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The Impact of Diabetes and Time on the Atherosclerotic Plaque and Cardiovascular Outcome in Patients Undergoing Iliofemoral Endarterectomy

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WHAT THIS PAPER ADDS

The current study shows how iliofemoral plaques changed over a period of 12 years for diabetic and non-diabetic patients. An important finding is that the percentage of calcified plaques did not decrease over time in patients with diabetes, whereas it did decrease in patients without diabetes. Moreover the secondary cardiovascular event rate remained high in patients with diabetes in contrast to a time dependent decrease in patients without diabetes. These findings stress the need for improvement of care in iliofemoral endarterectomy patients with diabetes.

Objective: The incidence of diabetes is rapidly increasing and diabetes is associated with an increased risk of peripheral artery disease. Recent studies have shown a time dependent decline in vulnerable plaque features and secondary cardiovascular events in iliofemoral endarterectomy (IFE) patients. IFE patients with diabetes have a high risk of cardiovascular events. It is not known, however, whether vulnerable plaque features and cardiovascular events reduce over time in IFE patients with diabetes.

Methods: Between 2003 and 2014, 691 atherosclerotic plaques were obtained by IFE, from 212 patients with and 479 patients without diabetes. Plaques were immunohistochemically stained and analysed for the presence of intraplaque haemorrhage, lipid core, calcification, collagen, smooth muscle cells, and macrophages. Patients were stratified according to their diabetic status and year of inclusion. All patients had a follow up of three years in which cardiovascular adverse events were recorded.

Results: A time dependent decrease was observed in intraplaque haemorrhage, plaque lipid core, and percentage of macrophages in IFE patients with diabetes. After multivariable correction for changes in risk factors over time, intraplaque haemorrhage (64.2% [2002—2005] vs. 39.6% [2012—2014], $p = .01$) became significantly less prevalent. Interestingly, the percentage of severely calcified plaques remained high over time. The number of secondary events decreased over time in patients without diabetes (HR 1.80, 95% CI 1.15—2.81 ($p = .010$) for 2002—2005 vs. 2012—2014), but remained high and unchanged in patients with diabetes.

Conclusion: In patients with diabetes undergoing IFE, a time dependent stabilisation of atherosclerotic plaque features was found in line with previous observations in patients with severe atherosclerosis. The presence of severely calcified lesions remained high and unchanged. The secondary event rate remained high in patients with diabetes in contrast to a significant decrease in patients without diabetes. These findings stress the need for improvement of care in IFE patients with diabetes.

Keywords: Diabetes mellitus, Plaque histology, Peripheral arterial disease, Cardiovascular outcomes
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INTRODUCTION

The number of individuals diagnosed with type 2 diabetes is rapidly increasing.[1—3] As diabetes is associated with an increased risk of peripheral artery disease (PAD), it is expected that vascular specialists will have to treat more limbs from diabetic patients in the near future.[4]

Although the incidence of diabetes is increasing, recent studies have shown a time dependent stabilisation of both

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carotid and iliofemoral endarterectomy (IFE) atherosclerotic plaques. Histological features of plaque instability (large lipid core, high lipid content, plaque macrophages, and intraplaque haemorrhage) were less prevalent within plaques obtained during the most recent years when compared with plaques obtained in earlier years. Furthermore a reduced incidence of cardiovascular events was reported during three years of follow up in IFE patients.[5,6]

A possible explanation for the observed plaque stabilisation and reduced incidence of secondary cardiovascular events is the dramatic changes made in cardiovascular risk management of patients with atherosclerotic disease.[7,8] Until the beginning of 2000, separate guidelines existed for each risk factor and patients were treated if their risk factor exceeded a certain threshold. Nowadays, all cardiovascular risk factors are treated aggressively despite these previously determined thresholds. This is especially true for non-diabetic patients as most diabetic patients have already been subjected to years of aggressive primary prevention regimens since their year of diagnosis.[9]

It is unknown, however, whether this reduction in vulnerable plaque features and secondary cardiovascular events also occurs in IFE patients with diabetes who are known to have more severely calcified plaques.[10] Moreover, in the Netherlands, patients with claudication are first treated conservatively with supervised walking therapy before invasive therapy. It is unclear whether these treatment strategies have influenced outcomes for diabetic patients.

Considering all the changes occurring in the diabetic PAD population, insight into the underlying reason for PAD and clinical follow up over the last decade is essential for understanding how the disease course changes over time. This study investigated atherosclerotic plaques and three year cardiovascular follow up of IFE patients with and without diabetes included over a 12 year period.

METHODS

Athero-express biobank study

Data from the Athero-Express Biobank Study (AE) were used for this study. The AE is an ongoing prospective study continuously collecting atherosclerotic plaques from patients undergoing IFE or carotid endarterectomy. The AE started in 2002 at the University Medical Centre Utrecht and the St. Antonius Hospital Nieuwegein in the Netherlands and has since collected over 2800 plaques, making it the largest plaque biobank in the world.[11] The University Medical Centre Utrecht is an academic centre and the St. Antonius Hospital Nieuwegein is a community centre, and both hospitals serve as tertiary care centres. The study design has been reported previously but, in brief, atherosclerotic plaques of patients undergoing IFE are harvested during surgical revascularisation and are directly processed for histological staining.[11,12] Between July 2002 and October 2014, 691 patients underwent IFE at one of the two institutions. Patients considered eligible for the current analysis did not have missing data on diabetic status or plaque histology. Furthermore, when patients had multiple entries in the AE (e.g. patients operated on both left and right iliofemoral arteries), only the first entry was used for analysis. All included patients provided written informed consent and medical ethics committees from both participating hospitals approved the study. Included patients completed an extensive questionnaire regarding medical history, cardiovascular history, medication, family history of cardiovascular disease, functional status, quality of life, and presence of general cardiovascular risk factors. Missing information was supplemented with information provided by the general practitioner or hospital data systems. The cohort was stratified based on diabetic status, and time dependent plaque changes were investigated separately for diabetic and non-diabetic patients.

Definition of diabetes

Diabetic disease status was defined as follows: patients receiving insulin or oral glucose inhibitors as stated in the questionnaire extracted from the hospital patient file, or diabetes in medical history obtained from the patient file, or self reported diabetes mellitus in the questionnaire. Moreover, the blood glucose measurement presented was the last pre-operative measurement within a one month range.

Follow up

All patients were contacted annually during a three year follow up for the occurrence of adverse cardiovascular events or hospitalisations for cardiovascular disease. When a patient indicated an event or hospitalisation had occurred, hospital data records were checked for validation of the event. When no hospital records were present for the indicated event, the general practitioner was contacted directly. In the case of no response, the general practitioner was contacted for the occurrence of adverse cardiovascular events during three year follow up. For the current analyses, the endpoint was a composite consisting of stroke, myocardial infarction, cardiovascular death, coronary interventions (percutaneous coronary intervention or coronary artery bypass grafting), peripheral intervention (percutaneous transluminal angioplasty or surgical endarterectomy), and leg amputation.

Atherosclerotic plaque processing

Plaque processing protocols have been reported previously, but, in brief, atherosclerotic plaques were immediately processed after IFE.[11] The culprit lesion was determined in the location with most severe atherosclerotic plaque burden. This culprit lesion was cut into 5 mm segments along the longitudinal axis, and then further processed for immunohistochemical staining.[13] Plaques were stained with haematoxylin eosin (HE) for a general overview including fat content and calcifications, alpha actin for smooth muscle cells (SMC), CD68 for macrophages, picro-sirius Red (PSR) for collagen, and HE and fibrin for intraplaque haemorrhage. Presence of calcifications, collagen content, and intraplaque haemorrhage were scored semi-quantitatively either as no/minor (0) or moderate/heavy staining (1). Furthermore, plaques were scored for lipid content in which a cut off of more or less than 10% fat was used stratifying between fibrous (less than 10% fat) and fibro-atheromatous plaques. Macrophage content and SMC content were scored quantitatively using computerised analysis software AnalySIS 3.2 (Soft Imaging Systems GmbH, Münster, Germany), and reported as percentage positive staining per plaque area. Intra- and inter-observer variability were examined previously and showed good reproducibility (κ 0.6—0.9).[12]

Statistical analyses

To study time dependent differences in diabetic patients, all diabetic patients were selected based on the previously determined diabetes definition. The cohort was then stratified in quartiles based on date of inclusion. Differences in baseline characteristics over time were tested using Pearson chi-square for dichotomous variables and one way ANOVA was used for normally distributed continuous variables. For parameters showing a non-parametric distribution, the Kruskal—Wallis test was applied to assess differences over time. The first quartile was then compared with the other quartiles using multivariable logistic regression analyses. To test the association between operation year and binary plaque characteristics, multivariable logistic regression analyses were performed with correction for possible confounders. Confounders for time dependent differences were determined as follows: baseline characteristics which changed over time ($p < .2$) and associated in univariable analyses with the plaque characteristic of interest ($p < .2$). Possible confounders added to the multivariable models were operation type, serum cholesterol and high density lipoprotein (HDL) cholesterol for lipid core, and surgery type for collagen. For smooth muscle cells, the type of surgery and operated artery was added; for macrophages, surgery type was added to the multivariable model. Cox regression was used for survival analyses. Baseline characteristics that changed over time, associated with the endpoint of interest ($p < .2$), were determined as possible confounders and added to the multivariable model. In non-diabetic patients, estimated glomerular filtration rate and surgery type were added to the multivariable model; in diabetic patients, operated artery, surgery type, and HDL cholesterol were added to the multivariable model. p values < 0.05 were considered to be statistically significant. Missing data were imputed using single imputation with R (R computing platform version 3.0.2), all statistical analyses were performed using SPSS version 21.0 (SPSS Inc, Chicago, IL, USA).

RESULTS

From 2002 until 2014, a total of 691 unique IFE plaques were collected in the AE. These plaques were obtained from 212 IFE patients with, and 479 without diabetes. A table showing the baseline characteristics of patients with and without diabetes has been added to the supplementary file (Table S1). Patients were first stratified on diabetic status and then further stratified into four groups of three years based on their date of inclusion.

In IFE patients with diabetes, no large differences were observed in baseline characteristics for diabetic patients (Table 1) within the 12 years of the study period. The number of patients with a previous history of coronary artery disease, stroke, or peripheral intervention remained unchanged. Furthermore, the indication for surgery did not alter over time. The only significant differences that could be observed were lower levels of total and HDL cholesterol within the last three time periods, whereas levels of low density lipoprotein (LDL) remained stable over time.

In IFE patients without diabetes more differences were observed (Table 2). First, there was a non-significant trend with a higher Fontaine classification over time. Furthermore, patients who had surgery within the last time periods compared with the first time period were older, more often treated for hypertension, and levels of both total as well as LDL cholesterol were significantly lower. The percentage of remote endarterectomy decreased over time for both diabetic and non-diabetic patients.

Plaque histology patients with diabetes

Over time, plaque morphology changed in the IFE population with diabetes. IFE patients with diabetes demonstrated a more stable plaque phenotype within the 2009—2012 and 2012—2014 time periods when compared with the 2002—2005 and 2006—2008 time periods (Table 3). The percentage of plaques with a lipid core, presence of intraplaque haemorrhage, and severe calcification decreased over time (Table 3). After correction for potential confounders, the presence of intraplaque haemorrhage was the only plaque feature that remained statistically significant (Table 4). The amount of quantitatively determined SMCs and macrophages did not show a gradual decline over time. Fig. 1 demonstrates the binary plaque features over time for both diabetic and non-diabetic IFE patients. Interestingly, IFE patients with diabetes and IFE patients without diabetes demonstrate the same pattern over time for the percentage of plaques with a lipid core, intraplaque haemorrhage, or moderate or heavy collagen staining. However, a different pattern was observed for the percentage of severely calcified lesions. Non-diabetic patients demonstrated a lower percentage of plaque calcifications in the 2009—2011 and 2012—2014 periods. In patients with diabetes, the high percentage of severely calcified lesions persisted throughout the first three periods (2002—2011) and only showed a decrease in the 2012—2014 period.

Cardiovascular outcomes

Overall, patients with diabetes had a higher incidence of secondary cardiovascular endpoints (any cardiovascular endpoint) compared with non-diabetic patients (Table 5). Although it was not the primary goal to directly compare endpoints between IFE patients with and without diabetes, the average incidence of all separate components of the

Table 1. Baseline characteristics of diabetic iliofemoral endarterectomy patients over time.

Year	2002–2005	2006–2008	2009–2011	2012–2014	*p* value[a]
Number of patients	*n* = 53	*n* = 53	*n* = 53	*n* = 53	
Male gender, *n* (%)	43 (81.1)	40 (75.5)	37 (69.8)	43 (81.1)	.453
Age in years, mean ± SD	67.3 ± 9.6	67.6 ± 9.0	68.7 ± 9.1	69.6 ± 7.6	.520
Body mass index (BMI), mean ± SD	27.3 ± 3.5	27.5 ± 4.8	27.9 ± 4.4	27.4 ± 4.5	.918
Current smoker, yes *n* (%)	21 (40.4)	17 (32.1)	14 (26.4)	16 (30.2)	.474
Hypertension/drug use, yes *n* (%)	41 (80.4)	39 (75.0)	42 (80.8)	43 (86.0)	.579
Hypercholesterolaemia, yes *n* (%)	38 (74.5)	39 (79.6)	34 (81.0)	32 (76.2)	.870
History of coronary artery disease (CAD), yes *n* (%)	25 (47.2)	30 (56.6)	23 (43.4)	26 (49.1)	.580
History of stroke, yes *n* (%)	4 (7.8)	1 (1.9)	3 (6.0)	4 (8.3)	.497
History of peripheral intervention, yes *n* (%)	30 (56.6)	22 (41.5)	26 (50.0)	32 (60.4)	.225
History of amputation, yes *n* (%)	5 (9.6)	6 (11.5)	4 (9.1)	4 (8.2)	.950
Fontaine classification					
Fontaine IIb	23 (47.9)	21 (42.0)	20 (50.0)	14 (35.0)	.823
Fontaine III	10 (20.8)	12 (24.0)	7 (17.5)	12 (30.0)	
Fontaine IV	15 (31.3)	17 (34.0)	13 (32.5)	14 (35.0)	
Operated artery					
femoral	46 (86.8)	49 (92.5)	53 (100.0)	50 (94.3)	.054
iliac	7 (13.2)	4 (7.5)	–	3 (5.7)	
Operation type					
remote endarterectomy (REA)	17 (32.1)	17 (32.1)	8 (15.1)	7 (13.2)	**.022**
thrombo-endarterectomy (TEA)	36 (67.9)	36 (67.9)	45 (84.9)	46 (86.8)	
Systolic blood pressure, mmHg, mean, ± SD	148 ± 29	144 ± 22	149 ± 18	143 ± 25	.599
Diastolic blood pressure, mmHg, mean, ± SD	79 ± 13	75 ± 11	77 ± 12	74 ± 16	.339
Glucose in mmol/L fasting, mean ± SD	8.7 ± 4.6	7.8 ± 2.5	8.6 ± 2.7	7.2 ± 1.8	.269
Estimated glomerular filtration rate (eGFR) in mL/min/1.73 m^2, mean ± SD	73.4 ± 23.3	74.3 ± 32.9	74.0 ± 32.8	77.1 ± 28.3	.932
Triglycerides in mmol/L, mean ± SD	2.4 ± 1.5	2.0 ± 1.3	2.1 ± 0.9	2.0 ± 1.5	.738
Total cholesterol in mmol/L, mean ± SD	5.0 ± 1.3	4.1 ± 1.3	4.1 ± 1.1	4.2 ± 1.1	**.023**
High density lipoprotein (HDL) in mmol/L, mean ± SD	1.4 ± 0.5	1.0 ± 0.2	1.0 ± 0.3	1.0 ± 0.4	**<.001**
Low density lipoprotein (LDL) in mmol/L, mean ± SD	2.2 ± 0.7	2.2 ± 1.0	2.1 ± 0.7	2.3 ± 1.0	.879
Statin use, yes *n* (%)	37 (69.8)	42 (79.2)	43 (81.1)	43 (81.1)	.439
Antiplatelet use, yes *n* (%)	44 (83.0)	38 (71.7)	41 (77.4)	45 (84.9)	.328
Insulin use, yes *n* (%)	18 (34.0)	21 (39.6)	14 (26.4)	17 (32.1)	.545
Oral glucose inhibitor, yes *n* (%)	36 (67.9)	34 (64.2)	41 (77.4)	38 (71.8)	.491
Anticoagulant use, yes *n* (%)	13 (24.5)	15 (28.3)	8 (15.1)	8 (15.1)	.225

BMI = body mass index; CAD = coronary artery disease; eGFR = estimated glomerular filtration rate; HDL = high density lipoprotein; LDL = low density lipoprotein; REA = remote endarterectomy; SD = standard deviation; TEA = thrombo-endarterectomy. Bold values were considered statistically significant when $p < .05$.

[a] Comparison of the four groups by univariable analysis.

composite endpoint was higher in patients with diabetes. The occurrence of endpoints in IFE patients with diabetes did not change and remained high over time (Table 6). However, for patients without diabetes the percentage of patients with a composite endpoint was significantly lower during the 2002–2005 and 2006–2008 periods when compared with the 2012–2014 period. This was mainly caused by a significant decrease in peripheral interventions during the 2009–2011 and 2012–2014 periods. These differences remained significant after correction for confounders (Table 6, Fig. 2). Considering that peripheral interventions were an important part of the secondary interventions, insight into the specific target vessel revascularisation (TVR) is crucial. As presented in Table 5, TVR remained unchanged in IFE patients with diabetes but decreased over time in IFE patients without diabetes ($p = .002$). Considering the relatively small number of events and small groups, no multivariable analyses were performed on these secondary events. Sex can be an important factor influencing the number of secondary events in patients with atherosclerotic disease. This interaction for sex was tested by adding an interaction term to the multivariable analyses. Within IFE patients with diabetes no statistically significant interaction was seen between male and female patients on cardiovascular outcomes and this is probably because of insufficient power. However, female patients are known to have a worse outcome after surgical revascularisation and sex stratified outcomes have therefore been added to the supplementary tables (Tables S2 and S3).[14]

DISCUSSION

As the prevalence of type 2 diabetes is increasing, furthering knowledge of the effects of diabetes on atherosclerotic plaque morphology is of great importance. Primary

Table 2. Baseline characteristics of non-diabetic iliofemoral endarterectomy patients over time.

Year	2002–2005	2006–2008	2009–2011	2012–2014	p value[a]
Number of patients	n = 120	n = 120	n = 120	n = 119	
Male gender, n (%)	86 (72)	83 (69)	86 (72)	88 (74)	.880
Age in years, mean ± SD	66.5 ± 9.55	66.7 ± 9.46	67.9 ± 9.39	69.3 ± 8.94	.080
Body mass index (BMI), mean ± SD	25.4 ± 3.7	25.2 ± 4.2	25.5 ± 3.3	25.6 ± 4.0	.864
Current smoker, yes	58 (48)	50 (43)	49 (42)	45 (38)	.459
Hypertension/drug use, yes	70 (58)	78 (65)	91 (78)	90 (80)	**.001**
Hypercholesterolaemia, yes	74 (62)	74 (65)	72 (72)	77 (73)	.252
History of coronary artery disease (CAD), yes	37 (31)	51 (43)	49 (41)	50 (42)	.203
History of stroke, yes	8 (7)	4 (3)	4 (3)	9 (8)	.258
History of peripheral intervention, yes	53 (44)	58 (48)	53 (45)	56 (47)	.901
History of amputation, yes	2 (2)	4 (3)	4 (4)	2 (2)	.669
Fontaine classification					.262
Fontaine IIb	78 (66)	65 (57)	46 (51)	54 (55)	
Fontaine III	28 (24)	33 (29)	30 (33)	24 (24)	
Fontaine IV	13 (11)	16 (14)	15 (16)	20 (20)	
Operated artery					
femoral	102 (85)	102 (85)	114 (95)	116 (97)	<**.001**
iliac	18 (15)	18 (15)	6 (5)	3 (3)	
Operation type					
remote endarterectomy (REA)	55 (46)	29 (24)	17 (14)	22 (18)	<**.001**
thrombo-endarterectomy (TEA)	65 (54)	91 (76)	103 (86)	97 (82)	
Systolic blood pressure, mmHg, mean ± SD	153 ± 25	147 ± 22	146 ± 25	150 ± 26	.158
Diastolic blood pressure, mmHg, mean ± SD	80 ± 12	77 ± 11	77 ± 12	77 ± 12	.120
Glucose, mmol/L fasting, mean ± SD	5.8 ± 1.0	6.0 ± 2.0	5.9 ± 1.3	5.8 ± 0.8	.948
Estimated glomerular filtration rate (eGFR) in mL/min/1.73 m^2, mean ± SD	73.4 ± 23.6	80.9 ± 25.8	77.1 ± 26.2	83.3 ± 29.3	**.027**
Triglycerides, mmol/L, mean ± SD	2.0 ± 1.3	1.7 ± 0.9	1.9 ± 0.8	2.1 ± 1.2	.252
Total cholesterol, mmol/L, mean ± SD	5.1 ± 1.2	4.4 ± 0.9	4.5 ± 1.0	4.4 ± 1.0	<**.001**
High density lipoprotein (HDL), mmol/L, mean ± SD	1.2 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	1.2 ± 0.5	.397
Low density lipoprotein (LDL), mmol/L, mean ± SD	3.0 ± 1.1	2.5 ± 0.7	2.5 ± 0.8	2.3 ± 0.8	<**.001**
Statin use, yes	79 (66)	89 (74)	91 (76)	84 (71)	.325
Antiplatelet use, yes n (%)	102 (85)	98 (82)	102 (86)	103 (87)	.736
Anticoagulant use, yes n (%)	21 (18)	24 (20)	18 (15)	12 (10)	<**.001**

BMI = body mass index; CAD = coronary artery disease; eGFR = estimated glomerular filtration rate; HDL = high density lipoprotein; LDL = low density lipoprotein; REA = remote endarterectomy; SD = standard deviation; TEA = thromboendarterectomy. Bold values were considered statistically significant when $p < .05$.
[a] Comparison of the four groups by univariable analysis.

and secondary prevention of cardiovascular disease has changed within recent decades, especially for non-diabetic patients. Analysis of a large number of atherosclerotic plaques over a long period enabled investigation of trends in plaque development. A cohort of 691 IFE patients was analysed, in which 212 patients with diabetes were identified. The study showed a time dependent decrease in intraplaque haemorrhage in patients with diabetes. Other plaque features such as lipid content, collagen, calcifications, macrophage content, and SMC did not show a

Table 3. Histological atherosclerotic plaque characteristics of diabetic iliofemoral patients

Year	2002–2005	2006–2008	2009–2011	2012–2014	p value[a]
	n = 53	n = 53	n = 53	n = 53	
Semi-quantitative plaque features					
Presence of lipid core ≥10%, n (%)	19 (35.8)	11 (20.8)	6 (11.3)	14 (26.9)	**.03**
Moderate/heavy calcifications, n (%)	36 (67.9)	41 (77.4)	42 (79.2)	28 (52.8)	**.01**
Moderate/heavy collagen, n (%)	47 (90.4)	46 (86.8)	50 (94.3)	41 (78.8)	.10
Presence of intraplaque haemorrhage, n (%)	34 (64.2)	24 (45.4)	21 (39.6)	21 (39.6)	**.04**
Continuous plaque features					p value
% of positive macrophage staining per plaque, median (IQR)	0.036 (0.0100–0.235)	0.197 (0.0390–0.898)	0.029 (0.0080–0.113)	0.027 (0.0070–0.130)	<**.001**
% of positive smooth muscle cell (SMC) staining per plaque (median IQR)	1.317 (0.426–3.385)	2.377 (1.015–4.492)	1.597 (0.496–4.238)	1.543 (0.310–2.547)	.10

IQR = interquartile range; SMC = smooth muscle cell. Bold values were considered statistically significant when $p < 0.05$.
[a] Comparison of the four groups by univariable analysis.

Table 4. Multivariable plaque analysis of diabetic iliofemoral endarterectomy patients

Parameter	2006–2008 vs. 2002–2005		2009–2011 vs. 2002–2005		2012–2014 vs. 2002–2005	
	OR/Beta (95% CI)	*p* value	OR/Beta (95% CI)	*p* value	OR/Beta (95% CI)	*p* value
Binary plaque characteristic (OR)						
Presence of lipid core ≥ 10%	0.51 (0.21 1.24)	.14	0.22 (0.08–0.65)	.01	0.67 (0.28–1.65)	.39
Moderate/heavy calcifications	2.06 (0.78–5.46)	.15	1.93 (0.75–4.98)	.17	0.62 (0.26–1.42)	.25
Moderate/heavy collagen	0.70 (0.21–2.338)	.57	2.03 (0.45–9.01)	.36	0.45 (0.14–1.44)	.18
Presence of intraplaque haemorrhage	0.46 (0.21–1.01)	.05	0.37 (0.17–0.81)	.01	0.37 (0.17–0.81)	.01
Continuous plaque characteristics (Beta)						
% of positive macrophage staining per plaque	0.31 (0.11–0.35)	<.001	−0.08 (−0.19–0.06)	.31	−0.04 (−0.20–0.12)	.59
% of positive smooth muscle cell (SMC) staining per plaque	0.16 (−0.06–0.50)	.06	0.11 (−0.09–0.43)	.19	−0.02 (−0.36–0.30)	.84

OR = odds ratio; CI = confidence interval; SMC = smooth muscle cell. OR and betas are given for listed cohorts compared with the cohort 2002–2005.

gradual decline over time in patients with diabetes. The follow up data showed that occurrence of adverse cardiovascular events decreased in IFE patients who did not have diabetes but not in patients with diabetes. This decrease in adverse events in follow up was attributable to a decrease in peripheral interventions in IFE patients who did not have diabetes; in patients with diabetes, the percentage of peripheral interventions remained high during follow up. These findings stress the need for improved outcomes in IFE patients with diabetes.

The decline in intraplaque haemorrhage as found in previous work was also observed in patients with diabetes.[6] Diabetic patients are known to have a high percentage of calcified plaques, but no decline was detected over time in this diabetes specific plaque feature.[10] One possible explanation is that the persistent high prevalence of severely calcified lesions within patients with diabetes might be an effect of statin treatment. Statin use has been associated with increased calcifications of coronary arteries. Within a large post hoc analysis of more than 3400 patients with coronary artery disease undergoing serial coronary IVUS, statin therapy was significantly associated with an increase in calcification of the coronary atheroma. Moreover, high intensity statin treatment was compared with low intensity

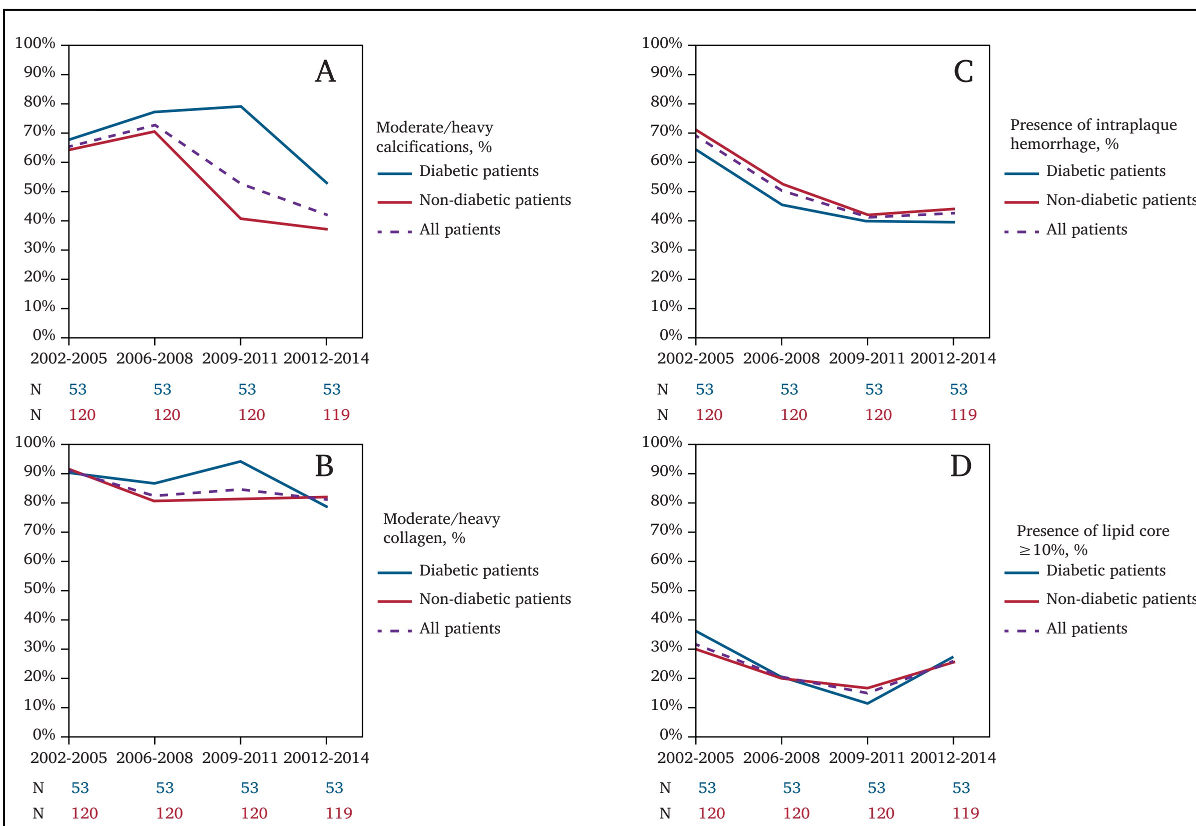

Figure 1. Percentage of patients per three year cohort with moderate/heavy plaque calcifications (panel A), intraplaque hemorrhage (Panel B), moderate/heavy collagen staining (Panel C) and lipid cores (D).

Table 5. Clinical outcomes of diabetic and non-diabetic iliofemoral patients at three year follow up

	Cohort 2002–2005	Cohort 2006–2008	Cohort 2009–2011	Cohort 2012–2014	Total cohort	*p* value[a]
Diabetic patients, (n)	52	53	52	50	207	
Endpoint (EP) composite, *n* (%)	30 (58)	29 (55)	33 (63)	29 (58)	121 (58)	.835
EP major, *n* (%)	3 (6)	7 (13)	13 (25)	8 (16)	31 (15)	.052
Death, *n* (%)	9 (17)	15 (28)	17 (33)	5 (10)	46 (22)	.024
Cardiovascular (CV) death, *n* (%)	3 (6)	4 (8)	10 (19)	3 (6)	20 (10)	.060
Peripheral interventions, *n* (%)	28 (54)	23 (43)	26 (50)	21 (42)	98 (47)	.586
Target vessel revascularisation (TVR), *n* (%)	10 (19)	8 (15)	7 (14)	10 (20)	35 (17)	.776
Percutaneous transluminal angioplasty (PTA), *n* (%)	25 (48)	18 (34)	22 (42)	21 (42)	86 (42)	.535
Leg amputations, *n* (%)	6 (12)	9 (17)	8 (15)	2 (4)	25 (12)	.185
Non-diabetic patients, (n)	119	116	115	116	466	
EP composite, *n* (%)	64 (54)	59 (51)	47 (41)	38 (33)	208 (45)	.004
EP major, *n* (%)	13 (11)	12 (10)	10 (9)	11 (9)	46 (10)	.945
Death, *n* (%)	20 (17)	20 (17)	16 (14)	17 (15)	73 (16)	.875
CV death, *n* (%)	9 (8)	8 (7)	8 (7)	6 (5)	31 (7)	.897
Peripheral interventions, *n* (%)	55 (46)	48 (41)	39 (34)	25 (22)	167 (36)	.001
TVR, *n* (%)	26 (22)	15 (13)	7 (6)	11 (10)	59 (13)	.002
PTA, *n* (%)	53 (45)	44 (38)	37 (32)	25 (22)	159 (34)	.002
Leg amputations, *n* (%)	6 (5)	7 (6)	4 (3)	2 (2)	19 (4)	.367

CI = coronary interventions; CV = cardiovascular death; EP = endpoint; MI = myocardial infarction; TVR = target vessel revascularisation; PTA = percutaneous transluminal angioplasty.
EP composite includes CV, stroke, MI, CI, and peripheral interventions. EP major includes all cardiovascular death, and all cerebral and myocardial infarctions.
[a] Comparison of the four groups by univariable analysis.

Table 6. Multivariate outcome analysis comparing individual time periods against 2012–2014 in diabetic and in non-diabetic patients, respectively.

Operation year	Composite endpoint in diabetic patients		Composite endpoint in non-diabetic patients	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value
2002–2005	0.87 (0.52–1.46)	.595	1.80 (1.15–2.81)	.010
2006–2008	1.05 (0.62–1.77)	.973	1.69 (1.08–2.64)	.022
2009–2011	1.18 (0.71–1.97)	.524	1.10 (0.68–1.78)	.669
2012–2014	Ref.	-	Ref.	-

CI: confidence interval; HR: hazard ratio.
The composite endpoint includes cardiovascular death, stroke, myocardial infarction, coronary interventions, and peripheral interventions.

treatment and no statin treatment, and the high intensity treatment was found to be associated with the largest increase in coronary atheroma calcification.[15] Patients with diabetes demonstrated a consistently high percentage of statin use throughout the years of inclusion. As a consequence, no significant differences in LDL levels were observed in patients with diabetes. In non-diabetic patients, however, an increase in statin use and subsequent lowering of LDL levels was observed. Another explanation might be that patients undergoing IFE have a longer duration of symptoms before the plaque is removed and plaques stabilise after an initial event. In a sample of 1455 CEA patients, no clear differences were observed in calcifications between the diabetic and non-diabetic patients.[16] This might be explained by the time between event and removal of the plaque being much shorter compared with IFE patients in whom surgical revascularisation is often postponed as long as possible, because more severely calcified lesions were found in the diabetic IFE cohort. This suggests that timing of surgical intervention could have had an effect on the plaque calcification and suggests that plaque remodelling in patients with diabetes is different from plaque remodelling in patients without diabetes.

Recent guideline changes favouring conservative therapy for patients with claudication as a first treatment and an increase in endovascular interventions has resulted in a delay between first symptoms and plaque harvesting. The number of remote endarterectomy procedures has significantly declined over time, probably because of an increase in endovascular revascularisation procedures. As patients only enter the AE biobank after an endarterectomy procedure, the exact number of patients treated endovascularly is unknown. In addition, currently, the superficial femoral artery is more often treated by endovascular procedures. As a consequence, relatively more plaques derived from the common femoral artery have been collected in

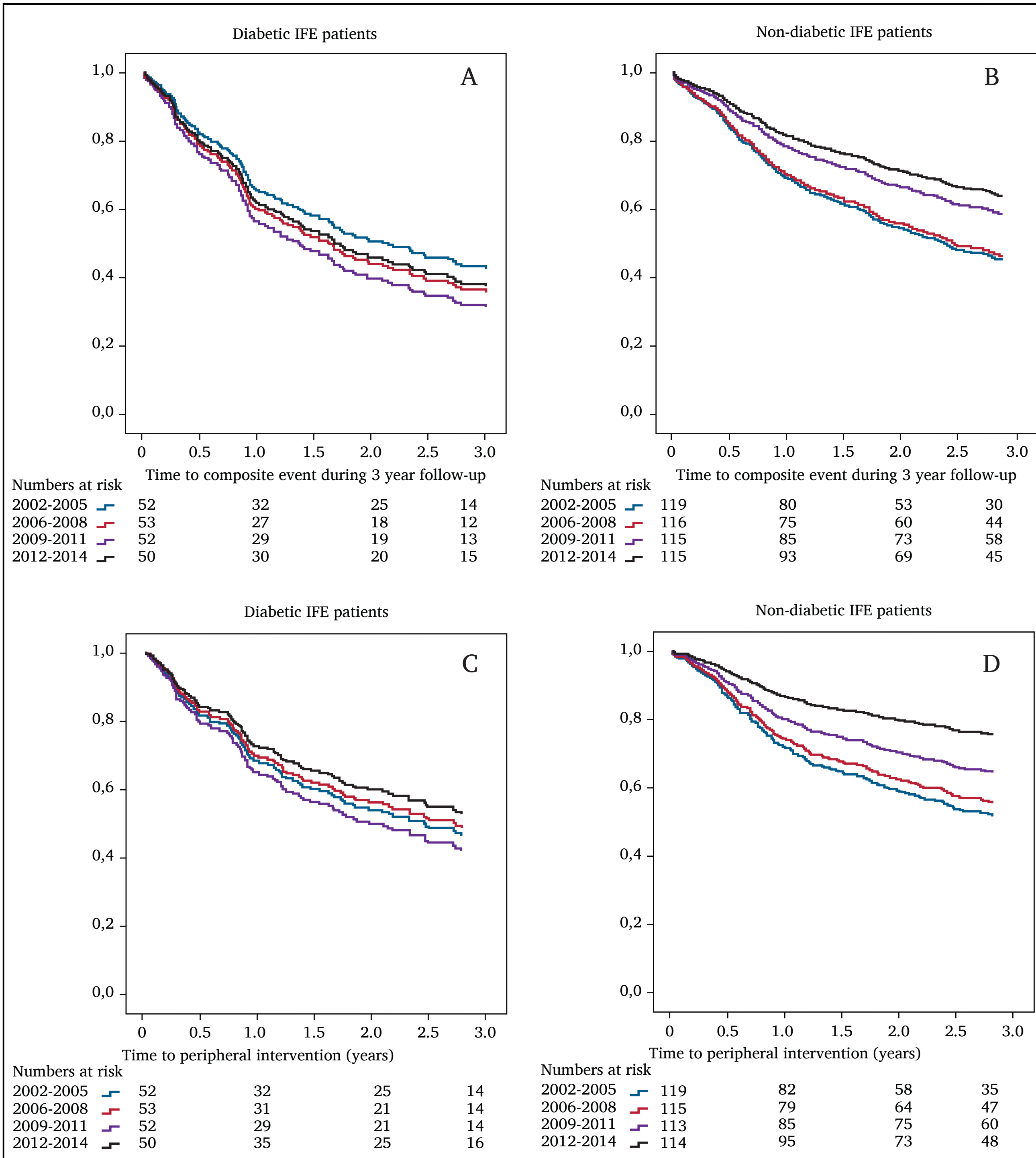

Figure 2. Hazard curves for composite events after iliofemoral endarterectomy in diabetic and non-diabetic patients. Panel A and B show hazard curves for the composite event which includes cardiovascular death, stroke, myocardial infarction, coronary interventions, and peripheral interventions. Panels C and D show hazard curves for all peripheral interventions alone. IFE = iliofemoral endarterectomy..

recent years. These effects were assessed and did not influence the plaque histology analyses.

The numbers of secondary peripheral interventions are declining over time, probably because of guideline changes which have increasingly advocated conservative therapy.[17] This could explain the decline in peripheral interventions found in IFE patients who did not have diabetes. Conservative therapy might, however, be less effective in diabetic patients. It is conceivable that therapies which strongly depend on functional status are less effective in diabetic patients because of higher body mass index (BMI) and a higher incidence of foot ulcers. Both of these features are increasingly present in the diabetic cohort with an overall BMI of 27.5 compared with 25.4 in non-diabetic patients ($p < .001$) and a Fontaine classification grade 4 in 33.1% of all diabetic patients compared with 15.2% in non-diabetic patients ($p < .001$). Overall, the higher Fontaine classifications in IFE patients with diabetes probably contributed to the overall higher incidence of secondary peripheral interventions. Moreover, in diabetic patients, disease progression is often characterised by neuro-ischaemic components such as diabetic neuropathy which warrant more aggressive re-intervention strategies as stated by the current guidelines.[18] In interaction models focussing on the combined effects of diabetic status and blood glucose levels on secondary events, only a small effect was found on outcome. These models showed that elevated blood glucose levels did not impose an additional effect on future events over diabetic disease status. This indicates that better glucose control might not provide better outcomes in this severely affected atherosclerotic population.

Clinical (treatment) perspectives

An important finding of the current study is that the percentage of plaque calcifications did not decrease over time in patients with diabetes undergoing IFE. Within more recent years, patients with diabetes had a higher prevalence of severely calcified plaques, compared with non-diabetic patients. The remaining high prevalence of severely calcified plaques is important in light of different treatment modalities applied to treat iliofemoral stenosis. It has been previously established that after angioplasty, severe calcifications are associated with poor outcome.[19–21] Calcifications are an important risk factor for stent fracture and consecutive vessel patency.[22] A negative relationship was previously described between circumferential calcification, assessed using computed tomography angiography (CTA), and vessel patency after paclitaxel coated balloon angioplasty.[23] These calcifications are believed to prevent formation of a reservoir of paclitaxel and affect drug penetration into the adventitial layer, thereby influencing outcome negatively.[23] Therefore (novel) debulking endovascular strategies are being developed in which severely calcified plaques are removed after which the lesion is treated with drug coated balloons.[24] One of the main messages of this study, however, is the great and unchanged percentage of secondary events in IFE patients with diabetes. The percentage of secondary peripheral interventions and three year mortality remained high and calls for improved treatment strategies.

Strengths and limitations

This study has some limitations. First, as guidelines have changed over the last decade, it is likely that different patients have been selected for surgical revascularisation. This is expected to have affected the number of re-interventions during follow up. The current study, unfortunately, could not provide information on conservative therapy applied in the cohorts. Second, because of the relatively small number of patients with diabetes, the study is less powered to find statistical significance for all time dependent plaque changes. Third, it was not possible to investigate disease specific markers such as HbA1c. It would be interesting to examine the severity of diabetes in relation to plaque morphology. Lastly, the relatively small sample size of diabetic patients in this population should be taken into consideration when interpreting these results. A major strength of the current study is the unique opportunity to study both the effect of diabetes and time dependency on plaque characteristics and follow up data. The 15 year long collections of plaques and clinical data have provided essential insights into changes in the pathophysiological substrate of cardiovascular disease.

In conclusion, in patients with diabetes undergoing IFE, a time dependent stabilisation of atherosclerotic plaque features including plaque haemorrhage was found, in line with previous observations in patients with severe atherosclerosis. The presence of severely calcified lesions remained high and unchanged. The secondary event rate decreased over time in patients without diabetes; however, this remained high in patients with diabetes. These findings stress the need for improvement of care in IFE patients with diabetes.

Take home messages

- Over time, iliofemoral plaques obtained from patients with diabetes showed signs of stabilisation and a decrease in the percentage of plaque haemorrhage.
- Secondary cardiovascular outcomes did not improve over time in patients with diabetes in contrast to the non-diabetic iliofemoral population who showed a decrease in the amount of secondary peripheral interventions.
- Plaque calcification is the feature strongest associated with diabetes, and plaques from patients with diabetes remained severely calcified over time.
- Changes in best medical treatment are likely to have contributed to these effects. Considering that in the past, patients with diabetes were treated aggressively with regards to blood pressure and lipid control. In patients without diabetes past treatment strategies have been less aggressive.

Multivariable model corrected for age, estimated glomerular filtration rate, hypertension, and Fontaine classification in non-diabetic patients, and operated artery, high density lipoprotein cholesterol, and type of surgery in diabetic patients.

Overview of semi-quantitatively scored iliofemoral plaque characteristics over time. Blue line: diabetic patients; red line: non-diabetic patients; green line: all patients.

Multivariable Cox regression survival analyses for both composite cardiovascular endpoints and peripheral events during three year follow up. Multivariable model corrected for age, estimated glomerular filtration rate, hypertension, and Fontaine classification in non-diabetic patients and operated artery and type of surgery in diabetic patients.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejvs.2018.10.017.

CONFLICTS OF INTEREST

None.

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