

Plasma Desmosine and Abdominal Aortic Aneurysm Disease

Ify R. Mordi, MD; Rachael O. Forsythe, PhD; Corry Gellatly, PhD; Zaid Iskandar, MBChB; Olivia M. McBride, MD; Athanasios Saratzis, PhD; Rod Chalmers, MD; Calvin Chin, MD; Matthew J. Bown, MD; David E. Newby, PhD; Chim C. Lang, MD; Jeffrey T. J. Huang, PhD; Anna-Maria Choy, MD

Background—It is recognized that factors beyond aortic size are important in predicting outcome in abdominal aortic aneurysm (AAA) disease. AAA is characterized by the breakdown of elastin within the aortic tunica media, leading to aortic dilatation and rupture. The aim of this study was to investigate the association of plasma desmosine (pDES), an elastin-specific degradation product, with disease severity and clinical outcome in patients with AAA.

Methods and Results—We measured pDES and serum biomarker concentrations in 507 patients with AAAs (94% men; mean age, 72.4±6.1 years; mean AAA diameter, 48±8 mm) and 162 control subjects (100% men; mean age, 71.5±4.4 years) from 2 observational cohort studies. In the longitudinal cohort study (n=239), we explored the incremental prognostic value of pDES on AAA events. pDES was higher in patients with AAA compared with control subjects (mean±SD: 0.46±0.22 versus 0.33±0.16 ng/mL; $P<0.001$) and had the strongest correlation with AAA diameter ($r=0.39$; $P<0.0001$) of any serum biomarker. After adjustment for baseline AAA diameter, pDES was associated with an AAA event (hazard ratio, 2.03 per SD increase [95% CI, 1.02–4.02]; $P=0.044$). In addition to AAA diameter, pDES provided incremental improvement in risk stratification (continuous net reclassification improvement, 34.4% [95% CI, –10.8% to 57.5%; $P=0.09$]; integrated discrimination improvement, 0.04 [95% CI, 0.00–0.15; $P=0.050$]).

Conclusions—pDES concentrations predict disease severity and clinical outcomes in patients with AAA.

Clinical Trial Registration—<http://www.isrctn.com>. Unique identifier: ISRCTN76413758. (*J Am Heart Assoc*. 2019;8:e013743. DOI: 10.1161/JAHA.119.013743.)

Key Words: abdominal aortic aneurysm • aortic rupture • desmosine • elastin

Abdominal aortic aneurysm (AAA) is a common life-threatening disease that affects 1% to 2% of men by the age of 65 years.¹ The most worrying complication of AAA is rupture, which is usually fatal, with a reported age-adjusted annual mortality of 15.1 per 1 million people in the United States.^{2,3} Randomized clinical trials of population screening in

men have demonstrated cost-effective reduction of AAA-related mortality by ≈40% and have provided the underpinning evidence for screening and surveillance programs for men aged at least 65 years with surgical referral when AAA diameter >5.5 cm.^{4,5} Strategies of repair of smaller aneurysms have shown no benefit, although there continues to be a considerable risk of rupture, especially in women.^{6,7} Conversely, the rupture rate of AAAs above the 5.5-cm threshold in the contemporary era is much lower than historically reported; and many large AAAs do not rupture at all.⁸ Furthermore, there is an increasing appreciation that growth of AAA is nonlinear,⁹ and the risk of rupture is time varying.

There is increasing recognition of the need to improve risk stratification in patients with AAA beyond measurement of the aortic diameter.^{10,11} Biomarkers, especially those related to pathophysiological processes of inflammation and aortic wall degradation, are attractive potential candidates.^{10,12} However, no biomarker has yet been shown to provide enough additional prognostic value to AAA diameter to enter into routine clinical use.^{13,14} A key pathophysiological process is loss of integrity of the extracellular matrix in the tunica media of the aortic wall, resulting from elastin breakdown.^{15–19} A recent review of biomarkers for prediction of AAA events

From the Division of Molecular and Clinical Medicine, University of Dundee, Dundee, United Kingdom (I.R.M., Z.I., C.C.L., A.M.C.); Division of Systems Medicine, University of Dundee, Dundee, United Kingdom (J.T.J.H.); British Heart 3 Foundation/University of Edinburgh Centre for Cardiovascular Science, Edinburgh, United Kingdom (R.O.F., O.M.M., R.C., D.E.N.); Department of Cardiovascular Sciences and National Institute for Health Research Leicester Biomedical Research Centre, University of Leicester, Glenfield Hospital, Leicester, United Kingdom (C.G., A.S., M.J.B.); and Department of Cardiovascular Science, National Heart Center, Singapore 20 (C.C.).

Correspondence to: Jeffrey T. J. Huang, PhD, and Anna-Maria Choy, MD, Division of Molecular and Clinical Medicine, University of Dundee, Dundee DD1 9SY, United Kingdom. E-mails: j.t.j.huang@dundee.ac.uk and a.choy@dundee.ac.uk

Received June 26, 2019; accepted September 6, 2019.

© 2019 The Authors. Published on behalf of the American Heart Association, Inc., by Wiley. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

Clinical Perspective

What Is New?

- Desmosine is a structural cross-link found in plasma after breakdown of mature elastin.
- Plasma desmosine levels were significantly higher in patients with abdominal aortic aneurysms and were strongly correlated with aortic diameter.
- Plasma desmosine levels were significantly associated with abdominal aortic aneurysm events after adjustment for abdominal aortic aneurysm diameter.

What Are the Clinical Implications?

- Plasma desmosine may have potential as an additional marker for assessing the risk of rupture in patients with abdominal aortic aneurysms.

suggested that serum elastin peptides, which are released in the circulation when there is breakdown of elastin-rich tissue, could hold promise for prediction of events in patients with AAA; however, the authors agreed that larger prospective studies are needed.²⁰ Studies evaluating serum elastin peptides have, however, been limited.^{21–23}

Desmosine is a structural cross-linking amino acid that is specifically released into the circulation when mature elastin is broken down.²⁴ We, therefore, hypothesized that plasma desmosine (pDES) concentrations may reflect disease activity in the aneurysm and could be used as a risk marker for AAA events. The aim of this study was, therefore, to determine the association of pDES concentrations with disease severity and determine if pDES provides incremental prognostic value for AAA-related clinical outcomes.

Methods

The data that support the findings of this study are available from the corresponding authors on reasonable request.

Patient Populations

This study was conducted using analysis from 2 independent study cohorts: the MA³RS (Magnetic Resonance Imaging for Abdominal Aortic Aneurysms to Predict Rupture or Surgery) study²⁵ and the UKAGS (UK Aneurysm Growth Study).²⁶ Full details of the MA³RS study design have been described previously.²⁷ In brief, the MA³RS study was a prospective, observational, multicenter cohort study of 342 patients with AAA designed to determine the relationship between mural ultrasmall superparamagnetic particles of iron oxide uptake and clinical outcomes. Patients with AAA diameter >40 mm

were recruited from 3 Scottish centers between 2012 and 2014. In the MA³RS study, baseline assessment, including computed tomography of the aorta and blood sampling, was performed within 6 weeks of the screening abdominal ultrasound. UKAGS is an ongoing, prospective, observational cohort study of patients with small AAA (<55 mm) identified from the National Health Service AAA screening programs in England and Wales. UKAGS is currently in a recruitment phase with ongoing longer-term follow-up. Details of the study have been published previously.²⁸ Screening participants from UKAGS provided both patients with AAA as well as controls (aortic diameter <30 mm). Baseline assessment, including blood sampling, was performed at the time of the screening abdominal ultrasound. For the purposes of this study, patients were randomly selected from the UKAGS cohort to reflect the range of aortic diameter. Both studies were approved by their institutional review boards, and ethical approvals have been previously sought for both the MA³RS study (East of Scotland Research Ethics Service [12/ES/0068]) and UKAGS,²⁹ with consent for further research studies.

Participant Characterization

In all participants, abdominal aortic diameter was defined as the maximal anteroposterior inner wall to inner wall diameter of the infrarenal aorta. Plasma samples from the MA³RS study cohort and the selected cohort from the UKAGS were analyzed for desmosine. pDES concentrations were analyzed using a validated stable isotope dilution liquid chromatography–tandem mass spectrometry method, as described previously.³⁰ The lower limit of quantification is 0.1 ng/mL. Other biomarkers of interest (interleukin-6, matrix metalloproteinase-2, matrix metalloproteinase-9, and tissue inhibitor of metalloproteinase-1) were obtained at baseline in the MA³RS study, as described previously.²⁷

Clinical Outcomes

Outcome data were available for the MA³RS study cohort. Patients underwent 6 monthly assessments and were followed up for at least 2 years for AAA events (AAA-related death, rupture, or urgent repair). No outcome data are available from the UKAGS as recruitment is ongoing and completion of follow-up is not anticipated for some time.

Statistical Analysis

Continuous variables are reported as mean±SD or median with interquartile range, whereas categorical variables are reported as number and percentage. As pDES concentrations were not normally distributed, they were log transformed for analysis of outcomes. Differences between groups were assessed using

unpaired *t* tests, analysis of variance, Kruskal-Wallis tests, or χ^2 tests, as appropriate. Correlations were assessed using Spearman's correlation and linear regression. Association between the earliest available pDES and clinical outcomes was assessed using Cox regression (hazard ratio per SD change in log pDES) and Kaplan-Meier analysis. Because of the limited number of events and to prevent statistical overfitting, our multivariable model only included variables associated with AAA events at $P < 0.05$ in univariable analysis. The optimal cutoff for pDES was obtained using the Youden index (sensitivity+specificity-1). The incremental predictive value of pDES in addition to AAA diameter was determined using the C-statistic, net reclassification improvement, and integrated discrimination improvement. All tests were 2 sided, and a $P < 0.05$ was considered significant. All statistical analysis was performed using R 3.4.3 (Foundation for Statistical Computing, Vienna, Austria).

Results

Participant Characteristics

In total plasma, desmosine concentrations were available from 669 individuals (507 patients with AAA and 162 controls without AAA). Baseline characteristics of both patients with AAA and controls are shown in Table 1. Overall, the cohort was typical of patients with AAA (94% men; mean age, 72.4 ± 6.1 years). Patients with AAA were more likely to be smokers, have cardiovascular risk factors (diabetes mellitus, hypertension, and hypercholesterolemia), and have had a prior myocardial infarction.

Baseline characteristics of the MA³RS study cohort have been reported previously.²⁷ From the MA³RS study cohort, 560 plasma samples were collected from 239 patients at different time points with contemporaneous abdominal ultrasound scans. Mean baseline AAA diameter in the index MA³RS study cohort was 51 ± 9 mm. Most patients were men, and there was a high proportion of patients with cardiovascular risk factors. Mean pDES in the MA³RS study cohort was 0.52 ± 0.23 ng/mL.

In the UKAGS cohort, plasma samples were available from 430 participants, including 268 patients with AAA and 162 controls without AAA. Patients with AAA in UKAGS were of a similar age to those in the MA³RS study and had a similar body mass index, although there was a lower prevalence of hypertension, smoking, hypercholesterolemia, and prior myocardial infarction. There was a higher prevalence of diabetes mellitus in UKAGS patients with AAA compared with MA³RS study patients. Mean AAA diameter in UKAGS was lower than in the MA³RS study (43 ± 8 versus 51 ± 9 mm; $P < 0.001$). Mean aortic diameter in the control group was 20 ± 5 mm.

pDES and Disease Severity

Overall, pDES levels in AAA patients was higher than controls (AAA patients, 0.46 ± 0.22 ng/mL; controls, 0.33 ± 0.16 ng/mL; $P < 0.001$) (Figure 1).

pDES concentrations correlated with the maximal AAA diameter, measured by ultrasound in both study cohorts (MA³RS study cohort, $r = 0.26$, $P < 0.001$; UKAGS cohort, $r = 0.15$, $P = 0.002$). This relationship was also seen in using

Table 1. Baseline Characteristics of the MA³RS Study and UKAGS Cohorts

Characteristics	All Patients With AAA (n=507)	MA ³ RS Patients With AAA (n=239)	UKAGS Patients With AAA (n=268)	Controls (n=162)	P Value Between AAA Patients and Controls
Age, y*	72.4±6.1	73.1±7.1	71.7±5.0	71.5±4.4	0.044 [†]
Men	477 (94.0)	209 (87.4)	268 (100)	162 (100)	<0.001 [†]
Body mass index, kg/m ² *	27.7±4.3	27.3±4.1	28.2±4.7	27.2±3.6	0.58
Current smoker	107 (21.1)	66 (27.6)	41 (15.3)	9 (0.1)	<0.001 [†]
COPD	69 (13.6)	22 (9.2)	47 (17.5)	12 (7.4)	<0.001 [†]
Hypertension	323 (63.7)	172 (72.0)	151 (56.3)	75 (46.3)	<0.001 [†]
Type 2 diabetes mellitus	86 (17.0)	33 (13.8)	53 (19.8)	15 (9.3)	0.025 [†]
Hypercholesterolemia	333 (65.7)	188 (78.7)	145 (54.1)	61 (37.7)	<0.001 [†]
Prior myocardial infarction	124 (24.5)	67 (28.0)	57 (21.2)	16 (9.9)	<0.001 [†]
Prior stroke	33 (6.5)	13 (5.4)	20 (7.5)	13 (8.0)	0.98
AAA ultrasound diameter, mm [‡]	48±8	51±9	45±8	20±5	<0.001 [†]

Data are given as mean±SD or number (percentage). AAA indicates abdominal aortic aneurysm; COPD, chronic obstructive pulmonary disease; MA³RS, Magnetic Resonance Imaging Using Ultrasound Superparamagnetic Particles of Iron Oxide to Predict Clinical Outcome in Patients Under Surveillance for Abdominal Aortic Aneurysms; UKAGS, UK Aneurysm Growth Study.

*Unpaired *t* test.

[†] $p < 0.05$.

[‡]Kruskal-Wallis test.

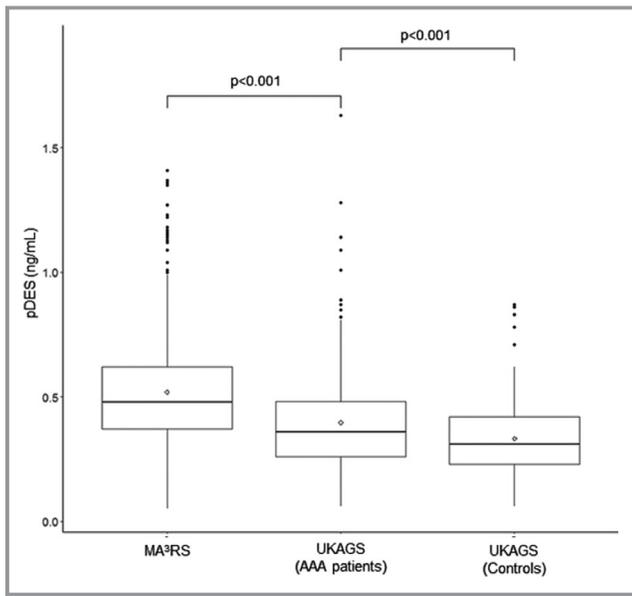


Figure 1. Box plot of plasma desmosine (pDES) at baseline in the MA³RS (Magnetic Resonance Imaging for Abdominal Aortic Aneurysms to Predict Rupture or Surgery) study and UKAGS (UK Aneurysm Growth Study) cohorts. Horizontal lines represent median plasma desmosine, whereas diamonds represent mean plasma desmosine. The mean plasma desmosine level for all patients with abdominal aortic aneurysm (AAA) was 0.46 ± 0.22 ng/mL; controls, 0.33 ± 0.16 ng/mL ($P < 0.001$, Kruskal-Wallis test).

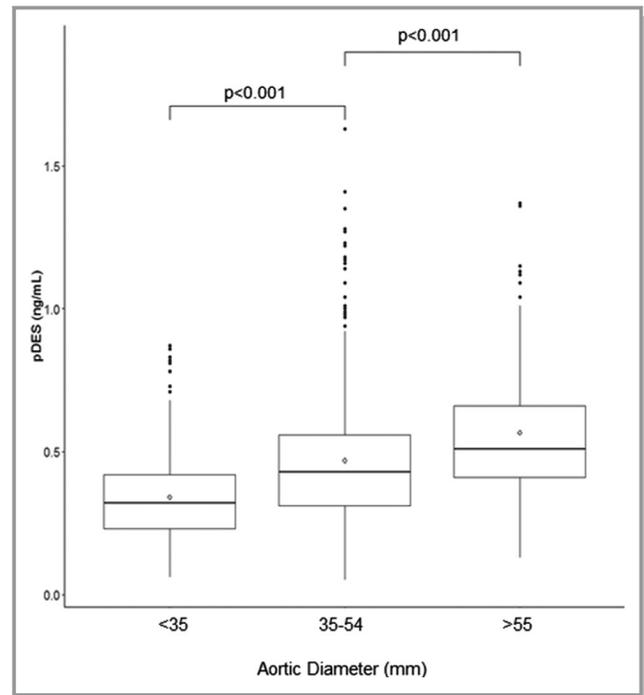


Figure 2. Box plot of plasma desmosine (pDES) for all patients, stratified by baseline abdominal aortic aneurysm diameter from the MA³RS (Magnetic Resonance Imaging for Abdominal Aortic Aneurysms to Predict Rupture or Surgery) study and UKAGS (UK Aneurysm Growth Study) cohorts. Horizontal lines represent median plasma desmosine, whereas diamonds represent mean plasma desmosine. Mean plasma desmosine at ≥ 55 mm, 0.57 ± 0.24 ng/mL; 35 to 54 mm, 0.47 ± 0.22 ng/mL; and < 35 mm, 0.34 ± 0.16 ng/mL (Kruskal-Wallis test).

computed tomography assessment of the aorta within the MA³RS study cohort ($r=0.20$; $P=0.003$). There was a stepwise incremental increase in pDES concentrations, stratified by aortic diameter, with those patients with large aneurysms having a higher median pDES concentration than those with smaller aneurysms (Figure 2). Across both cohorts, in multivariable linear regression analysis, log pDES concentrations were associated with AAA diameter after adjustment for age and history of hypertension, smoking status, and chronic obstructive pulmonary disease (COPD) (MA³RS study β , 3.36; SE, 1.34; $P=0.013$; UKAGS β , 0.33; SE, 0.12; $P=0.009$) (Table 2).

pDES concentrations correlated with matrix metalloproteinase-2 ($r=0.26$; $P < 0.001$), tissue inhibitor of metalloproteinase-1 ($r=0.27$; $P < 0.001$), and interleukin-6 ($r=0.30$; $P < 0.001$) but not matrix metalloproteinase-9 ($r=0.07$; $P=0.31$). However, pDES concentrations were more strongly associated with aortic diameter than the other measured biomarkers (interleukin-6, $r=0.28$, $P=0.001$; matrix metalloproteinase-2, $r=0.14$, $P=0.053$; matrix metalloproteinase-9, $r=0.01$, $P=0.90$; tissue inhibitor of metalloproteinase-1, $r=0.16$, $P=0.027$).

pDES and Disease Progression

From the MA³RS study cohort, 186 patients from the 239 had >1 plasma sample available. There was no correlation

between change in pDES and change in AAA diameter at the following visit ($r=0.05$; $P=0.53$). Patients with pDES higher than the median did not have a significantly higher AAA diameter at 1 year than those with desmosine lower than the median (pDES above median AAA increase, 2.56 versus 2.01 mm; $P=0.15$). In those patients with serial measurements, desmosine levels were fairly stable, with patients whose baseline desmosine level was below the median remaining significantly lower than those whose baseline desmosine levels were higher over the 24-month follow-up period (Figure 3).

pDES and Clinical Outcomes

In total, 13 (5.5%) of the 239 patients had an AAA event (11 AAA deaths, 11 AAA ruptures, and 4 urgent repairs). Among baseline clinical variables, AAA diameter was the only significant predictor of an AAA event (hazard ratio, 1.07 per mm increase; 95% CI, 1.03–1.12; $P=0.002$) (Table 3). In univariable analysis, log pDES was associated with increased likelihood of an AAA event (hazard ratio per SD increase, 2.43; 95% CI, 1.29–4.57; $P=0.006$); and this remained the case after adjustment for AAA

Table 2. Multivariable Linear Regression for Association With AAA Diameter in MA³RS Study and UKAGS

Study	β Estimate	SE	P Value
MA³RS study			
Age	0.19	0.09	0.023*
History of hypertension	1.56	1.24	0.21
History of diabetes mellitus	-2.50	1.52	0.10
Current smoker	0.67	1.22	0.58
Chronic obstructive pulmonary disease	2.87	1.85	0.12
Log plasma desmosine	3.36	1.34	0.013*
UKAGS			
Age	0.01	0.01	0.48
History of hypertension	0.12	0.12	0.32
History of diabetes mellitus	0.23	0.17	0.17
Current smoker	0.83	0.19	<0.001*
Chronic obstructive pulmonary disease	0.67	0.18	<0.001*
Log plasma desmosine	0.33	0.12	0.009*

AAA indicates abdominal aortic aneurysm; MA³RS, Magnetic Resonance Imaging Using Ultrasound Superparamagnetic Particles of Iron Oxide to Predict Clinical Outcome in Patients Under Surveillance for Abdominal Aortic Aneurysms; UKAGS, UK Aneurysm Growth Study. * $p < 0.05$.

diameter (hazard ratio, 2.03 per SD increase; 95% CI, 1.02–4.02; $P = 0.044$) (Table 4). Similarly, after adjustment for both AAA diameter and current smoking status (as smoking was the variable most strongly associated with AAA diameter after

pDES), log pDES remained associated with the likelihood of having an AAA event (hazard ratio per SD increase, 2.06; 95% CI, 1.03–4.12; $P = 0.040$). The optimal cutoff of pDES (determined using Youden's index) for prediction of an AAA event was 0.56 ng/mL. Patients with pDES concentrations ≥ 0.56 ng/mL were more likely to have an AAA event (unadjusted hazard ratio, 6.77; 95% CI, 1.86–24.62; $P = 0.004$; adjusted for AAA diameter hazard ratio, 4.97; 95% CI, 1.31–18.90; $P = 0.019$) (Figure 4). pDES concentrations showed better discrimination for prediction of an AAA event than other biomarkers (C-statistic: pDES, 0.70; interleukin-6, 0.49; matrix metalloproteinase-2, 0.52; matrix metalloproteinase-9, 0.60; and tissue inhibitor of metalloproteinase-1, 0.51).

The area under the curve for pDES for prediction of AAA events was 0.70 (sensitivity, 0.77; specificity, 0.64; positive predictive value, 0.11; negative predictive value, 0.98). When combined with abdominal ultrasound AAA diameter, the addition of pDES improved reclassification for prediction of AAA events (continuous net reclassification improvement, 34.4%; 95% CI, -10.8% to 57.5%; $P = 0.09$; integrated discrimination improvement, 0.04; 95% CI, 0.00–0.15; $P = 0.05$).

Discussion

This study is the largest study of a circulating biomarker in AAA to date, and we have identified 3 novel findings. First, pDES concentrations are higher in patients with AAA than in control subjects, suggesting a potential role as a disease marker. Second, it correlated with AAA diameter in 2 independent cohorts of patients. Third, it predicted clinical

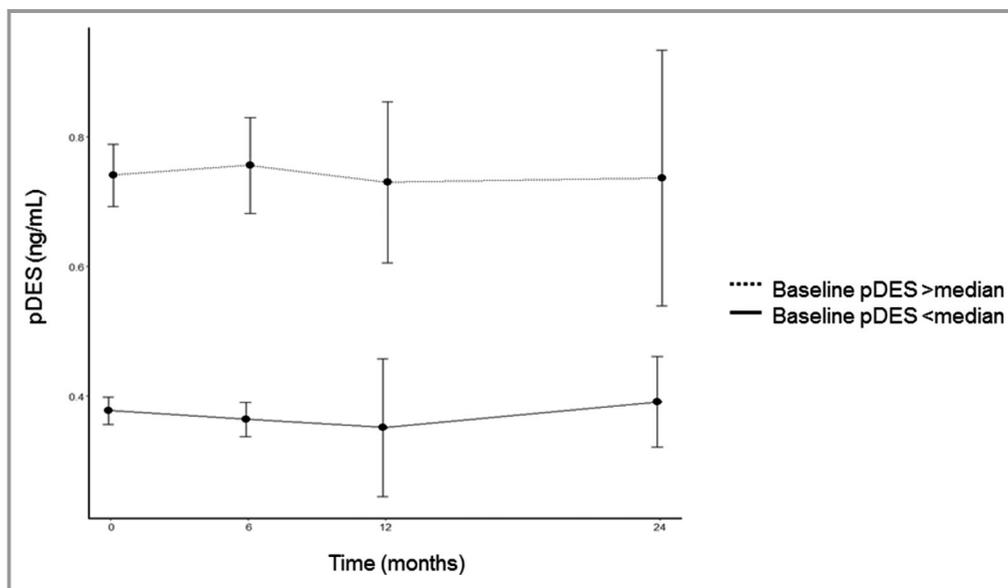


Figure 3. Serial plot of mean plasma desmosine (pDES) over the 24-month follow-up period, stratified by baseline desmosine. Points represent mean plasma desmosine in each group (stratified by baseline desmosine levels). Error bars represent 95% CIs.

Table 3. Univariable Cox Regression Analysis for Association With AAA Events

Variable	Hazard Ratio (95% CI)	P Value
Age (per year)	1.06 (0.98–1.15)	0.12
Female sex	1.26 (0.28–5.69)	0.76
Current smoker	1.82 (0.60–5.57)	0.29
History of hypertension	1.86 (0.41–8.39)	0.42
Diabetes mellitus	0.47 (0.06–3.61)	0.47
Hypercholesterolemia	2.89 (0.38–22.26)	0.31
History of angina	2.02 (0.55–7.33)	0.29
History of COPD	1.71 (0.38–7.71)	0.49
Previous myocardial infarction	0.69 (0.19–2.51)	0.57
Previous stroke	1.22 (0.16–9.41)	0.85
BMI (per kg/m ² increase)	0.99 (0.87–1.13)	0.89
Baseline systolic blood pressure (per mm Hg increase)	1.00 (0.97–1.04)	0.82
AAA diameter (per mm increase)	1.07 (1.03–1.12)	0.002*
pDES (per SD increase)	2.43 (1.29–4.58)	0.006*

AAA indicates abdominal aortic aneurysm; BMI, body mass index; COPD, chronic obstructive pulmonary disease; pDES, plasma desmosine.

* $p < 0.05$.

outcome even after adjustment for AAA diameter, providing incremental improvements in risk prediction. Finally, when compared with other measured plasma biomarkers, pDES demonstrated the strongest associations with aortic diameter and prediction of AAA events. These findings suggest that pDES may be a promising biomarker for prediction of adverse clinical events in patients with AAA (Figure 5).

There has been intense interest in the utility of biomarkers to monitor AAA size and their role in predicting rupture.^{13,14} However, none has been recommended for clinical use. A recent systematic review of blood, imaging, and genetic markers evaluated their prognostic value for the prediction of AAA growth and rupture.²⁰ Among them, serum elastin peptides were the only plasma biomarker considered to have clinical promise. Previous studies of serum elastin peptides in patients

with AAA have reported an association with AAA size and risk of rupture^{21–23,31}; however, the previously published studies have been relatively small, with cohort sizes ranging from 100 to 150 patients, and 3 of the 4 studies of serum elastin peptides have been from the same cohort of patients. Our study advances these interesting exploratory results by using a single analyte, pDES, as a specific marker of mature elastin breakdown, rather than the more nonspecific serum elastin peptides. The results of our study not only show a relationship between pDES and AAA diameter, but also an association between baseline pDES and AAA rupture, which is stronger than that reported with serum elastin peptides,^{21,22} supporting our hypothesis that pDES may be a more clinically useful biomarker. In our study, other plasma biomarkers, such as MMP (matrix metalloproteinase)-9, were not significantly associated with AAA diameter, as has been found in several other studies, strengthening the case for the potential utility of desmosine.²⁰

Although intervention in patients with small aneurysms (30–55 mm) has not been shown to be superior to surveillance in randomized trials or meta-analyses,³² AAA ruptures can still occur in patients who do not meet the criteria for AAA repair, particularly in women who account for a third of deaths caused by rupture; they remain at risk from cardiovascular events.^{28,33} Given this, our finding that pDES was associated with AAA events independent of aortic diameter suggests that pDES may be useful as an additional risk marker, particularly given the improvements in net reclassification improvement and integrated discrimination improvement. The independent prognostic value of pDES suggests that it may be providing an additional pathophysiological insight beyond AAA diameter.

In our study, there was interestingly no association between pDES and AAA diameter progression, and this contrasts with prior work using serum elastin peptides.³¹ The reason for this discrepancy is unknown, although the recognized nonlinear growth of aneurysmal progression may partly account for this.^{9,34} We did see, however, that pDES levels remained stable over time compared with baseline, suggesting that single measurement of pDES at any time point may be enough to provide a prediction of risk beyond AAA diameter. We hypothesize that pDES is related to other

Table 4. Cox Regression Analysis of Association of pDES With AAA Diameter

Variable	No. of Events	Univariable Hazard Ratio (95% CI) per SD Increase	P Value	Hazard Ratio (95% CI) per SD Increase Adjusted for AAA Diameter	P Value
AAA event	13	2.43 (1.29–4.58)	0.006*	2.03 (1.02–4.02)	0.044*
AAA death	11	2.46 (1.23–4.91)	0.011*	1.97 (0.92–4.21)	0.08
AAA rupture	11	2.46 (1.23–4.91)	0.011*	1.97 (0.92–4.21)	0.08
Urgent repair	4	3.58 (1.03–12.45)	0.044*	2.73 (0.73–10.17)	0.13

AAA indicates abdominal aortic aneurysm; pDES, plasma desmosine.

* $p < 0.05$.

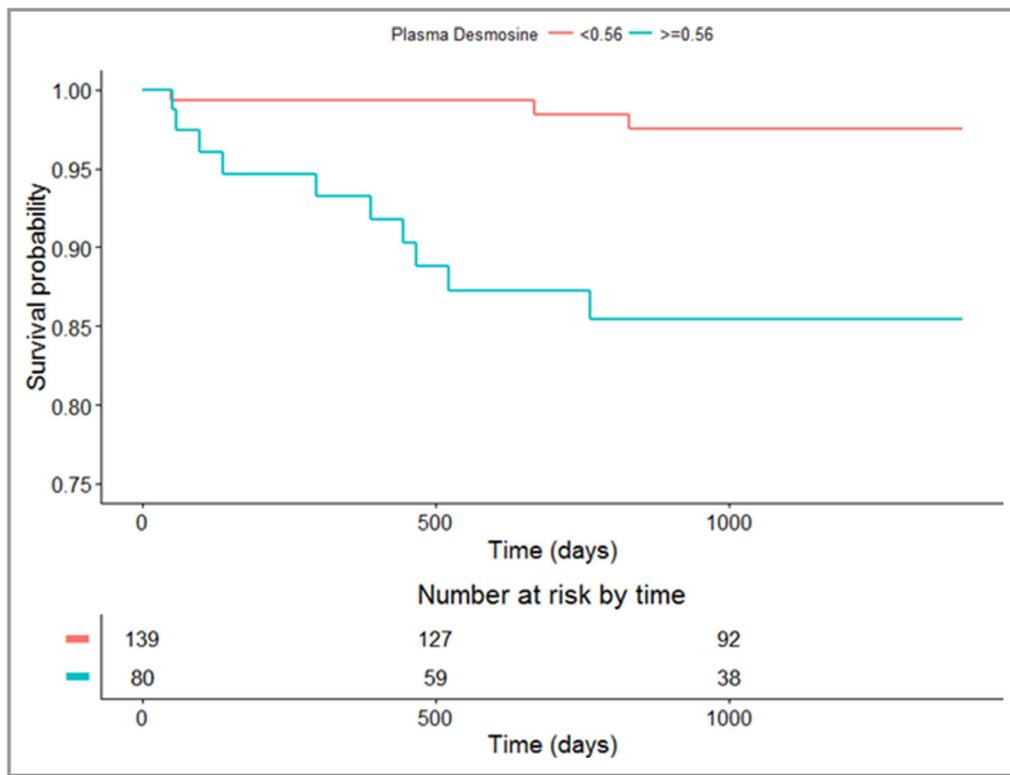


Figure 4. Kaplan-Meier curve for emergency abdominal aortic aneurysm events based on the optimal cutoff for plasma desmosine (log-rank $P < 0.001$).

parameters of risk beyond AAA diameter, perhaps related to vessel wall integrity.¹⁰ Using this assay specific for desmosine rather than general serum elastin peptides, we have shown the independent and incremental value of pDES in predicting clinical events when added to AAA diameter, which has never been demonstrated with previously studied biomarkers.^{35,36} Follow-up data in our study were also only available in the MA³RS study cohort, in which patients had a larger AAA diameter at baseline compared with those in the studies of serum elastin peptides, which largely focused on patients with smaller AAAs.^{21,22,37}

Although pulmonary elastin breakdown is a component of conditions such as COPD, a large study of 1177 stable patients with COPD found that pDES was not a predictor of emphysema progression or lung function decline but that pDES was increased in those patients with COPD and underlying cardiovascular disease. A similar lack of association between pDES and lung function decline was found in patients with bronchiectasis.³⁸ This suggests that pDES appears to be more specific to vascular elastin breakdown as opposed to lung elastin and, hence, could be useful in monitoring patients with AAA.²⁸ In addition, we have previously shown that smoking, although associated with AAA diameter in our study, is not associated with pDES levels.³⁹ In our study, pDES was associated with aortic diameter independent of COPD and smoking status.

The main limitations of this study were as follows. First, although we have used 2 prospectively recruited cohorts of reasonable size, we still have relatively few events in our outcome MA³RS study cohort, limiting the ability to adjust for other confounders. We were, however, able to adjust for AAA diameter (which was the only other significant predictor of outcome in our study), and pDES remained predictive of adverse outcome. In addition, both cohorts were obtained from multiple centers, increasing generalizability of the results. Although assessment of AAA diameter using ultrasound does have limitations on intraobserver and interobserver variability, it is the main method used in clinical practice. In addition, we also found a significant correlation between pDES and computed tomographic aorta diameter within the MA³RS study cohort. Second, few women were included in our study, typical of studies in patients with AAA. This is particularly important as female sex is itself an important predictor of AAA rupture,⁴⁰ and a third of all deaths caused by AAA rupture are in women.^{41,42} This does, however, highlight a potential need for a biomarker, as on the basis of size and high surgical risk, women are often less likely to undergo AAA repair,⁴³ and screening programs for women based on aortic size, similar to that for men, do not appear to be cost-effective.⁴⁴ Finally, as recruitment to UKAGS is ongoing, we do not have follow-up data in this group of patients with smaller AAAs, and outcomes are not anticipated

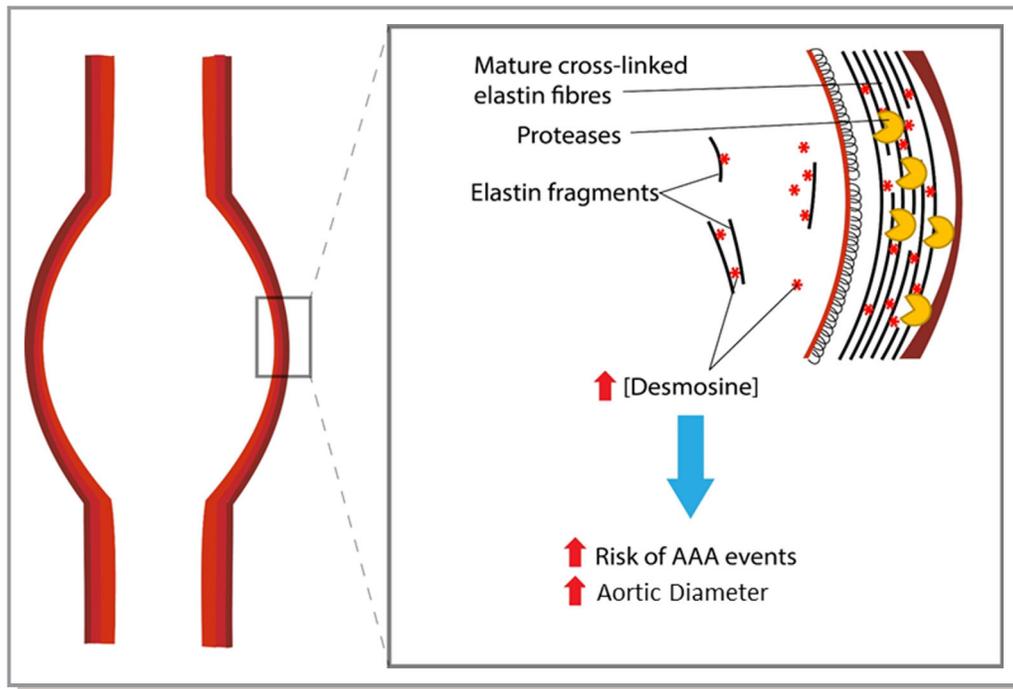


Figure 5. Desmosine is released into the circulation only when there is breakdown of mature elastin within the aortic vessel wall. Increased plasma desmosine reflects a loss of aortic structural integrity and is associated with increased abdominal aortic aneurysm (AAA) size and AAA events.

to be imminently available. Given the relatively small number of events, these results must be seen as preliminary, although intriguing.

In conclusion, we have found that pDES concentrations are increased in patients with AAA disease and correlate with disease severity. pDES concentrations also appear to predict adverse clinical outcomes, even after adjustment for AAA diameter. pDES is a promising new marker of AAA risk that is independent and incremental to AAA diameter. Larger studies are warranted to validate optimal cutoff thresholds and allow integration into clinical use.

Acknowledgments

The authors acknowledge the support of the MA³RS (Magnetic Resonance Imaging for Abdominal Aortic Aneurysms to Predict Rupture or Surgery) and UKAGS (UK Aneurysm Growth Study) Investigators.

Sources of Funding

This study was supported by a Tenovus Scotland Major Research Grant (T17/22) and a Chief Scientist Office Catalytic Grant (CGA/17/07). Dr Mordi is supported by a National Health Service Education for Scotland/Chief Scientist Office Postdoctoral Clinical Lectureship (PCL 17/07). Dr Iskandar is supported by a Tenovus Scotland Major Research Grant (T17/22). The MA³RS (Magnetic Resonance Imaging for

Abdominal Aortic Aneurysms to Predict Rupture or Surgery) study was funded by the Medical Research Council and managed by the National Institute of Healthcare Research on behalf of the Medical Research Council–National Institute of Healthcare Research partnership (National Institute of Healthcare Research Efficacy and Mechanism Evaluation Program: funding reference 11/20/03). Dr Newby is supported by the British Heart Foundation (CH/09/002, RE/13/3/30183, and RM/13/2/30158) and is the recipient of a Wellcome Trust Senior Investigator Award (WT103782AIA). Dr McBride is supported by the Academic Department of Military Surgery and Trauma. The UKAGS (UK Aneurysm Growth Study) was funded by the British Heart Foundation (CS/14/2/30841) and the Circulation Foundation. Dr Saratzis is funded by the National Institute for Health Research and Academy of Medical Sciences (SGCL13). Dr Bown is funded by the British Heart Foundation, National Institute for Health Research, and The Dunhill Trust.

Disclosures

None.

References

1. Benson RA, Poole R, Murray S, Moxey P, Loftus IM. Screening results from a large United Kingdom abdominal aortic aneurysm screening center in the context of optimizing United Kingdom National Abdominal Aortic Aneurysm Screening Programme protocols. *J Vasc Surg*. 2016;63:301–304.

2. Abdulameer H, Al Taii H, Al-Kindi SG, Milner R. Epidemiology of fatal ruptured aortic aneurysms in the United States (1999–2016). *J Vasc Surg*. 2019;69:378–384.e2.
3. Weintraub NL. Understanding abdominal aortic aneurysm. *N Engl J Med*. 2009;361:1114–1116.
4. Cosford PA, Leng GC. Screening for abdominal aortic aneurysm. *Cochrane Database Syst Rev*. 2007;CD002945.
5. Lederle FA. The last (randomized) word on screening for abdominal aortic aneurysms. *JAMA Intern Med*. 2016;176:1767–1768.
6. Brown LC, Powell JT. Risk factors for aneurysm rupture in patients kept under ultrasound surveillance: UK Small Aneurysm Trial participants. *Ann Surg*. 1999;230:289–296; discussion 296–297.
7. Lo RC, Lu B, Fokkema MT, Conrad M, Patel VI, Fillinger M, Matyal R, Schermerhorn ML. Relative importance of aneurysm diameter and body size for predicting abdominal aortic aneurysm rupture in men and women. *J Vasc Surg*. 2014;59:1209–1216.
8. Parkinson F, Ferguson S, Lewis P, Williams IM, Twine CP. Rupture rates of untreated large abdominal aortic aneurysms in patients unfit for elective repair. *J Vasc Surg*. 2015;61:1606–1612.
9. Kurvers H, Veith FJ, Lipsitz EC, Ohki T, Gargiulo NJ, Cayne NS, Suggs WD, Timaran CH, Kwon GY, Rhee SJ, Santiago C. Discontinuous, staccato growth of abdominal aortic aneurysms. *J Am Coll Surg*. 2004;199:709–715.
10. Forsythe RO, Newby DE, Robson JM. Monitoring the biological activity of abdominal aortic aneurysms beyond ultrasound. *Heart*. 2016;102:817–824.
11. Kontopodis N, Pantidis D, Dedes A, Daskalakis N, Ioannou CV. The - not so - solid 5.5 cm threshold for abdominal aortic aneurysm repair: facts, misinterpretations, and future directions. *Front Surg*. 2016;3:1.
12. Fleming C, Whitlock EP, Beil TL, Lederle FA. Screening for abdominal aortic aneurysm: a best-evidence systematic review for the U.S. Preventive Services Task Force. *Ann Intern Med*. 2005;142:203–211.
13. Golledge J, Tsao PS, Dalman RL, Norman PE. Circulating markers of abdominal aortic aneurysm presence and progression. *Circulation*. 2008;118:2382–2392.
14. Moris DN, Georgopoulos SE. Circulating biomarkers for abdominal aortic aneurysm: what did we learn in the last decade? *Int Angiol*. 2013;32:266–280.
15. Dale MA, Xiong W, Carson JS, Suh MK, Karpisek AD, Meisinger TM, Casale GP, Baxter BT. Elastin-derived peptides promote abdominal aortic aneurysm formation by modulating M1/M2 macrophage polarization. *J Immunol*. 2016;196:4536–4543.
16. Nakamura M, Tachieda R, Niinuma H, Ohira A, Endoh S, Hiramori K, Makita S. Circulating biochemical marker levels of collagen metabolism are abnormal in patients with abdominal aortic aneurysm. *Angiology*. 2000;51:385–392.
17. Saraff K, Babamusta F, Cassis LA, Daugherty A. Aortic dissection precedes formation of aneurysms and atherosclerosis in angiotensin II-infused, apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol*. 2003;23:1621–1626.
18. Zampetaki A, Attia R, Mayr U, Gomes RS, Phinikaridou A, Yin X, Langley SR, Willeit P, Lu R, Fanshawe B, Fava M, Barallobre-Barreiro J, Molenaar C, So PW, Abbas A, Jahangiri M, Waltham M, Botnar R, Smith A, Mayr M. Role of miR-195 in aortic aneurysmal disease. *Circ Res*. 2014;115:857–866.
19. Botnar RM, Wiethoff AJ, Ebersberger U, Lacerda S, Blume U, Warley A, Jansen CH, Onthank DC, Cesati RR, Razavi R, Marber MS, Hamm B, Schaeffter T, Robinson SP, Makowski MR. In vivo assessment of aortic aneurysm wall integrity using elastin-specific molecular magnetic resonance imaging. *Circ Cardiovasc Imaging*. 2014;7:679–689.
20. Groeneveld ME, Meekel JP, Rubinstein SM, Merckestein LR, Tangelder GJ, Wisselink W, Truijers M, Yeung KK. Systematic review of circulating, biomechanical, and genetic markers for the prediction of abdominal aortic aneurysm growth and rupture. *J Am Heart Assoc*. 2018;7:e007791. DOI: 10.1161/JAHA.117.007791.
21. Lindholt JS, Heickendorff L, Vammen S, Fasting H, Henneberg EW. Five-year results of elastin and collagen markers as predictive tools in the management of small abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg*. 2001;21:235–240.
22. Lindholt JS, Ashton HA, Heickendorff L, Scott RA. Serum elastin peptides in the preoperative evaluation of abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg*. 2001;22:546–550.
23. Petersen E, Gineitis A, Wagberg F, Angquist KA. Serum levels of elastin-derived peptides in patients with ruptured and asymptomatic abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg*. 2001;22:48–52.
24. Luisetti M, Ma S, Iadarola P, Stone PJ, Viglio S, Casado B, Lin YY, Snider GL, Turino GM. Desmosine as a biomarker of elastin degradation in COPD: current status and future directions. *Eur Respir J*. 2008;32:1146–1157.
25. Forsythe RO, Syed M, Newby DE. Response to letter regarding article, “aortic wall inflammation predicts abdominal aortic aneurysm expansion, rupture, and need for surgical repair.” *Circulation*. 2018;137:1295–1296.
26. University of Leicester. The United Kingdom Aneurysm Growth Study (UKAGS)—Study Information. Available at: <http://www.2le.ac.uk/projects/ukags>. Accessed September 23, 2019.
27. MA³RS Study Investigators. Aortic wall inflammation predicts abdominal aortic aneurysm expansion, rupture, and need for surgical repair. *Circulation*. 2017;136:787–797.
28. Bath MF, Saratzis A, Saedon M, Sidloff D, Sayers R, Bown MJ; UKAGS investigators. Patients with small abdominal aortic aneurysm are at significant risk of cardiovascular events and this risk is not addressed sufficiently. *Eur J Vasc Endovasc Surg*. 2017;53:255–260.
29. Bath MF, Sidloff D, Saratzis A, Bown MJ. Impact of abdominal aortic aneurysm screening on quality of life. *Br J Surg*. 2018;105:203–208.
30. Albarbarawi O, Barton A, Miller D, McSharry C, Chaudhuri R, Thomson NC, Palmer CN, Devereux G, Huang JT. Characterization and validation of an isotope-dilution LC-MS/MS method for quantification of total desmosine and isodesmosine in plasma and serum. *Bioanalysis*. 2013;5:1991–2001.
31. Lindholt JS, Heickendorff L, Henneberg EW, Fasting H. Serum-elastin-peptides as a predictor of expansion of small abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg*. 1997;14:12–16.
32. Filardo G, Powell JT, Martinez MA, Ballard DJ. Surgery for small asymptomatic abdominal aortic aneurysms. *Cochrane Database Syst Rev*. 2015;2:CD001835.
33. Golestani R, Sadeghi MM. Emergence of molecular imaging of aortic aneurysm: implications for risk stratification and management. *J Nucl Cardiol*. 2014;21:251–267; quiz 268–270.
34. Sharp MA, Collin J. A myth exposed: fast growth in diameter does not justify precocious abdominal aortic aneurysm repair. *Eur J Vasc Endovasc Surg*. 2003;25:408–411.
35. Urbonavicius S, Urbonaviciene G, Honore B, Henneberg EW, Vorum H, Lindholt JS. Potential circulating biomarkers for abdominal aortic aneurysm expansion and rupture: a systematic review. *Eur J Vasc Endovasc Surg*. 2008;36:273–280; discussion 281–282.
36. Wanhainen A, Mani K, Golledge J. Surrogate markers of abdominal aortic aneurysm progression. *Arterioscler Thromb Vasc Biol*. 2016;36:236–244.
37. Lindholt JS, Vammen S, Fasting H, Henneberg EW, Heickendorff L. The plasma level of matrix metalloproteinase 9 may predict the natural history of small abdominal aortic aneurysms: a preliminary study. *Eur J Vasc Endovasc Surg*. 2000;20:281–285.
38. Chalmers JD, Moffitt KL, Suarez-Cuartan G, Sibila O, Finch S, Furrie E, Dicker A, Wrobel K, Elborn JS, Walker B, Martin SL, Marshall SE, Huang JT, Fardon TC. Neutrophil elastase activity is associated with exacerbations and lung function decline in bronchiectasis. *Am J Respir Crit Care Med*. 2017;195:1384–1393.
39. Huang JT, Chaudhuri R, Albarbarawi O, Barton A, Grierson C, Rauchhaus P, Weir CJ, Messow M, Stevens N, McSharry C, Feuerstein G, Mukhopadhyay S, Brady J, Palmer CN, Miller D, Thomson NC. Clinical validity of plasma and urinary desmosine as biomarkers for chronic obstructive pulmonary disease. *Thorax*. 2012;67:502–508.
40. Sweeting MJ, Thompson SG, Brown LC, Powell JT. Meta-analysis of individual patient data to examine factors affecting growth and rupture of small abdominal aortic aneurysms. *Br J Surg*. 2012;99:655–665.
41. Nelissen BG, Herwaarden JA, Pasterkamp G, Moll FL, Vaartjes I. Shifting abdominal aortic aneurysm mortality trends in The Netherlands. *J Vasc Surg*. 2015;61:642–647.e2.
42. Office for National Statistics. Deaths registered in England and Wales (series DR). 2016; 2018. Available at: <https://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/deaths/bulletins/deathsregistrationsummarytables/2016>. Accessed September 23, 2019.
43. Starr JE, Halpern V. Abdominal aortic aneurysms in women. *J Vasc Surg*. 2013;57:3S–10S.
44. Sweeting MJ, Masconi KL, Jones E, Ulug P, Glover MJ, Michaels JA, Bown MJ, Powell JT, Thompson SG. Analysis of clinical benefit, harms, and cost-effectiveness of screening women for abdominal aortic aneurysm. *Lancet*. 2018;392:487–495.