of the American Heart Association put forward in 2000 [8].

Definition of PAD onset

ABI was measured at baseline after one year, three years, five years, and seven years. The onset of PAD could be symptomatic or asymptomatic. Symptomatic PAD was defined as a history of peripheral revascularization, necrosis/gangrene, and/or amputation due to PAD. Because of its considerably low specificity, intermittent claudication was omitted from the definition of symptomatic PAD.

Because of a mean error of 8–9 % within as well as between observers for repeated measurements of the ABI [9], we defined the onset of PAD as any peripheral symptom (only the first event was taken into account) or by means of linear regression. Rather than basing the diagnosis of PAD on a single ABI value < 0.9, PAD onset was set by the following criteria: measurement points over the course of the study were used for linear regression, and the time at which the straight regression line crossed the 0.9 level was defined as PAD onset. At least one measurement after baseline had to exist for linear regression; missing values in between were ignored and interpolated. The regression line was not extended beyond the points with ABI values. In comparison to single point assessment, the standard error of interpolated ABI values is expected to be reduced up to a factor of $\sqrt{n}$, where n is the number of available ABI measurements per participant.

Table I. Participant characteristics at baseline (asymptomatic PAD defined by the regression method)

	All participants (n = 6880)	No PAD (n = 5735)	PAD (n = 1145)
PAD symptoms, no. (%)	170 (2.5)	–	170 (14.8)
ABI<0.9, no. (%), no symptoms	975 (14.2)	–	975 (85.2)
ABI>1.5, no. (%)	60 (0.9)	60 (1.0)	0 (0.0)
Female sex, no. (%)	3975 (57.8)	3390 (59.1)	585 (51.1)
Mean age, years (SD)	72.5 (5.29)	72.2 (5.14)	74.0 (5.76)
65-69 years, no. (%)	2365 (34.4)	2066 (36.0)	299 (26.1)
70-74 years, no. (%)	2220 (32.3)	1892 (33.0)	328 (28.6)
75-79 years, no. (%)	1497 (21.8)	1185 (20.7)	312 (27.2)
80-84 years, no. (%)	646 (9.4)	491 (8.6)	155 (13.5)
≥85 years, no. (%)	152 (2.2)	101 (1.8)	51 (4.5)
ISCED 0-3, no. (%)	5947 (86.4)	4908 (85.6)	1039 (90.7)
Current smoker, no. (%)	638 (9.3)	439 (7.7)	199 (17.4)
Diabetes, no. (%)	1762 (25.6)	1328 (23.2)	434 (37.9)
Antihypertensive medication, no. (%)	3544 (51.5)	2781 (48.5)	763 (66.6)
Statin use, no. (%)	1401 (20.4)	1089 (19.0)	312 (27.2)
Other CVD, no. (%)	1103 (16.0)	786 (13.7)	317 (27.7)
BMI ≥ 30 kg/m², no. (%)	1584 (23.0)	1296 (22.6)	288 (25.2)
Systolic blood pressure ≥ 140 mm Hg (%)	4408 (64.1)	3581 (62.4)	827 (72.2)
LDL ≥ 3.4 mmol/l (%)	2937 (42.7)	2462 (42.9)	475 (41.5)
Vitamin D < 50 nmol/l (%)	4783 (69.5)	3905 (68.1)	878 (76.7)
Homocysteine ≥ median (%)	3438 (50.0)	2769 (48.3)	669 (58.4)
GFR < 60 ml/min/1.73 m² (%)	1353 (19.7)	1014 (17.7)	339 (29.6)
sCRP > 3 mg/l (%)	2667 (38.8)	2114 (36.9)	553 (48.3)

Abbr.: PAD, peripheral artery disease; CVD, cardiovascular disease; ABI, ankle brachial index; ISCED, international standard classification of education (range 0-6); BMI, body mass index; LDL, low-density lipoprotein; GFR, glomerular filtration rate; sCRP, sensitive C-reactive protein; SD, standard deviation

Participants with only one ABI value or with an ABI >1.5 (due to linear regression) were defined as "no PAD" unless they exhibited PAD symptoms. The minimum time of peripheral symptom observation, intersection of the regression line with the 0.9 level, and 7.25 years (in participants without PAD) was used as the time at risk.

Definition of risk factors

Participants were defined as having diabetes if they had been clinically diagnosed with diabetes by their physician, if their HbA1c was ≥6.5%, or if they were receiving any type of oral anti-diabetic drug or insulin at baseline.

The use of angiotensin-1 receptor antagonists, ACE inhibitors, or diuretics at baseline was classified as antihypertensive medication. Systolic blood pressure of the ABI measurement (with a standardized Doppler ultrasonic device) of the left arm was used for determining a state of arterial hypertension. If this value was missing (n=19), the results from the right arm were used. If these results were also missing (n=2), results obtained by conventional blood pressure measurement were used instead.

Pre-existing cardiovascular diseases (CVDs) were defined as myocardial infarction, stroke, coronary revascularization, or revascularization of the carotid arteries before baseline.

The definition of "smoker" was restricted to participants who smoked cigarettes. Participants smoking pipes were defined as non-smokers.

The Cockcroft-Gault formula [10] was used to estimate the glomerular filtration rate (GFR).

Statistical models

The characteristics of the participants at baseline are presented descriptively.

Cumulative incidence rates for overall and symptomatic PAD were calculated for the entire follow-up period (seven years and three months). Incidence rates of overall PAD per 1000 person years were determined in total as well as by age and sex.

If values of low-density lipoprotein (LDL) (n=62), body mass index (n=5), glomerular filtration rate (n=64), C-reactive protein (n=64), 25-OH vitamin D (n=77), or homocysteine (n=136) were missing, they were replaced by randomly selected values of the cohort.

To assess the impact of risk factors on PAD incidence, we used univariable as well as multivariable logistic regression. The multivariable model employed 15 parameters:

- age (≤75 versus [vs] >75 years),
- sex
- education (according to the International Standard Classification of Education [ISCED] [11], 0–3: preprimary up to upper secondary education vs 4–6: post-secondary non-tertiary education up to second stage of tertiary education),
- smoker status (never or past vs currently smoking),
- body mass index (<30 vs ≥30 kg/m²),
- diabetes,
- systolic blood pressure (<140 vs ≥140 mm Hg),
- antihypertensive medication,
- statin use,
- LDL level (<3.4 vs ≥3.4 mmol/l),
- pre-existing comorbid cardiovascular conditions,
- 25-OH vitamin D levels (<50 vs ≥50 nmol/l),
- sensitive C-reactive protein (sCRP) (≤3 vs >3 mg/l),
- homocysteine (lower vs. higher than median [14.1 mol/l]), and
- GFR (<60 vs ≥60 ml/min/1,73r).

All p-values are two-sided. A p-value <0.05 was considered statistically significant. Statistical analyses were performed using SAS, version 9.2 (SAS Institute, Cary, North Carolina, USA).

The getABI trial was approved by the institutional review board of the University of Heidelberg; an amendment of this post-hoc analysis was provided by the ethics committee of the University of Bochum. Before entering the trial, each participant provided written informed consent. The study was conducted in accordance with the recommendations of Good Epidemiological Practice.

Results

Prevalence of PAD at baseline

Initially, 6880 participants were enrolled in the study. At baseline, 170 of them had symptoms of PAD (as defined by history of peripheral revascularization, necrosis/gangrene, and/or limb amputation due to PAD). Of the remaining participants, 975 had PAD at baseline as defined by the regression method (Table I). Thus, there were 1145 participants (16.6%) with prevalent PAD at baseline.

The percentage of participants with PAD at baseline increased with age. The baseline characteristics of these participants are shown in Table I, column 3: 37.9% had diabetes, 72.2% systolic blood pressure ≥140 mm Hg, 66.6% used antihypertensive medication, and 27.2% used statins. Vitamin D was below 50 nmol/l in 76.7%, GFR was <60 ml/min in 29.6% of these participants.

At baseline, 5735 participants had no symptomatic or asymptomatic PAD and therefore comprised the cohort for this analysis. The baseline characteristics of this group are shown in Table I, column 2.

Incidence of PAD

During the observation period of a maximum of 7.25 years, 740 out of 5735 (12.9%) participants experienced PAD on-

Table II. Age-specific PAD incidence rates per 1000 person years

Age group (years)	No.	Rate/1000 years (95% CI)
65 – 69	214	15.5 (13.4 to 17.6)
70 – 74	214	17.5 (15.1 to 19.8)
75 – 79	202	28.3 (24.4 to 32.2)
80 – 84	91	32.1 (25.5 to 38.7)
>84	19	38.5 (21.2 to 55.9)

Abbr.: PAD, peripheral artery disease; no., number of participants with incident PAD in a period of seven years, CI, confidence interval

set (incidence rate 20.3, 95% CI 18.8 to 21.7, per 1000 person years), of whom 77 (10.4%) had symptoms (1.3% of the cohort) as defined by peripheral revascularization, necrosis/gangrene, or amputation due to PAD (incidence rate 2.0, 95% CI 1.5 to 2.4, per 1000 person years). An additional 646 participants would have met the traditional definition of PAD with at least one ABI value below 0.90, but were not classified as PAD patients according to the regression-based definition.

The course of the cumulative PAD incidence was fairly linear. There were no major differences in incidence rates per 1000 person years between men (21.3, 95% CI 18.9 to 23.6) and women (19.6, 95% CI 17.8 to 21.5).

Older age was not only a risk factor for prevalent PAD at baseline, but also for the incidence rates in the seven-year follow-up period (Table II).

Differences between age groups were especially apparent during the first four years (Figure 1).

After seven years of follow-up, 878 participants were deceased, yielding a mortality rate of 22.6 (95% CI 21.1 to 24.0) per 1000 person years.

Risk factors

In the univariable analyses, age >75 years (odds ratio 1.62, 95% CI 1.37 to 1.91), current smoker status (2.37, 95% CI 1.88 to 3.00), diabetes (1.45, 95% CI 1.22 to 1.72), antihypertensive medication (1.55, 95% CI 1.33 to 1.81), systolic blood pressure ≥140 mm Hg (1.21, 95% CI 1.03 to 1.42), and CVD comorbidity (1.74, 95% CI 1.43 to 2.12) at baseline were associated with overall PAD incidence. Regarding novel risk factors, 25-OH vitamin D deficiency (1.33, 95% CI 1.12 to 1.58), elevated sCRP (1.39, 95% CI 1.19 to 1.62), elevated homocysteine (1.40, 95% CI 1.20 to 1.63), and impaired re-

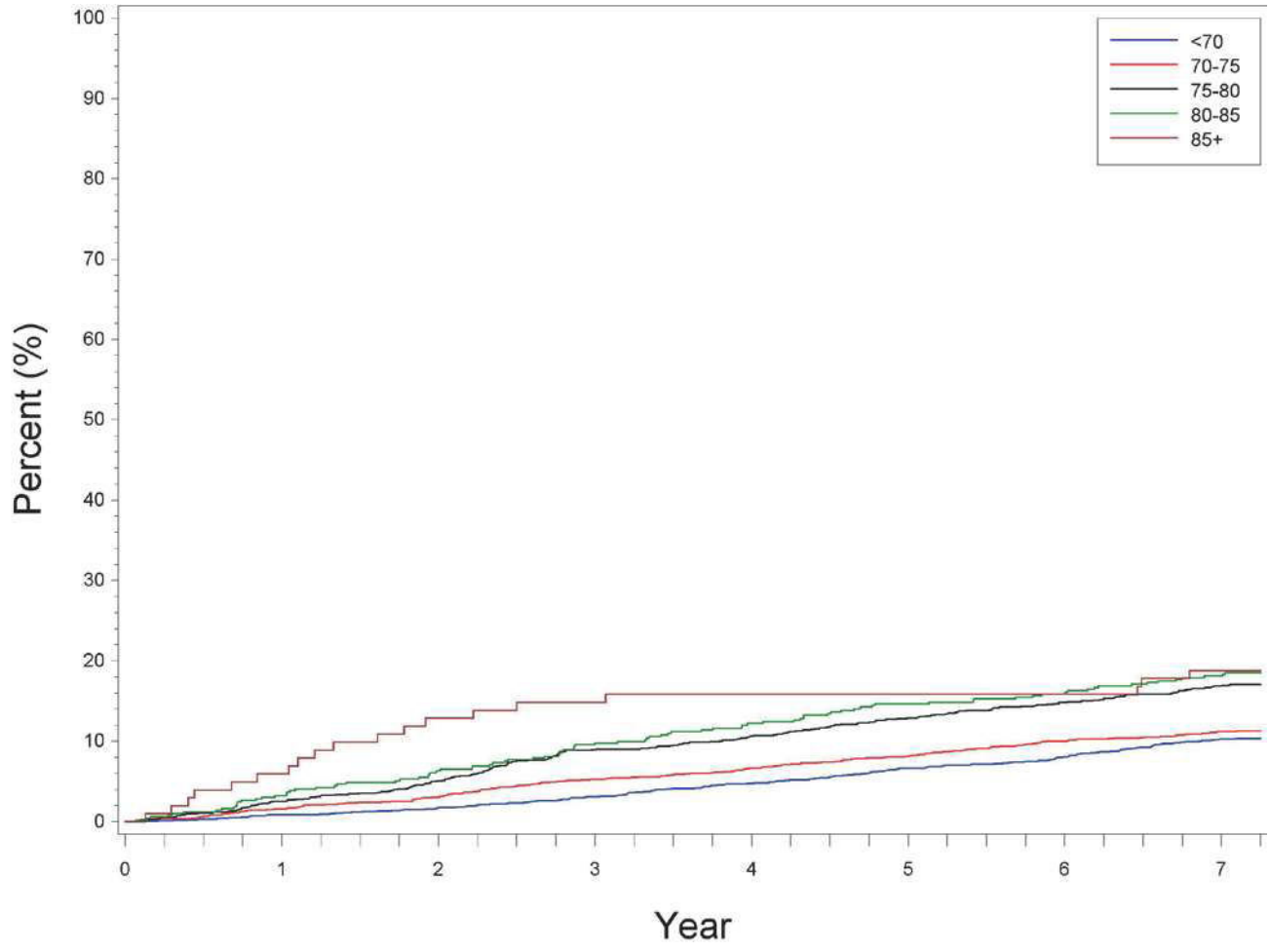

Figure 1. Cumulative incidence of PAD according to age.
Abbr.: PAD, peripheral artery disease

Table III. Risk factors at baseline and seven-year incidence of overall and symptomatic peripheral arterial disease, univariable analyses

Risk factor	Overall PAD (n=740)		Symptomatic PAD (n=77)	
	Odds ratio (95% CI)	Pr > chi square	Odds ratio (95% CI)	Pr > chi square
Male sex	1.04 (0.89 to 1.22)	0.596	2.29 (1.45 to 3.63)	<0.001
Age >75 years	1.62 (1.37 to 1.91)	<0.001	1.02 (0.61 to 1.70)	0.950
Education, ISCED 0-3	1.17 (0.93 to 1.47)	0.190	0.83 (0.45 to 1.51)	0.536
Current smoker	2.37 (1.88 to 3.00)	<0.001	2.04 (1.07 to 3.89)	0.031
BMI ≥30 kg/m²	1.15 (0.96 to 1.37)	0.138	1.05 (0.62 to 1.78)	0.869
Diabetes	1.45 (1.22 to 1.72)	<0.001	2.81 (1.79 to 4.43)	<0.001
Antihypertensive medication	1.55 (1.33 to 1.81)	<0.001	1.59 (1.00 to 2.51)	0.049
Systolic blood pressure ≥140 mm Hg	1.21 (1.03 to 1.42)	0.023	1.12 (0.70 to 1.79)	0.649
Statin use	1.08 (0.89 to 1.31)	0.452	1.72 (1.05 to 2.83)	0.033
LDL ≥3.4 mmol/l	1.16 (0.99 to 1.35)	0.064	0.76 (0.47 to 1.21)	0.243
CVD comorbidity	1.74 (1.43 to 2.12)	<0.001	3.09 (1.91 to 5.02)	<0.001
Vitamin D <50 nmol/l	1.33 (1.12 to 1.58)	0.001	1.34 (0.80 to 2.24)	0.263
sCRP > 3 mg/l	1.39 (1.19 to 1.62)	<0.001	1.51 (0.96 to 2.38)	0.072
Homocysteine ≥ median	1.40 (1.20 to 1.63)	<0.001	1.52 (0.96 to 2.39)	0.075
GFR <60 ml/min/1.73 m²	1.58 (1.31 to 1.89)	<0.001	1.13 (0.64 to 1.99)	0.677

Abbr.: PAD, peripheral artery disease; CI, confidence interval; Pr, probability; ISCED, international standard classification of education; BMI, body mass index; LDL, low-density lipoprotein; CVD, cardiovascular disease; GFR, glomerular filtration rate; sCRP, sensitive C-reactive protein

nal function (1.58, 95% CI 1.31 to 1.89) showed significant associations with overall PAD onset (Table III).

In the multivariable model, age >75 years (1.49, 95% CI 1.23 to 1.80), current smoker status (2.65, 95% CI 2.08 to 3.37), diabetes (1.35, 95% CI 1.13 to 1.58), antihypertensive medication (1.34, 95% CI 1.13 to 1.62), and CVD comorbidity (1.62, 95% CI 1.30 to 2.02), as well as elevated sCRP (1.23, 95% CI 1.05 to 1.45), elevated homocysteine level (1.19, 95% CI 1.01 to 1.41), and impaired renal function (1.27, 95% CI 1.03 to 1.56) at baseline remained significant predictors. LDL ≥3.4 mmol/l was significant only in the multivariable model (1.26, 95% CI 1.07 to 1.48) (Table IV).

Regarding symptomatic PAD, factors male sex (2.29, 95% CI 1.45 to 3.63), current smoker status (2.04, 95% CI 1.07 to 3.89), diabetes (2.81, 95% CI 1.79 to 4.43), antihypertensive medication (1.59, 95% CI 1.00 to 2.51), statin use (1.72, 95% CI 1.05 to 2.83), and CVD comorbidity (3.09, 95% CI 1.91 to 5.02) were significant predictors in the univariable analyses (Table III), whereas male sex (1.86, 95% CI 1.13 to 3.06), current smoker status (2.00, 95% CI 1.03 to 3.88), diabetes mellitus (2.45, 95% CI 1.53 to 3.94), and CVD comorbidity (2.21, 95% CI 1.29 to 3.79) showed a significant association in the multivariable model (Table IV).

Discussion

In this large-scale prospective cohort study with a follow-up of seven years, 740 participants out of 5735 older adults (12.9%) had incident PAD. This corresponds to an incidence rate of about 20.3 (95% CI 18.8 to 21.7) per 1000 person years. Of these participants, 77 (10.4%) had symptoms (1.3% of the whole cohort) as defined by a history of peripheral revascularization, necrosis/gangrene, and/or amputation due to PAD.

Traditional risk factors such as age, current smoker status, diabetes, and elevated LDL level, proved to significantly increase the risk of PAD in this cohort. The same was true for the history of CVD comorbidities at baseline and antihypertensive medication, whereas elevated systolic blood pressure did not predict PAD. This finding may indicate delayed or insufficient antihypertensive treatment in the past leading to vascular damage and thus increasing the risk of PAD. Concerning novel risk factors, a significant contribution to the risk of PAD incidence was found for low vitamin D, elevated sCRP, impaired renal function, and high homocysteine level in the univariable analyses, whereas low vitamin D did not reach significance in the multivariable analysis.

Table IV. Risk factors at baseline and seven-year incidence of overall and symptomatic peripheral arterial disease, multivariable analyses

Risk factor	Overall PAD (n=740)		Symptomatic PAD (n=77)	
	Odds ratio (95% CI)	Pr > Chi-Square	Odds ratio (95% CI)	Pr > Chi-Square
Male	1.01 (0.85 to 1.10)	0.914	1.86 (1.13 to 3.06)	0.015
Age >75 years	1.49 (1.23 to 1.80)	<0.001	0.98 (0.56 to 1.73)	0.947
Education, ISCED 0-3	1.09 (0.86 to 1.39)	0.463	0.87 (0.47 to 1.61)	0.651
Current smoker	2.65 (2.08 to 3.37)	<0.001	2.00 (1.03 to 3.88)	0.041
BMI ≥30 kg/m²	1.10 (0.90 to 1.33)	0.347	0.86 (0.49 to 1.50)	0.582
Diabetes mellitus	1.35 (1.13 to 1.58)	0.001	2.45 (1.53 to 3.94)	<0.001
Antihypertensive medication	1.34 (1.13 to 1.62)	0.001	1.16 (0.71 to 1.90)	0.544
Systolic blood pressure ≥140 mm Hg	1.13 (0.96 to 1.34)	0.154	1.12 (0.69 to 1.81)	0.643
Statin use	0.99 (0.80 to 1.22)	0.922	1.30 (0.75 to 2.26)	0.342
LDL ≥3.4 mmol/l	1.26 (1.07 to 1.48)	0.006	0.98 (0.60 to 1.59)	0.929
CVD comorbidity	1.62 (1.30 to 2.02)	<0.001	2.21 (1.29 to 3.79)	0.004
Vitamin D <50 nmol/l	1.15 (0.96 to 1.38)	0.137	1.42 (0.83 to 2.42)	0.196
sCRP > 3 mg/l	1.23 (1.05 to 1.45)	0.013	1.40 (0.88 to 2.22)	0.159
Homocysteine ≥ median	1.19 (1.01 to 1.41)	0.034	1.31 (0.81 to 2.10)	0.270
GFR <60 ml/min	1.27 (1.03 to 1.56)	0.029	1.01 (0.54 to 1.91)	0.974

Abbreviations: PAD, peripheral artery disease; CI, confidence interval; Pr, probability; ISCED, international standard classification of education; BMI, body mass index; LDL, low-density lipoprotein; CVD, cardiovascular disease; GFR, glomerular filtration rate; sCRP, sensitive C-reactive protein

Strengths and limitations

This study involved one of the largest PAD cohorts in a primary care setting. Compared to population-based cohorts, this setting may provide results that are more likely to be directly applicable to routine care. In order to increase the generalizability of these results, an effort was made to contact GPs and vascular physicians from all parts of Germany. The recruitment of primary care attendees over three pre-specified weeks was intended to reduce selection bias. Nonetheless, the inclusion criteria (age ≥ 65 years) may limit the applicability of results to this age group. Moreover, in the course of the study the number of missing values increased.

Different methods for determining the ankle brachial index may influence the estimate of PAD prevalence and incidence, with a method using the lowest ankle pressure yielding a higher sensitivity for PAD [12–14]. In order to achieve comparability with other studies, we used the method recommended by the American Heart Association [9].

While in cross-sectional studies, PAD assignment is determined by a single ABI value, the availability of several ABI values in longitudinal studies renders the opportunity to assess the individual course. In so doing, a low ABI value that would have led to the diagnosis of PAD in a cross-sectional study may be followed by an ABI value above the cut-off for PAD assignment several years later due to the substantial inter-observer and intra-observer variability of repeated ABI measurements. Therefore, instead of relying on a single measurement, we used the entire course of available data for linear regression. Based on this notion, PAD was defined by the declining linear regression line crossing the 0.9 level (between points with ABI values). Thus, 646 of 1386 participants (47%) with at least one ABI-value below 0.9 did not fulfill our regression definition of PAD. Our definition of PAD may therefore render our results only partially comparable to other studies.

Comparison with other studies

Although the definition of PAD differs among various studies, the reported incidence rates of overall PAD are fairly similar. A prospective study with 2327 subjects from 18 general practice centers in the Netherlands (Limburg PAOD Study) [15] with measurements repeated after 7.2 years found an incidence rate of PAD of 17.8 (95% CI 7.3 to 51.7) for men and 22.9 (95% CI 10.1 to 55.6) for women in the age group ≥65 years. In the Limburg PAOD study, PAD was defined by an ABI of less than 0.95, measured twice at intervals of one week, whereas in our study a defining limit of 0.90 was used.

In a population-based cohort of 468 men from Spain aged 55 to 74 years, measurements could be performed at baseline and after 5 years. Among these subjects, 56 (12%) developed PAD as defined by ABI < 0.9, yielding an annual incidence rate of about 2.4% [16]. Thus, our incidence rate as found by a regression method of single measurements at different time points corresponds to incidence rates from studies with repeated measurements.

In the Oxford Vascular Study, incidence rates of acute vascular events increased with age in all arterial territories, with 147 peripheral vascular events in 12 886 individuals aged 65 years or older (1.14 % in three years) and 105 in 5919 individuals aged 75 years or older (1.78 % in three years), respectively [17]. These incidence rates of peripheral vascular events are about twice as high as our cumulative incidence of 1.3 % in seven years. On the other hand, the Oxford Vascular Study peripheral events also included aortic events and visceral ischemia. Out of 188 participants with peripheral vascular events, 92 had critical limb ischemia. Thus, the incidence of critical limb ischemia was similar to our findings.

Various studies have shown that traditional cardiovascular risk factors (smoking, hypertension, hypercholesterolemia, and diabetes) are important predictors of incident PAD [16, 18]. Among patients with diabetes, this was confirmed in three large trials, the most recent one with 1 921 260 individuals, yielding a hazard ratio (HR) of 2.98 (95 % CI 2.76 to 3.22) for the association of type 2 diabetes and symptomatic incident PAD [19]. A large prospective study of 44 985 men in the United States examined the combined effects of conventional risk factors of PAD (smoking, hypertension, hypercholesterolemia, and diabetes). Each of these risk factors was significantly and independently associated with a higher risk of PAD after adjustment for the other three risk factors. The multivariable-adjusted HR for each additional risk factor was 2.06 (95 % CI 1.88 to 2.26). The population-attributable risk associated with these four risk factors was 75 % [5]. In the Limburg PAOD Study, multivariable analyses showed that older age, smoking, hypertension, and diabetes were the most important risk factors [15]. This is closest to our results that yielded a significant increase in PAD risk for age, smoking, diabetes, antihypertensive treatment, the history of CVD comorbidities at baseline, and hypercholesterolemia (not significant in the univariate analysis).

The D.E.S.I.R. study reported on 3591 participants, 2139 of whom had a healthy weight and 1453 were overweight or obese. Overweight or obesity did not increase the risk of developing peripheral arterial disease [20]. This is in accordance with our study that found no impact of obesity on the incidence of PAD.

In the Edinburgh Artery Study, there was no significant association between metabolic syndrome and incident symptomatic PAD [21]. However, this association was shown by a larger cohort from the Women's Health Study (HR 1.48, 95 % CI 1.01 to 2.18) [22]. Recently, this association was confirmed in the Cardiovascular Health Study for both symptomatic (HR 1.47, 95 % CI 1.11 to 1.94) and asymptomatic PAD (risk ratio 1.26, 95 % CI 1.00 to 1.58) [23].

Novel risk factors have been discovered in recent years. In a cross-sectional study of 2174 participants aged 40 years or older from the 1999–2000 National Health and Nutrition Examination Survey, traditional risk factors of PAD, as well as low kidney function, elevated fibrinogen, and C-reactive protein levels were associated with PAD [24]. In a nested case-control study using plasma samples from initially healthy American male physicians aged 40 to 84 years with a nine-year follow-up period, eleven atherothrombotic biomarkers were assessed at baseline. The total cholesterol-HDL-C ratio and CRP were the strongest independent predictors of development of peripheral arterial disease. CRP provided additive prognostic information over standard lipid measures [25]. Similar results were found in a cohort in San Diego, California, [26] and the EPIC-Norfolk cohort [27]. In a nested matched case-control study using data from the Nurses' Health Study (NHS, 1990–2010) and the Health Professionals Follow-up Study (HPFS, 1986–2010), higher homocysteine levels were positively associated with risk of PAD in men [28]. Recently, vitamin D deficiency has been considered an independent risk factor for the development of PAD [29].

In our cohort, the four novel risk factors selected (vitamin D deficiency, elevated sCRP, high homocysteine levels, and impaired renal function) showed significant associations with incident overall PAD in the univariate model. In the multivariate model, vitamin D deficiency was no longer a significant predictor (in contrast to other observations [6, 29]), but elevated sCRP, high homocysteine levels, and impaired renal function remained significant.

Unanswered questions and future research

Apart from traditional risk factors (smoking, hypertension, hypercholesterolemia, diabetes, history of cardiovascular diseases), this study investigated novel risk factors. We found significant associations between the incidence of PAD and elevated sensitive CRP levels, impaired renal function, as well as high homocysteine levels in the univariate and multivariate models. The association of PAD incidence with vitamin D deficiency was significant only in the univariate analysis. Future research may confirm this association of novel risk factors with incident PAD, clarify the role for vitamin D deficiency, and identify novel risk factor candidates which will facilitate the identification of patients at risk for PAD. The assessment of therapeutic measures against these risk factors, as recently shown for the effect of smoking cessation on cardiovascular disease in general and PAD in particular [30], will be of paramount importance.

Conclusions

The results of this large PAD cohort study in a primary care setting from GPs all over Germany indicate that one-point PAD assessment by one ABI value below 0.9 may lead to biased PAD incidence rates. To adjust these figures, it is helpful to repeat measurements.

Novel risk factors such as elevated C-reactive protein, impaired renal function, and elevated homocysteine levels may be helpful for identifying patients at risk of PAD.

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Full Length Article

Bisphosphonate drug holidays: Risk of fractures and mortality in a prospective cohort study

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ABSTRACT

Purpose: This study examined osteoporotic fractures and mortality in patients pretreated with bisphosphonates (BPs) during BP holidays and ongoing BP use.

Methods: Interview-based prospective observational study in a cohort of 1973 patients with BP treatment for at least 80% of the total time of the preceding 4 years. Patients were recruited from 146 primarily endocrinological, orthopedic and rheumatological practices and clinics across Germany between May 2013 and June 2015. Outcomes were analyzed by Cox proportional hazards regression in relation to treatment status at the time of the first interview (model 1) or using time-dependent treatment variables (model 2). Temporal changes in fracture risk during BP holidays were evaluated by comparisons among 3 incremental levels of simple moving averages of BP treatment during the preceding 12 months (BP-SMA levels 0%, > 0% to < 50%, and ≥50%).

Results: For an observation period of up to 25 months, the adjusted hazard ratios (HRs) in model 1 for BP holidays compared to ongoing BP use were 0.87 (95% confidence interval [CI] 0.59–1.28) for major osteoporotic fractures (MOFs), 0.95 (95% CI 0.70–1.28) for any clinical osteoporotic fracture, 0.96 (95% CI 0.55–1.68) for clinical vertebral fractures, and 0.86 (95% CI 0.50–1.48) for mortality. The risk of MOFs was higher for the BP-SMA level 0%, corresponding to a time > 12 months since the start of a BP holiday, than for the BP-SMA level > 0% to < 50%, corresponding mainly to a time > 6 to ≤12 months since the start of a BP holiday (adjusted HR 2.28, 95% CI 1.07–4.86). We found an interaction between prevalent vertebral fractures (PVFs) and BP-SMA-related time to first MOF for BP-SMA as a continuous variable (p for interaction 0.046 in the adjusted model). The adjusted HR for MOFs for the BP-SMA level 0% compared to the BP-SMA level > 0% to < 50% was 3.53 (95% CI 1.19–10.51) with a PVF but was 1.44 (95% CI 0.49–4.22) without a PVF.

Conclusions: Fracture risk and mortality in patients with preceding BP treatment did not significantly differ between BP holidays and ongoing BP use for an observation period up to 25 months when outcomes were analyzed in relation to treatment at the time of the first interview. However, in the presence of a PVF, the risk of MOFs was higher for a BP-SMA level corresponding to a time > 12 months since the start of a BP holiday than for a BP-SMA level corresponding mainly to a time > 6 to ≤12 months since the start of a BP holiday. The presence of a PVF may increase the relative risk of MOFs associated with a longer BP holiday.

1. Introduction

The efficacy of bisphosphonates (BPs) for reducing osteoporotic fracture risk has been established in large randomized controlled trials [1,2]. Long-term BP treatment is, however, associated with an increase in rare, but serious adverse effects, such as atypical femoral fractures (AFFs) and osteonecrosis of the jaw (ONJ) [3–11]. Because BPs have residual effects on bone metabolism after discontinuation [12–14], it has been suggested that for some patients with osteoporosis who have been treated with oral BPs for 5 years or with zoledronate for 3 years, a BP holiday with periodic reassessment may be considered to minimize adverse events while potentially maintaining some skeletal benefits [15,16]. However, the current evidence regarding the risks and benefits of BP holidays is limited [6,13,14,17–22]. To expand our knowledge of BP holidays, we prospectively compared fracture risk in patients with preceding BP treatment between BP holidays and ongoing BP use and

Abbreviations: AFF, atypical femoral fracture; BMD, bone mineral density; BP, bisphosphonate; BP-SMA, simple moving average of bisphosphonate treatment during the preceding 12 months; CCI, Charlson comorbidity index; DVO, Dachverband Osteologie; DXA, dual X-ray absorptiometry; IQR, interquartile range; IV, interview; SMA, simple moving average; MOF, major osteoporotic fracture; ONJ, osteonecrosis of the jaw; PVF, prevalent vertebral fracture; TH, total hip

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analyzed the fracture risk in relation to BP holiday length. Because BPs have been associated with reduced mortality [23–26], we also compared mortality between BP holidays and ongoing BP treatment.

2. Methods

2.1. Study population

Included in the analysis were men aged ≥59 years and postmenopausal women, all of whom had been treated with BPs over a period of time of at least 4 years and for ≥80% of the total time of the preceding 4 years at doses and dosing intervals approved for the treatment of osteoporosis. Further inclusion criteria were either a dual X-ray absorptiometry (DXA) T-score of ≤ −2.0 at the lumbar spine, femoral neck, or total hip (TH) before or during BP treatment, or one moderate or severe low-trauma prevalent vertebral fracture (PVF) or multiple low-trauma PVFs, regardless of bone mineral density (BMD).

Patients were recruited from primarily endocrinological, orthopedic and rheumatological practices and clinics across Germany. In order to target physicians likely to frequently encounter patients on long-term BP treatment, we send a letter to all physicians in Germany certified as "Osteologe Dachverband Osteologie (DVO)", and to all orthopedics, endocrinologists, and rheumatologists in Germany, whose addresses were available, inviting them to participate in the recruitment of eligible patients seen in these practices and clinics. Invitations were also sent by email to the members of the German Professional Association for Orthopaedics and Trauma Surgery, the German Society of Endocrinology, and the German Society of Rheumatology. Two hundred and fifty-two of the contacted physicians agreed to participate. Physicians from 146 practices and clinics contributed to the inclusion of patients between May 2013 and June 2015. We excluded patients with prevalent bone metastases, those who had used osteoporosis pharmacotherapies other than BPs in the previous 4 years or who intended to use them, and patients for whom the time since the beginning of a continuous BP holiday would have exceeded 6 months at the beginning of the observation.

Baseline information on fracture risks and comorbidity was obtained from the recruiting physicians and the patients either at the beginning of the observation or, for some of the patients, based on data from the 1–2 years prior to the start of the observation period. Eighty-two patients had participated earlier in a randomized controlled trial that compared the effect of alendronate or a BP holiday on fracture risk in patients with preceding BP treatment, and that was early terminated because of a low recruitment rate (EudraCT Number: 2011-000290-31). For 15 of these patients, baseline information was based on data from the previous study. In another 64 patients with insufficient BP pretreatment at the time of the initial recruitment, inclusion in the observation was delayed > 1 month until the required pretreatment was achieved. For these patients, baseline information was based on the assessment at the time of the initial recruitment. Follow-up information was obtained from the patients by structured telephone interviews (IV1 to IV5) approximately 3, 6, 12, 18 and 24 months after recruitment. The telephone IVs were conducted centrally by trained staff of a research call center located at the Department of Medical Informatics, Biometry and Epidemiology of the Ruhr University Bochum. From September 2014 onwards, we alternatively offered mailed questionnaires (also termed IVs in the following analysis) to patients who were not able to or did not want to participate in telephone IVs. Patients were followed with regard to fractures and ONJ as outcomes until 'recent death' (defined as death either within the first 6 months of the observation without an IV or within 6 months after the last conducted IV, except for IV5), the last conducted IV (except for patients with 'recent death'), or 25 months of continuous observation, whichever came first. With regard to death as outcome, patients were followed until the date of the last known vital status or for 25 months, whichever came first. The vital status of participants who could no longer be reached during follow-up was primarily assessed through primary care physicians and registration offices.

The study was approved by the Ethics Committee of the Faculty of Medicine at the Ruhr University Bochum. All participants provided written informed consent. The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

2.2. Outcomes

The outcomes of interest in this analysis were incident major osteoporotic fractures (MOFs), any clinical osteoporotic fracture, clinical osteoporotic vertebral fractures, AFFs, ONJ and death.

2.3. Assessment of osteoporosis medication and BP holidays

Information on osteoporosis treatment prior to and at the time of recruitment was obtained from the recruiting physicians. Follow-up information on current treatment was obtained from patient self-reports at each IV. We attempted to obtain further information from the treating physicians if the patients were uncertain about their current medication. Missing dates of changes in treatment status between the start of the observation and IV1 or between one IV and the next IV were defined as the midpoint of the respective time intervals. In patients with 'recent death', the latest self- or physician-reported treatment status was carried forward until death. Throughout the analysis, a BP holiday was defined as any period of time without osteoporosis pharmacotherapy beyond the completion of the last dosing interval of a preceding BP treatment. Cumulative BP exposure was determined by summing all periods of previous BP treatment, excluding times of intermittent BP holidays.

2.4. Assessment of fracture risks and comorbidity

We asked the recruiting physicians for information on risks factors recommended for fracture risk assessment in the 2009 DVO guideline on the prevention, diagnosis, and therapy of osteoporosis [27]. Persons were defined as immobile in the guideline if their mobility was limited to such an extent that they were, for example, confined to their home, unable to perform housework, or have a maximal walking distance of < 100 m. Information on alcohol use and secondary osteoporosis was additionally assessed as defined in FRAX© [28]. In the patient questionnaire, patients were asked to answer the question "Has a doctor ever diagnosed one of the following diseases?". Information was obtained on hypertension, coronary heart disease, myocardial infarction, heart failure, peripheral artery disease, stroke, chronic obstructive airways disease, liver disease, kidney disease or renal failure, gastrointestinal hemorrhage, inflammatory bowel disease, thyroid dysfunction, impaired balance, Parkinson's disease, multiple sclerosis, diabetes mellitus, anemia, joint disease, visual impairment, hearing impairment, depression, confusion, and cancer. Comorbidity was estimated by means of a modified Charlson comorbidity index (CCI) without age adjustment [29], and the information was based mostly on the self-reported questionnaire. Information for the index was not available on AIDS and (with very few exceptions) on disease severity, metastasis (other than bone) and hemiplegia. With one exception, a history of gastrointestinal bleeding was included in the index instead of a gastrointestinal ulcer. A history of a solid tumor was only assigned a weight of 1.

2.5. Assessment of fractures

Incident fractures were recorded at each interview based on self-report. Patients were asked to provide the names of the hospitals or physicians involved in the diagnosis or treatment of incident fractures. In cases of uncertainty, the patients' general practitioners and/or the

physicians involved in osteoporosis care were asked for further information about the fractures. Incident fractures were considered confirmed if diagnosed as new fractures in medical records from these hospitals or physicians. Three new osteoporotic fractures were confirmed by telephone calls to the involved physicians. Only confirmed fractures were included in the analysis. Fractures not identified through the interviews but documented in medical reports (for example in patients who had died shortly after a fracture) were also included in the analysis depending on the model specifications described below. Information on the circumstances of the fractures obtained from the patient interviews and from the medical records was included in the assessment of the trauma level. High-impact fractures, fractures of the skull, face, fingers, toes, and AFFs were not regarded as osteoporotic. Osteoporotic fractures of the hip, spine, proximal humerus or forearm were classified as MOFs. For the majority of incident fractures, information was available on the date of their likely occurrence (for example, a fall or sudden new or aggravated back pain). Otherwise, the date of diagnostic imaging was used as the fracture date.

2.6. Assessment of AFFs and ONJ

AFFs were classified according to the criteria of the ASBMR Task Force [10]. Radiographs of all incident fractures in the subtrochanteric region or diaphysis of the femur were reviewed by a radiologist from the Ruhr University Bochum. Information on ONJ was obtained through the interviews. If patients reported impaired wound healing in the jaw area for > 4 weeks, reported the persistence of denture pressure points for > 4 weeks or provided other information that might indicate the presence of ONJ, we attempted to obtain further information from the treating dentists, oral surgeons, or other physicians.

2.7. Statistical methods

Differences in baseline characteristics between the patients using BPs at the time of IV1 and those on a BP holiday at the time of IV1 were evaluated with the use of the chi-square test for categorical variables and *t*-tests or the Wilcoxon rank sum test for continuous and ordinally scaled variables. Time to first fracture and time to death were analyzed by means of Cox proportional hazards regression. Fractures were analyzed accounting for death as a competing risk [30]. Only osteoporotic fractures were included as outcomes in the fracture analyses. Outcomes were analyzed either in relation to treatment status at the time of IV1 (model 1) or using time-dependent treatment variables (model 2). Unless mentioned otherwise, patients with 'recent death' without an interview are included in all statements and analyses related to treatment at the time of IV1 using their last recorded treatment prior to death for analysis. In model 2a, outcomes were analyzed in relation to current treatment assessed as a time-dependent categorical variable. In model 2b, time to first fracture was additionally analyzed in relation to the current percentage of time on BP treatment during a 12-month retrospective period, calculated as simple moving average (SMA). An SMA is often used in economics to calculate mean data values during a rolling, finite period, but this technique has also been applied to similar analyses in medical research [31]. SMAs calculated as described above (BP-SMAs) were categorized into 3 levels ($\geq$50%, > 0% to < 50%, and 0%). Since not all shorter interruptions of treatment are likely to be "true" BP holidays, we designed the BP-SMA levels in a way that the first level corresponded to times of ongoing treatment and the first up to 6 months following discontinuation of BP treatment, whereas the second (with few exceptions) and third level corresponded to times > 6 to $\leq$12 months and > 12 months since the start of a BP holiday, respectively. This allowed a comparison of fracture risk between earlier and later times since the start of a BP holiday while limiting the interference by the more ambiguous short treatment breaks. The comparison of fracture risk between the latter two levels is also less likely to suffer from a potential selection bias than the comparison between BP holidays and ongoing BP treatment. Since there are no established thresholds for temporal changes in fracture risk with time since the beginning of a BP holiday, and since the median length of the first BP holiday longer than 6 months by the end of the observation was 1.71 years, the threshold between the second and third BP-SMA level was set to correspond to 12 months since the beginning of a BP holiday.

Except for patients with 'recent death' without an interview, analyses were restricted in the above models to patients with one or more interviews. Patients whose osteoporosis treatment at the time of IV1 was either unknown or who received osteoporosis pharmacotherapy other than BPs at the time of IV1 were excluded from model 1. Observations were censored in the fracture models at death, 25 months, or the date of the last conducted interview except for patients with 'recent death', whichever came first. Observations in model 2 were additionally censored at the time of a switch to osteoporosis pharmacotherapies other than BPs, the resumption of BP use after a BP holiday exceeding 6 months and, in the case of BP-SMA analyses, also upon transition to an unknown treatment status. In model 1, first incident particular fractures in patients who had died during the observation period were included in the analyses independent of the date of death, if they had occurred within the first 6 months of the observation in patients without interview or within 6 months after the last conducted interview other than IV5. For mortality as the endpoint, observations were censored in model 1 at the date of the last recorded vital status, in model 2 at the date of the last conducted interview except for patients with 'recent death', or at 25 months, whichever came first.

Individual estimates of TH T-scores for 475 patients for whom measurements at this skeletal site were either missing or dated back > 2 years were imputed using a linear regression model constructed by the stepwise selection of predictors of the TH T-score based on 1498 available other measurements (SAS procedure GLMSELECT). Clinical fracture risks and comorbidities were assumed to be absent if missing or reported as unknown. Predictors supposed to nonlinearly affect outcomes were either logarithmically transformed (number of PVFs) or trimmed (modified CCI) before being modeled as continuous variables. Sex was pre-specified as covariate. Baseline variables with a prevalence in the study population of > 5%, and which had been shown to be associated with fracture risk and/or mortality in previous studies, were selected as additional covariates based on their respective ranking in explaining MOF risk or mortality in univariate analysis. In the mortality models, the modified CCI was used for adjustment instead of individual impairments and comorbidities. We limited the number of covariates in the fracture models to 8 and in the mortality models to 5 to avoid overfitting. All covariates were checked for collinearity. Unless mentioned otherwise, models with fractures as endpoint were adjusted for sex, TH T-score, either a history of PVF (SMA models) or the number of PVFs (all other models), the use of oral glucocorticoids for $\geq$3 months, a self-reported history of depression, immobility, $\geq$2 falls within the last 12 months, and a self-reported history of chronic obstructive airways disease. The modifying effect of a PVF on BP-SMA-related fracture risk was examined through an interaction term in the Cox regression analysis. The modifying effect of cumulative BP exposure at baseline (dichotomized at the median) on BP-SMA-related MOF risk was analyzed in an analogous manner. We additionally examined interaction terms of the median-dichotomized cumulative BP exposure at baseline and the treatment variables in model 1 and 2a with respect to MOF risk. Models with death as endpoint were adjusted for sex, age, modified CCI, current smoking and TH T-score. Regression analyses were performed with SAS, version 9.4 (SAS Institute, Cary, NC). A two-sided p-value < 0.05 or a hazard ratio (HR) with a 95% confidence interval (CI) excluding 1 were considered significant.

2.8. Sensitivity analyses

As a sensitivity analysis, we compared fracture risk between BP-SMA levels without 50 patients for whom treatment during the

Table 1
Frequencies of ever and exclusive use of different BP treatment regimens prior to the observation.

BP type, dose, route of administration, dosing interval	Patients with ever use		Patients with exclusive use	
	n	%	n	%
Alendronate 10 mg oral once daily	7	0.35	0	0.00
Alendronate 70 mg oral once weekly	1207	61.18	801	40.60
Ibandronate 150 mg oral once monthly	111	5.63	56	2.84
Ibandronate 3 mg intravenous every 3 months	385	19.51	149	7.55
Risedronate 5 mg oral once daily	11	0.56	0	0.00
Risedronate 35 mg oral once weekly	645	32.69	345	17.49
Risedronate 75 mg oral on 2 consecutive days each month	13	0.66	7	0.35
Zoledronate 5 mg intravenous once yearly	232	11.76	78	3.95

Absolute number (n) and percentage (%) of all participants who had ever or exclusively used one of the respective BP treatment regimens before the start of the observation.

observation could not be completely ascertained.

3. Results

3.1. Cohort characteristics

A total of 1973 patients were included in the analysis. Of these, 80 patients died during the observation period. In another 266 patients, observation with regard to fractures or both fractures and death ended prematurely because of loss to follow-up (n = 95) or withdrawal (n = 171). One or more interviews were missing in another 50 patients. Patients were observed for incident fractures and ONJ as outcomes for a median of 23.95 months (interquartile range [IQR] 23.85–24.05 months), for a total of 3438.40 person-years. The median cumulative BP exposure at the beginning of the observation was 5.17 years (IQR 4.40–6.59 years) for the full cohort and 5.16 years for the patients included in model 1, 2a and 2b. Alendronate 70 mg once weekly was the most commonly used BP treatment prior to the observation period (Table 1). Baseline characteristics are summarized in Table 2. Patients using BPs at the time of IV1 had higher baseline frequencies of nonvertebral fractures after the age of 50 and PVFs, a lower TH DXA T-score, and a higher mean modified CCI compared to the patients on a BP holiday at the time of IV1.

The median cumulative BP exposure at the end of the observation period was 5.50 years (IQR 4.75–6.91 years) for the patients on a BP holiday at the time of IV1 and 6.74 years (IQR 5.95–8.16 years) for the patients using BPs at the time of IV1. During a period of time between 6 months before the start of the observation and the end of the observation, 884 patients had taken a BP holiday for longer than 6 months. The median length of the first BP holiday longer than 6 months during this period (including the first 6 months) was 1.71 years (IQR 1.03–2.02 years). In model 2a, 50 BP holidays ended within 6 months after the beginning either by resumption of BP treatment (n = 37) or initiation of treatment with a different osteoporosis medication (n = 13), and 113 BP holidays were either censored within 6 months after the beginning by the end of the observation (n = 112) or were of unknown duration due to incomplete follow-up information (n = 1).

The following numbers refer to treatment at the time of IV1 and the first treatment changes during the observation period following IV1 and do not include patients with 'recent death' without an interview: at the time of IV1, 482 patients were treated with alendronate 70 mg once weekly, 246 patients with intravenous ibandronate 3 mg every 3 months, 56 patients with oral ibandronate 150 mg once monthly, 277 patients with risedronate 35 mg once weekly, 9 patients with risedronate 75 mg on 2 consecutive days each month, and 173 patients with zoledronate 5 mg intravenously once yearly. Of these 1243 patients using BPs at the time of IV1, 432 subsequently took a BP holiday either transiently or until the end of follow-up. Another 55 patients switched to an alternative osteoporosis treatment. Among the 601 patients on a BP holiday at the time of IV1, 99 subsequently resumed BP treatment, and another 41 patients started treatment with a different osteoporosis medication. For 20 of the patients on a BP holiday at the time of IV1 who resumed BP treatment and for 3 of the patients on a BP holiday at the time of IV1 who started treatment with a different osteoporosis medication, the duration of the BP holiday was 6 months or less.

3.2. Association between treatment and time to first fracture

Except for the hazard rates of MOFs in the subgroup of patients with a PVF, the unadjusted hazard rates of MOFs, any clinical osteoporotic fracture and clinical vertebral fractures were (not significantly) lower for BP holidays compared to ongoing BP use, with outcomes analyzed in relation to treatment at the time of IV1 (Table 3 and Fig. 1). Relative differences in hazard rates between BP holidays and BP use in the unstratified analyses were attenuated after adjustment for fracture risks. The results for the unstratified analysis tended to be similar with outcomes analyzed in relation to current treatment assessed as a time-dependent categorical variable (Table 4). The HR for MOFs per year of increase in cumulative BP exposure at baseline for the patients included in model 1 was 1.03 (95% CI 0.93–1.13) with adjustment for treatment at the time of IV1 and 1.00 (95% CI 0.90–1.11) with additional adjustment for baseline fracture risks. No interaction was observed for time to first MOF between the median-dichotomized cumulative BP exposure at baseline and treatment at the time of IV1 or current treatment (p for interaction 0.98 and 0.99 in the adjusted models, respectively).

3.3. Association between BP holiday length and time to first fracture

Fracture risk related to BP holiday length was compared among 3 incremental BP-SMA levels. The BP-SMA level 0% corresponded to a time > 12 months since the start of a BP holiday, the BP-SMA level ≥50% corresponded to current BP use or a time ≤6 months since the start of a BP holiday, whereas the BP-SMA level > 0% to < 50% largely corresponded to a time > 6 to ≤12 months since the start of a BP holiday with the exception of 27 patients in whom a short BP holiday either during the observation or the preceding 12 months was succeeded within 6 months or less by a second BP holiday (n = 24) or in whom pauses in BP treatment exceeded 6 months in the year prior to the observation (n = 3).

The HR for MOFs for the BP-SMA level > 0% to < 50% compared to the BP-SMA level ≥50% was 0.62 (95% CI 0.31–1.24) in univariable analysis and 0.67 (95% CI 0.34–1.35) after adjustment (Table 5). Conversely, the HR for MOFs for the BP-SMA level 0% compared to the BP-SMA level > 0% to < 50% was 2.20 (95% CI 1.03–4.68) in univariable analysis and 2.28 (95% CI 1.07–4.86) after adjustment. A similar pattern of (nonsignificant) relative differences in adjusted fracture risk across the 3 BP-SMA levels was observed for incident clinical

Table 2
Baseline characteristics of the full cohort and of the patients using BPs or on a BP holiday at the time of IV1.

Variable	Full cohort (n = 1973)	Treatment at the time of IV1	
		BP use[a] (n = 1250)	BP holiday (n = 601)
Female (%)	96.60 (1906/1973)	96.00 (1200/1250)	97.50 (586/601)
Age, years (mean, SD)	75.69, 7.33 (1973)	75.65, 7.27 (1250)	74.95, 7.24 (601)
Height, cm (mean, SD)	158.99, 7.11 (1972)	158.96, 7.09 (1250)	159.58, 6.87 (600)
Weight, kg (mean, SD)	66.39, 11.85 (1971)	66.42, 12.03 (1250)	66.71, 11.63 (600)
Current smoking (%)	5.47 (108/1973)	5.76 (72/1250)	5.66 (34/601)
Nonvertebral fracture(s) after age 50 years (%)	41.41 (817/1973)	44.08 (551/1250)	36.61 (220/601)*
PVF(s) (%)	39.48 (779/1973)	41.20 (515/1250)	32.45 (195/601)*
TH DXA T-score (mean, SD)[b]	−1.74, 0.86 (1498)	−1.78, 0.85 (931)	−1.64, 0.86 (481)*
Parental hip fracture (%)	12.06 (238/1973)	11.76 (147/1250)	14.48 (87/601)
Immobility (%)[c]	8.92 (176/1973)	8.48 (106/1250)	7.82 (47/601)
Two or more falls in the previous 12 months (%)	14.09 (278/1973)	14.40 (180/1250)	12.81 (77/601)
Use of drugs associated with increased fall risk (%)	11.25 (222/1973)	11.28 (141/1250)	10.65 (64/601)
Use of oral glucocorticoids for ≥3 months (%)	12.16 (240/1973)	13.20 (165/1250)	10.98 (66/601)
Rheumatoid arthritis (%)	12.01 (237/1973)	12.88 (161/1250)	10.98 (66/601)
Antihormonal therapy (%)	3.55 (70/1973)	3.76 (47/1250)	3.00 (18/601)
Epilepsy or anti-epileptic drugs (%)	1.12 (22/1973)	1.60 (20/1250)	0.17 (1/601)*
Hypertension (%)[d]	58.95 (1163/1973)	60.00 (750/1250)	55.57 (334/601)
Diabetes mellitus (%)[d]	9.98 (197/1973)	10.40 (130/1250)	8.65 (52/601)
Coronary heart disease (%)[d]	11.66 (230/1973)	11.52 (144/1250)	10.82 (65/601)
Myocardial infarction (%)[d]	3.80 (75/1973)	3.84 (48/1250)	2.83 (17/601)
Heart failure (%)[d]	15.10 (298/1973)	15.84 (198/1250)	14.14 (85/601)
Peripheral artery disease (%)[d]	8.72 (172/1973)	8.72 (109/1250)	8.82 (53/601)
Stroke (%)[d]	6.13 (121/1973)	5.92 (74/1250)	5.16 (31/601)
Chronic obstructive airways disease (%)[d]	13.08 (258/1973)	13.92 (174/1250)	12.31 (74/601)
Impaired balance (%)[d]	22.55 (445/1973)	21.76 (272/1250)	22.80 (137/601)
Depression (%)[d]	8.41 (166/1973)	8.00 (100/1250)	8.32 (50/601)
Cancer (%)[d]	13.53 (267/1973)	13.60 (170/1250)	13.81 (83/601)
Modified CCI (mean, median, IQR)	0.93, 1, 0–1 (1973)	0.97, 1, 0–2 (1250)	0.85, 1, 0–1 (601)*
Cumulative BP exposure at the start of the observation, years (median, IQR)	5.17, 4.40–6.59 (1973)	5.11, 4.29–6.63 (1250)	5.25, 4.55–6.50 (601)

Data are presented as percentages (%) for categorical data, and as mean and standard deviation (SD) or as the median and IQR for continuous and ordinally scaled variables, respectively. The numbers in parenthesis show the respective absolute numbers and/or total number of patients.

[a] Including patients without an interview who had died during the first 6 months of the observation with BP treatment as their last known medication.

[b] Values refer only to measurements performed between 24 months before and 7 weeks after the start of the observation.

[c] See definition of immobility in Section 2.4.

[d] Self-reported data based on the question: 'has a doctor ever diagnosed one of the following diseases?'

* $p < 0.05$ compared to BP use at the time of IV1.

vertebral fractures. In contrast, no major difference in adjusted fracture risk was observed among the 3 BP-SMA levels for any clinical osteoporotic fracture as the outcome. Excluding patients with incomplete ascertainment of treatment during the observation from the SMA-analyses had no major effect on these estimates (results not shown).

We found an interaction between PVFs and BP-SMA-related time to first MOF for BP-SMA as a continuous variable (p for interaction 0.046 in the adjusted model). The adjusted HR for MOFs for the BP-SMA level 0% compared to the BP-SMA level > 0% to < 50% was 1.44 (95% CI 0.49–4.22) without a PVF but was 3.53 (95% CI 1.19–10.51) with a PVF. Similarly, the adjusted HR for MOFs for the BP-SMA level 0% compared to the BP-SMA level ≥50% was 0.93 (95% CI 0.46–1.88) without a PVF but was 2.42 (95% CI 1.30–4.51) with a PVF (Table 5). The pattern of modification of the relative differences in the adjusted

Table 3
Fracture incidences and HRs for fractures for patients on a BP holiday at the time of IV1 compared to patients using BPs at the time of IV1.[a]

Fracture type	Subgroup	Treatment	Number of patients	Number of first incident fractures	Person-years (PJ)	Incidence rate/1000 PJ	HR[b] (95% CI) Univariable	HR (95% CI) Adjusted[c]
MOFs	–	BP holiday	601	36	1091.8	32.97	0.78	0.87
		BP use	1250	93	2191.2	42.44	(0.53–1.15)	(0.59–1.28)
	With a PVF	BP holiday	195	20	339.2	58.96	1.06	1.20
		BP use	515	49	869.7	56.34	(0.63–1.78)	(0.71–2.01)
	Without a PVF	BP holiday	406	16	752.6	21.26	0.64	0.65
		BP use	735	44	1321.5	33.30	(0.36–1.13)	(0.37–1.14)
Any clinical osteoporotic fracture	–	BP holiday	601	61	1062.4	57.42	0.87	0.95
		BP use	1250	143	2142.4	66.75	(0.64–1.17)	(0.70–1.28)
Clinical vertebral fractures	–	BP holiday	601	18	1105.6	16.28	0.80	0.96
		BP use	1250	46	2240.0	20.54	(0.46–1.38)	(0.55–1.68)

[a] Patients without an interview who had died during the first 6 months of the observation were included in the analysis according to their last known treatment.

[b] HR for BP holiday compared to BP use.

[c] Adjustments were performed as described in the methods section with the exception of no adjustment for the number of PVFs in the subgroup analyses of the time to first MOF. Furthermore, no adjustment was made for sex in the analysis of the time to first MOF for the subgroup of patients without a PVF and in the vertebral fracture model, because of the lack of particular fracture outcomes in men in these models.

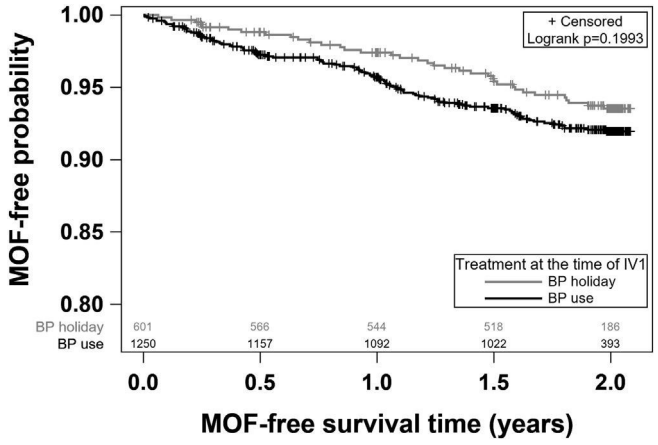

Fig. 1. Kaplan-Meier plot expressed as the probability of remaining free of MOFs among the patients with BP use at the time of IV1 (black line) or on a BP holiday at the time of IV1 (grey line). Patients without IVs who had died during the first 6 months of the observation were included in the analysis according to their last known treatment.

fracture risk among the BP-SMA levels by PVFs tended to be similar to that of MOFs for any clinical osteoporotic fracture (p for interaction 0.24 in the adjusted model with BP-SMA as a continuous variable). The interaction analysis with respect to clinical vertebral fractures was limited by the small number of incident clinical vertebral fracture events, and the results should therefore be viewed with caution. No interaction was observed for time to first MOF between the median-dichotomized cumulative BP exposure at baseline and BP-SMA as a continuous variable (p for interaction 0.47 in the adjusted model).

3.4. Association between treatment and mortality

The numbers of death events analyzed in the mortality models related to treatment status at the time of IV1 and as a time-dependent variable were 71 and 53, respectively. The HR for mortality for BP holidays compared to ongoing BP use with deaths analyzed in relation to the treatment status at the time of IV1 was 0.69 (95% CI 0.41–1.18) in univariable analysis and 0.86 (95% CI 0.50–1.48) after multiple adjustments. With deaths analyzed in relation to treatment as a time-dependent categorical variable, the HR for mortality for current BP holidays compared to current BP use was 0.70 (95% CI 0.40–1.23) in univariable analysis and 0.77 (95% CI 0.44–1.34) after adjustment. Mortality in the patients included in model 1, adjusted for treatment at the time of IV1, was positively associated with the cumulative BP exposure at baseline (HR per year of increase in cumulative BP exposure 1.12 [95% CI 1.003–1.25]). Mortality was 1.11-fold (not significantly) higher per year of increase in cumulative BP exposure at baseline (95% CI 0.99–1.23) when all patients, irrespective of their treatment during follow-up, were included in the analysis. With multiple adjustments, the HRs for mortality per year of increase in cumulative BP exposure at baseline were slightly attenuated to 1.10 (95% CI 0.98–1.23) in the former and 1.08 (95% CI 0.96–1.20) in the latter analysis, respectively.

3.5. AFFs and ONJ

Two femoral shaft fractures occurred during follow-up that fulfilled the criteria for BP-associated AFFs. One of the 2 AFFs was preceded by treatment with intravenous ibandronate for 6.1 years, the other by treatment with alendronate, followed by intravenous ibandronate. The total duration of BP treatment prior to this AFF was 5.5 years. Three cases of ONJ occurred during the observation period, based on clinical features and histological findings. None of the 3 patients had a history of cancer-related radiation therapy of the head or neck region. One of the 3 ONJ cases was preceded by treatment with intravenous ibandronate for 4.8 years, followed by treatment with denosumab for 0.85 years. The second ONJ occurred subsequent to treatment with alendronate for 4.3 years. The third ONJ was preceded by BP treatment with first risedronate and then intravenous ibandronate. The total duration of BP treatment prior to the third ONJ case was 11.4 years.

4. Discussion

In this observational cohort study, we prospectively compared fracture risk in patients with preceding BP treatment between BP holidays and ongoing BP use and between different times since the beginning of a BP holiday. The latter was modeled by comparing fracture risk among 3 different levels of BP-SMA: the BP-SMA level 0% corresponded to a time > 12 months since the start of a BP holiday; the BP-SMA level > 0% to < 50%, with few exceptions, corresponded to a time > 6 to ≤12 months since the start of a BP holiday; and the BP-SMA level ≥50% corresponded to current BP use, short BP treatment breaks or the initial period of a longer BP holiday.

Although not significantly different, the point estimates of the hazard rates of MOFs in the adjusted unstratified models were lower for a BP holiday compared to an ongoing BP use with outcomes analyzed in relation to treatment at the time of IV1 or in relation to current treatment. Numerically (nonsignificant) lower point estimates of the hazard rates of MOFs were also observed for the BP-SMA level corresponding largely to a time > 6 to ≤12 months since the beginning of a BP holiday compared to the BP-SMA level corresponding to current BP treatment or a time ≤6 months since the start of a BP holiday. It is conceivable that this might have been due to confounding by indication, with only partial accounting in our adjusted models for a higher baseline fracture risk in patients with an ongoing BP use compared to patients on a BP holiday. Theoretically, the observed hazard rates of MOFs in the above comparisons might also be explained by changes in the bone matrix composition following BP discontinuation, as detrimental effects of long-term BP use on bone quality have been discussed [32,33]. However, the examinations of bone biopsies in the FLEX study failed to reveal major changes in bone matrix composition for up to 5 years after the discontinuation of alendronate [34].

In contrast, we observed an approximately 2.3-fold higher adjusted risk of MOFs for the BP-SMA level corresponding to a time > 12 months since the start of a BP holiday (BP-SMA 0%) compared to the BP-SMA level corresponding largely to a period of time > 6 to ≤12 months since the start of a BP holiday (BP-SMA > 0% to < 50%). Though the confidence limits of our estimate were large, this finding suggests that a

Table 4
HRs for fractures for a current BP holiday compared to current BP use with treatment as a time-dependent variable.

Fracture type	Number of first incident fractures	HR[a] (95% CI) Univariable	HR (95% CI) Adjusted[b]
MOFs	127	0.79 (0.55–1.13)	0.88 (0.61–1.27)
Any clinical osteoporotic fracture	201	0.85 (0.64–1.13)	0.93 (0.70–1.25)
Clinical vertebral fractures	64	0.77 (0.46–1.29)	0.91 (0.55–1.52)

[a] HR for BP holiday compared to BP use.
[b] Adjustments were performed as described in the methods section with the exception of no adjustment for sex in the vertebral fracture model because no incident clinical vertebral fractures occurred in men.

Table 5
Adjusted HRs for fractures among different BP-SMA levels and modification by the presence of a PVF.

Fracture type	Pairwise comparison of different BP-SMA levels			Adjusted HR (95% CI) for fractures[a]		
				Without interaction	Interaction between BP-SMA levels and PVF	
	BP-SMA level				PVF = yes	PVF = no
MOFs	0%	vs.	> 0% to < 50%	2.28 (1.07–4.86)	3.53 (1.19–10.51)	1.44 (0.49–4.22)
	0%	vs.	≥50%	1.54 (0.94–2.52)	2.42 (1.30–4.51)	0.93 (0.46–1.88)
	> 0% to < 50%	vs.	≥50%	0.67 (0.34–1.35)	0.69 (0.25–1.90)	0.65 (0.25–1.65)
Any clinical osteoporotic fracture	0%	vs.	> 0% to < 50%	1.17 (0.68–2.01)	1.98 (0.85–4.63)	0.77 (0.37–1.58)
	0%	vs.	≥50%	1.20 (0.78–1.84)	1.60 (0.91–2.83)	0.91 (0.51–1.64)
	> 0% to < 50%	vs.	≥50%	1.02 (0.66–1.60)	0.81 (0.39–1.67)	1.19 (0.68–2.08)
Clinical vertebral fractures	0%	vs.	> 0% to < 50%	1.93 (0.71–5.25)	1.65 (0.49–5.54)	3.30 (0.41–26.82)
	0%	vs.	≥50%	1.60 (0.78–3.29)	2.01 (0.78–5.17)	1.15 (0.43–3.09)
	> 0% to < 50%	vs.	≥50%	0.83 (0.34–2.02)	1.22 (0.44–3.40)	0.35 (0.05–2.54)

[a] HR for the BP-SMA level on the left side compared to that on the right side of column 2. Adjustments were performed as described in the methods section with the exception of no adjustment for sex in the vertebral fracture models because no incident clinical vertebral fractures occurred in men.

BP holiday in excess of 12 months was associated with an at least partial loss of BP anti-fracture efficacy in our cohort.

PVFs, especially when recent, are a strong risk factor for subsequent fractures [35-37]. Analyses in the FLEX study did not suggest a difference in the relative efficacy of alendronate in reducing the clinical vertebral fracture risk between patients with and without a PVF [13]. Our present study, however, suggests that the presence of a PVF may modify the relative risk of MOFs associated with a BP holiday. The adjusted risk of MOFs for the BP-SMA level corresponding to a time > 12 months compared to the BP-SMA level corresponding largely to a time > 6 to ≤12 months since the start of a BP holiday was 3.53-fold higher in the presence of a PVF, but the risk was only 1.44-fold higher and not significantly different in the absence of a PVF, emphasizing the relevance of a PVF as a risk factor of MOFs during a longer BP holiday. It should be noted, however, that our study was not sufficiently powered to enable the detection of clinically relevant changes in fracture risk without a PVF.

Throughout the entire analysis, a BP holiday was defined as any period of time without osteoporosis medication use beyond the completion of the prior dosing interval and may thus also include gaps in treatment due to a lower adherence level. However, of the 601 patients on a BP holiday at the time of the first IV, only 23 (3.83%) resumed BP treatment or initiated treatment with a different osteoporosis medication within 6 months after the start of a BP holiday. Likewise, 95.22% of the total number of BP holidays in model 2a were either longer than 6 months, were censored by the end of the observation within 6 months of the beginning of the BP holiday, or were of unknown duration due to incomplete follow-up information. Only 50 BP holidays (4.78%) in model 2a ended within 6 months after the beginning by resumption of BP treatment or starting treatment with a different osteoporosis medication. Assuming that pauses in BP treatment exceeding 6 months are more likely to be “true” BP holidays than those up to and including 6 months, these findings suggest that the potential misclassification of a less-than-ideal adherence level as BP holidays did not appear to be a major issue in our cohort. This may be due to the fact that a BP use of at least 80% of the total time of the preceding 4 years was required for inclusion in the observation, and that our cohort was thus highly selected for patients with a high adherence level.

BP treatment prior to a BP holiday in our cohort varied in terms of compounds, routes of administration and dosing regimens, and consisted of monotherapy with various BPs, as well as sequential treatment with more than one type of BP. BPs are thought to have different skeletal retention times. As a consequence, the temporal resolution of their skeletal effects may vary after discontinuation [13,14,38–41]. It is, therefore, important to note that our observations reflect the association of a mix of different BP therapies with fracture risk and may not be representative of individual BP therapies. The offset of skeletal alendronate effects during BP holidays as assessed by BMD or bone turnover markers did not appear to be closely related to fracture risk in the FLEX study [42]. Further large studies with fracture endpoints may thus be needed to examine possible differences in the magnitude or temporal pattern of BP holiday-related changes in fracture risk with different BP therapies.

In the FLEX study, fewer clinical vertebral fractures were observed in patients receiving 10 years of treatment with alendronate compared to those who discontinued alendronate after 5 years [13], whereas in the HORIZON extension study, fewer morphometric vertebral fractures occurred in the patients receiving 6 years of treatment with zoledronate compared to those who discontinued zoledronate after 3 years [14]. A post-hoc analysis of the FLEX study additionally suggested a reduction in the nonvertebral fracture risk with the continuation of alendronate for 10 years instead of stopping after 5 years in a specific subgroup of women without a PVF who also had a femoral neck T-score of −2.5 or less [17]. However, MOFs were not reported as an outcome measure in these studies, and conversely, the number of clinical vertebral fractures in our study was small, and information on morphometric vertebral fractures was missing, making site-specific comparisons of the observed fracture risk with BP holidays between these studies and our study difficult. In a recent large observational cohort study of women aged 65 years and above, Curtis et al. observed an increased risk of hip, humerus, and clinical vertebral fractures with discontinuation of alendronate beyond 2 years in comparison to continuing treatment. An increased risk of hip and clinical vertebral fractures was also observed with discontinuation of risedronate beyond 2 years. Results were consistent in the subgroup of women using alendronate who had a prior fragility fracture, but BP holiday-associated fracture risk in the presence or absence of PVFs was not specifically reported in this study [18]. It should be noted that in another large retrospective cohort study, a slightly lower risk of osteoporosis-related and vertebral fractures was observed for women with ≥3 years of exposure to BPs who took a BP holiday in comparison to the women with persistent BP use, defined in the study as an ongoing BP use with ≥50% adherence [19]. The reasons for the different results between this study and the above studies remain unclear, but possible factors may include differences in the study populations and potential unmeasured confounders.

Several previous studies have observed decreased mortality with BP use [23–26,43–45]. Our study differs from these studies in that we examined patients during BP holidays and with ongoing BP use, all of whom had received prior BP treatment over a period of at least 4 years. Although not significantly different, the point estimates of the adjusted hazard rates of death were 14% and 23% lower for a BP holiday compared to ongoing BP use with mortality analyzed in relation to treatment status at the time of IV1 or in relation to current treatment, respectively. One explanation for this may be that baseline differences

in comorbidity due to a treatment selection bias between the patients taking and not taking a BP holiday may have only been partially accounted for by our adjustments. The number of deaths was too small for a more detailed analysis of the relationship between mortality and BP holiday length. Adjusted for treatment at the time of IV1, mortality in our cohort increased with the duration of cumulative BP exposure at baseline. This was slightly attenuated after accounting for baseline differences in mortality risk. Patients with a cumulative BP exposure at or above the median at the baseline were somewhat older and somewhat more likely to have experienced 2 or more falls in the preceding year compared to those with a cumulative BP exposure below the median at the baseline (results not shown), suggesting that the observed higher mortality in the patients with a longer cumulative BP exposure at the baseline in the treatment-adjusted model may have been in part attributable to a higher level of comorbidity in these patients. However, our findings do not exclude direct effects of cumulative BP treatment duration on mortality, and a large randomized controlled trial would be necessary to further clarify the association between long-term BP use and mortality.

Although AFFs and ONJ are rare in patients with BP use for osteoporosis [10,46], the risks of these adverse effects appear to increase with increased BP therapy duration [3–11]. In our study, 2 cases of AFFs and 3 cases of ONJ occurred during a median follow-up of approximately 24 months in a cohort of patients who had used BPs for a median cumulative duration of approximately 5.2 years at the beginning of the observation. One of the 3 observed ONJ cases was diagnosed after a switch from BP treatment to denosumab. All 5 cases of AFFs and ONJ in our study occurred with continued antiresorptive treatment. It is noteworthy that 2 of the 3 patients with ONJ in our study and one of the 2 patients with AFFs had a history of partial BP treatment with intravenous ibandronate and that the other patient who experienced an AFF had a history of exclusive BP treatment with intravenous ibandronate. ONJ risk is much more common in oncology patients receiving high cumulative doses of intravenous BPs than in patients treated with oral BPs for osteoporosis [46]. Evidence is, however, scarce for the incidence of ONJ with the long-term non-oncological use of intravenous BPs, though in a recent study, no cases of AFFs or ONJ were observed during follow-up for 5940 person-years in women with osteopenia who had received 4 infusions of zoledronate at a dose of 5 mg at 18-month intervals [47]. In light of our present findings, further studies may be warranted to explore potential differences in the long-term risks for ONJ and AFFs among various BP therapies with doses used for the treatment of osteoporosis.

Our study has several limitations apart from those already discussed. A major drawback is the lower than intended number of participants. The CIs of the reported HRs are accordingly wide. Because fracture assessment was mostly interview-based, the underestimation of fracture incidence due to recall bias is possible. Information on prior BP usage was provided by the recruiting physicians. We did not assess whether the information was based on medical records, patient reports, or a combination of both. It is therefore possible that the cumulative time of BP exposure prior to the observation may have been subject to some misreporting. Self-reports of medications with long dosing intervals may be particularly susceptible to misclassification with regard to current exposure. In patients who reported a holiday from zoledronate we therefore attempted to ascertain the date of the last infusion. For those receiving intravenous ibandronate, the reported date of discontinuation was for most patients assumed to correspond to the date of the last application, but for some of these patients, it may have corresponded to the end of the last dosing interval. Conversely, for some patients taking oral BPs, the date of the assumed beginning of a BP holiday may have rather been the date of the last prescription. However, it is unlikely that such potential misclassifications of the time of BP discontinuation for up to 3 months would have caused major distortions with regard to the analysis of fracture risk in relation to BP holiday length.

There was no indication from the interviews that patients who discontinued BP treatment might have instead initiated menopausal hormone therapy as osteoporosis treatment. However, we did not specifically assess menopausal hormone therapy prior or during the observation, and menopausal hormone therapy for reasons other than for osteoporosis was no explicit exclusion criterion. Some of the patients may, therefore, have used menopausal hormone therapy for the treatment of estrogen deficiency symptoms. Based on the data of a national health survey, conducted in Germany by the Robert Koch Institute between 2003 and 2004, the number of women using menopausal hormone therapy in our cohort was, however, likely to be low [48].

As already noted above, it is possible that our results may have been affected by risks that were either unmeasured or incompletely accounted for. Fracture risks and comorbidities were only assessed at baseline. Some covariates may therefore have been incompletely accounted for as confounders due to potential changes during the observation.

In some patients, a BP-SMA $> 0\%$ to $< 50\%$ did not or not completely coincide with a time > 6 months to ≤ 12 months since the beginning of a BP holiday, because a short BP holiday either during the observation or the preceding 12 months was succeeded within 6 months or less by a second BP holiday or because pauses in BP treatment, albeit not exceeding 20% of the total time of the preceding 4 years, exceeded 6 months in the year prior to the observation. However, in only $< 2\%$ of the observed person-years with a BP exposure during the preceding 12 months at a BP-SMA level $> 0\%$ to $< 50\%$, this level did not correspond to a time > 6 months and ≤ 12 months since the beginning of a BP holiday.

It should also be noted that not all patients included in our observation had osteoporosis prior to the beginning of the observation according to the WHO definition of a T-score ≤ -2.5 [49]. The rational for using a DXA T-score threshold of -2.0 as an inclusion criterion was that in the 2009 (German, Austrian and Swiss) DVO guideline, this was the threshold for pharmacological treatment for patients with primary osteoporosis and a high clinical fracture risk as outlined in the guideline [27]. However, results for MOFs as outcome were consistent in a subgroup of patients with a minimal T-score ≤ -2.5 at the lumbar spine, TH, or femoral neck at any measurement time prior to the beginning of the observation. For the 1508 patients in the BP-SMA analysis with a T-score ≤ -2.5, the HR for MOFs for the BP-SMA level corresponding to a time > 12 months since the start of a BP holiday compared to the level corresponding largely to a time > 6 to ≤ 12 months since the start of a BP holiday was numerically higher than that in the main cohort, both in the presence or absence of a PVF (results not shown).

The maximal duration of a BP holiday at the end of follow-up was approximately 2.5 years. We therefore cannot make statements regarding the fracture risk with longer BP holidays. No interaction was observed between the median-dichotomized cumulative BP exposure at baseline and BP holiday-related MOF risk, thus providing no evidence for a different BP holiday-related MOF risk in our cohort for a cumulative baseline BP exposure below or at/above the median of approximately 5.2 years. However, only a minor portion of the patients in our cohort had used BPs for 10 or more years prior to the observation period. Our study, therefore, does not provide information on fracture risk in patients with a very long cumulative BP exposure. Furthermore, the results of our study are predominantly derived from postmenopausal women and therefore may not apply to men.

In conclusion, in this prospective interview-based observational study we (1) compared fracture risk and mortality in patients with preceding BP treatment between BP holidays and ongoing BP use and (2) analyzed temporal changes in fracture risk during BP holidays by comparing fracture risk among 3 incremental levels of SMAs of BP treatment during the preceding 12 months corresponding to different times since the start of a BP holiday. We did not find a significant difference in facture risk or mortality between BP holidays and ongoing BP use for an observation period up to 25 months when outcomes were

analyzed in relation to treatment at the time of IV1 or in relation to current treatment. However, in the presence of a PVF, we observed a higher risk of MOFs for a BP-SMA level corresponding to a time > 12 months since the start of a BP holiday in comparison to a BP-SMA level corresponding mainly to a period > 6 to ≤12 months since the start of a BP holiday and a BP-SMA level corresponding to current BP use or a time ≤6 months since the start of a BP holiday. The presence of a PVF may increase the relative risk of MOFs associated with a longer BP holiday.

CRediT authorship contribution statement

Johannes Pfeilschifter: Conceptualization, Investigation, Writing - original draft, Writing - review & editing. **Inga Steinebach:** Investigation, Writing - review & editing. **Hans J. Trampisch:** Conceptualization, Writing - review & editing. **Henrik Rudolf:** Formal analysis, Writing - review & editing.

Declaration of competing interest

Johannes Pfeilschifter has received lecture fees from GWT-TUD GmbH and travel support from OmniaMed. Inga Steinebach has received honoraria from AMGEN and Novartis. Hans J. Trampisch and Henrik Rudolf declare that they have no conflict of interest.

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