Original article

Insights from a thermography-based method suggesting higher carotid inflammation in patients with diabetes mellitus and coronary artery disease

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Abstract

Aim. Diabetes mellitus (DM) is an independent risk factor for stroke. In a DM population, carotid atheromatosus is a major cause of stroke. The role of carotid plaque inflammation remains conflicting. Microwave radiometry (MWR) is a new non-invasive method allowing in vivo measurement of the temperature of tissues, so reflecting inflammation. The aim of this prospective study was to evaluate the impact of DM on carotid artery inflammation in patients with documented coronary artery disease (CAD).

Methods. Consecutive patients (n = 300) with significant CAD were evaluated by: (1) ultrasound study of both carotid arteries; and (2) the temperature difference (ΔT) along each carotid artery on MWR. ΔT ≥ 0.90 °C was considered high ΔT. Vessel- and patient-based analyses were performed to determine the impact of DM on morphological and functional characteristics of carotid arteries.

Results. Out of 300 patients, 113 (37.7%) had DM. Patients with DM had similar carotid plaque thickness compared with patients without DM in both vessel- and patient-based analyses. In contrast, patients with DM exhibited higher ΔT values in both vessel- and patient-based analyses. On multivariate logistic regression analysis, DM was an independent predictor of high ΔT both unilaterally and bilaterally (OR: 1.66, 95% CI: 1.06–2.58, P = 0.03 and OR: 1.96, 95% CI: 1.01–3.81, P = 0.05, respectively).

Conclusion. In patients with CAD, DM was an independent predictor of local carotid plaque inflammatory activation. Whether or not the assessment of functional plaque characteristics by MWR can be an additional prognostic tool independent of structural factors now needs to be further investigated.

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1. Introduction

Diabetes mellitus (DM) is independently associated with a two- to six-fold increased rate of stroke after adjusting for all other known risk factors [1,2]. Carotid atherosclerosis is a major cause of stroke [3]. Inflammation has been suggested to play a pivotal mechanistic role in the atherogenesis in DM [4]. Nevertheless, data regarding the role of systemic inflammatory status remain conflicting. While several studies have shown that levels of high-sensitivity C-reactive protein (CRP) is a prognostic biomarker for stroke along with cardiovascular events [5], other studies have failed to show such a relationship [6–8].

Ultrasoundography constitutes an invaluable tool for the assessment of carotid atherosclerosis in DM. Intima–media thickness (IMT) and plaque score are independent predictors of stroke in patients with DM [9,10]. However, ultrasonography provides no information on the inflammatory status of the carotid arteries, whereas differences in their morphological and functional characteristics have already been demonstrated in patients with DM [11]. New methods have shown that local inflammatory activation, as quantified by 18-fluorodeoxyglucose positron emission...
tomography ($^{18}$FDG-PET), is increased in patients with DM and has prognostic implications [12–14]. Although $^{18}$FDG-PET is the most accurate non-invasive method for detection of local inflammation, its wider application is still not feasible, especially for serial evaluations. Thus, new non-invasive methods are required for assessment of inflammatory activation to stratify patients with carotid atheromatoses [15,16]. Microwave radiometry (MWR) allows in vivo non-invasive measurement of the temperature of carotid atherosclerotic plaques, thereby reflecting their inflammatory status, although, in fact, there is no evidence of a direct etiological relationship between inflammation and heat generation in atherosclerosis [17–20].

The aim of the present study was to investigate the inflammatory status of carotid atherosclerotic plaques in patients with both DM and coronary artery disease (CAD), as assessed non-invasively by MWR.

2. Methods

2.1. Study population

Consecutive patients undergoing coronary angiography for evaluation of either stable angina (SA) or acute coronary syndrome (ACS) were prospectively enrolled in the study. Patients with CAD, as documented by coronary angiography ($\geq 50\%$ stenosis in one major epicardial vessel), underwent standard carotid ultrasound examination followed by MWR measurement carried out by different blinded specialists.

The study population was divided into two groups: patients with type 2 diabetes mellitus (DM group) and patients without diabetes mellitus (non-DM group). The definition of DM was based on American Diabetes Association (ADA) diagnostic criteria [21].

Conventional risk factors for CAD, current medical therapy and laboratory data were recorded for all patients. Exclusion criteria for MWR were previous stroke or transient ischemic attacks, vasculitis, non-atherosclerotic carotid artery disease and/or intermittent inflammatory, infectious or neoplastic conditions. All participants gave their informed consent, and our institution’s ethics committee approved the study.

2.2. Ultrasound imaging

Carotid artery ultrasound imaging was performed as previously described [17,19,20]. More specifically, extracranial (common, internal, external) carotid arteries were examined with a high-resolution B-mode ultrasound unit (iE33 xMA-TRIX, Philips Healthcare, Bothell, WA, USA), using a 7.5 MHz transducer. All data were collected and interpreted by two experienced ultrasonographers (D.T., C.A.).

The ultrasound protocol included scanning of both carotid arteries from their point of origin throughout their whole length. Both the internal and external carotid arteries were investigated in transverse and longitudinal sections. The vertebral arteries were also examined and evaluated with color and power Doppler imaging.

IMT measurements were performed as previously described [22]. In particular, IMT was measured at the far wall of the distal 10 mm of the common carotid segment of each carotid artery and designated “ccIMT”. The highest value for both carotid arteries was termed “ccIMT$_{\text{max}}$”. Carotid plaques were identified as focal structures encroaching at least 0.5 mm into the arterial lumen, with focal wall thickening $\geq 50\%$ than the surrounding vessel wall or a thickness $>1.5$ mm, as measured from the intima–lumen interface to the media–adventitia interface [22]. The following parameters were evaluated in all carotid plaques: thickness; echogenicity; texture; and surface contour.

Plaque thickness measurements were performed in three 20 mm segments along each carotid artery. In each case, the middle segment was the region of common carotid bifurcation (bifurcation segment) and served as a marker. The regions 20 mm proximal and 20 mm distal to the bifurcation region defined the two other segments—namely, the common carotid artery segment and the internal carotid artery segment, respectively. Maximum plaque thickness for each segment was calculated from three preselected images. The segment of each carotid artery with the highest plaque thickness was designated the “target segment” for the following MWR measurements. The greatest plaque thickness in the target segment was also used in the vessel-based analysis. For the carotids in which no plaques were identified, the bifurcation segment was the “target segment”. The greatest plaque thickness of both carotid arteries was defined as the “max plaque thickness”.

For plaque echogenicity assessment, the Gray–Weale classification was used as previously described (Types I–V) [19,20,23–25]. Type I and II plaques were considered fatty, Type III and IV as mixed, and Type V as calcified. According to the classification previously described, fatty plaques were considered heterogeneous and mixed, and calcified plaques were homogeneous. The plaque surface was defined as regular if smooth or irregular if a variation $\geq 0.3$ mm was observed on the surface of any plaque with a thickness of 1 mm [26].

2.3. Microwave radiometry measurements

RTM-01-RES, a microwave computer-based system (Bolton, UK), was used for the MWR measurements. The system measures the temperature of internal tissues at microwave frequencies. To avoid any influence on temperature from palpation or ultrasonography, MWR measurements were obtained at least 10 min after ultrasound examination. The basic principles of MWR have been previously described [17–20]. In brief, the MWR system has an antenna with two sensors: one for microwaves and the other for infrared. The microwave sensor filters all possible microwaves or radiofrequency waves that may be present in the room vicinity and cause interference with the sensor. The antenna is 3.9 cm in diameter and detects microwave radiation at 2–5 GHz with an accuracy of 0.20 °C. The “volume under investigation” is a rectangular area 3 cm wide, 2 cm long and 3–7 cm in depth, depending on the water content of the body. The second sensor takes infrared measurements from the skin for calibrating the microwave sensor readings.
Temperature measurements were taken at each carotid artery over the segments previously defined on ultrasound imaging, including the “target segment”. The segments analyzed were 20 mm in length, starting from the proximal common carotid artery and moving distally, thus avoiding overlapping or missing areas by MWR. To do these measurements, the microwave antenna of the device was placed at a 90° angle to the skin (with contact). The antenna was held at this position for 10 s, the time required for the receiver’s microprocessor to integrate the microwave emissions and convert the measured signals to temperature. All measurements for each of the three segments were performed three times to assess the reproducibility of the method (nine measurements in total by each operator) at a room temperature between 20°C and 24°C. The mean of the three temperatures for each segment was used for further analyses. Measurements were also compared to assess intra-observer variability, while differences in the mean temperature for each segment measured by each operator were compared to assess inter-observer variability. This method has been validated as previously described [20]. The temperature difference (∆T) for each carotid artery was defined as the temperature of the “target segment” minus the lowest temperature for each carotid (reference temperature), as previously described [17,20]. In the patient-based statistical analysis, “ΔT_{max}” was defined as the maximum ∆T value of both carotid arteries, while ∆T ≥ 0.90°C was considered as high ∆T according to previous observations [20].

To evaluate the impact of microvascular dysfunction in DM on MWR measurements, right humeral artery measurements were performed in selected patients from both DM and non-DM patient groups.

2.4. Statistical analysis

Statistical analyses were performed using commercially available SPSS software (version 20, SPSS Inc., Chicago, IL, USA). Quantitative data were presented as rates or mean values ± SD. Probability values were two-sided by Student’s t test or by the Wilcoxon test for continuous variables, depending on the normal or non-normal distribution of the variables. Non-continuous values were compared by chi-square test. A value of P < 0.05 was considered significant. Spearman’s correlation coefficient was used to analyze the correlation of ∆T between left and right carotid arteries. Stepwise multiple logistic regression analysis was used to determine independent factors predicting the presence of high local inflammation, as measured by ∆T, in the carotid arteries. Correlation for intra-observer and inter-observer measurements was assessed by Pearson’s correlation test. A value of two-tailed P < 0.05 was considered statistically significant.

3. Results

3.1. Baseline demographic and clinical characteristics

A total of 352 patients were included in the screening process: 300 patients had significant CAD and, of these patients, 113 (37.7%) were in the DM group and 187 (62.3%) were in the non-DM group. Their baseline demographic and clinical characteristics as well as laboratory data and coronary angiographic results are summarized in Table 1. The mean duration of known DM was 7.94 ± 7.73 years. Mean HbA1c for the DM patients was 7.06% (54 mmol/mol).

For temperature measurements with MWR, both intra-observer (0.06°C ± 0.08°C; range: 0–0.20°C) and inter-observer (0.08°C ± 0.1°C; range: 0–0.30°C) differences were lower than the accuracy of the microwave sensor (r = 0.94 for intra-observer and r = 0.89 for inter-observer variability). Distribution of the ∆T_{max} values in both study groups is shown in Fig. 1.

3.2. Carotid ultrasound analysis

Carotid plaques were identified in 390 (65%) carotid arteries and in 235 patients with CAD. Ultrasound plaque characteristics in the two study populations are presented in Table 2.
3.2.1. Plaque texture

When only carotid arteries with carotid plaques were analyzed (n = 390), those of the DM patients had similar ccmIMT compared with carotid arteries of non-DM patients (0.88 ± 0.18 mm vs 0.89 ± 0.23 mm; P = 0.80). Moreover, carotid arteries in patients with DM had similar plaque thicknesses compared with those of the non-DM patients (2.49 ± 0.83 mm vs 2.60 ± 1.02 mm; P = 0.30).

3.2.2. Patient-based analysis

Patients in the DM group had similar ccmIMT (0.95 ± 0.17 mm vs 0.94 ± 0.17 mm; P = 0.62) and similar max plaque thickness in comparison to patients in the non-DM group (2.22 ± 0.99 mm vs 2.48 ± 1.26 mm; P = 0.07).

When only patients with carotid plaques were included in the analysis (n = 235), patients in the DM group had similar ccmIMT compared with those in the non-DM group (0.94 ± 0.18 mm vs 0.95 ± 0.17 mm; P = 0.69). DM patients (n = 83) also had similar max plaque thickness vs non-DM patients (n = 152; 2.62 ± 0.84 mm vs 2.81 ± 1.17 mm; P = 0.21, Fig. 2A). In addition, plaque thickness was similar between left and right carotid arteries in both DM and non-DM groups (2.20 ± 0.95 mm vs 2.28 ± 0.91 mm; P = 0.47 and 2.25 ± 0.90 mm vs 2.44 ± 1.27 mm; P = 0.09, respectively).

3.3. Microwave radiometry measurements

3.3.1. Vessel-based analysis

The carotid arteries of DM patients had higher ΔT compared with non-DM patients (0.85 ± 0.50°C vs 0.74 ± 0.46°C; P = 0.007). When only carotid arteries with carotid artery disease were analyzed (n = 390), those of patients in the DM group (n = 140) had higher ΔT vs those in the non-DM group (n = 250; 0.90 ± 0.50°C vs 0.79 ± 0.47°C; P = 0.04).

Multivariate analysis revealed that DM was an independent predictor of high ΔT (≥ 0.90°C) in at least one carotid artery when adjusted for gender, age, established risk factors (arterial hypertension, dyslipidemia, smoking, family history of CAD) and statin use (OR: 1.66, 95% CI: 1.06–2.58; P = 0.03).

In both DM and non-DM groups, fatty carotid plaques showed higher ΔT compared with mixed and calcified plaques (1.21 ± 0.48°C vs 0.92 ± 0.46°C vs 0.66 ± 0.47°C and 1.04 ± 0.54°C vs 0.81 ± 0.43°C vs 0.55 ± 0.35°C, respectively; P < 0.001 for both). Similarly, in both study groups, irregular plaques had higher ΔT compared with regular plaques (1.22 ± 0.51°C vs 0.78 ± 0.20°C and 1.16 ± 0.47°C vs 0.68 ± 0.42°C, respectively; P < 0.001 for both), and heterogeneous plaques had higher ΔT values than homogeneous plaques (1.19 ± 0.57°C vs 0.82 ± 0.45°C and 1.03 ± 0.53°C vs 0.71 ± 0.42°C, respectively; P < 0.001 for both).

3.3.2. Patient-based analysis

DM patients had higher ΔTmax compared with non-DM patients (1.03 ± 0.51°C vs 0.91 ± 0.46°C; P = 0.03). Patients with SA (n = 132) had similar ΔTmax compared with patients

![Graph](image-url)
patients with ACS \( (n = 168; 0.97 \pm 0.51 \, ^\circ\mathrm{C} \text{ vs } 0.95 \pm 0.46 \, ^\circ\mathrm{C}; P = 0.65) \).

In the group with carotid plaques \( (n = 235) \), DM patients \( (n = 83) \) had higher \( \Delta T_{\text{max}} \) than non-DM patients \( (n = 152; 1.07 \pm 0.52 \, ^\circ\mathrm{C} \text{ vs } 0.93 \pm 0.47 \, ^\circ\mathrm{C}; P = 0.05; \text{ Fig. 2B}) \). Also, DM was found to be an independent predictor of a high \( \Delta T \) \( (\geq 0.90 \, ^\circ\mathrm{C}) \) in both carotid arteries when adjusted for gender, age, established risk factors and statin use \( (\text{OR: } 1.96, 95\% \text{ CI: } 1.01 - 3.81; P = 0.05) \).

There was a correlation between left and right \( \Delta T \) in both DM and non-DM groups. A stronger correlation between left and right \( \Delta T \) was observed in patients with DM compared with non-DM patients \( (r = 0.57, P < 0.001 \text{ vs } r = 0.37, P < 0.001, \text{ respectively; z score: } 2.66; P = 0.008; \text{ Fig. 3}) \).

There was no statistically significant difference between left and right carotid artery \( \Delta T \) in both our DM and non-DM groups \( [\Delta T(L) = 0.82 \pm 0.47 \, ^\circ\mathrm{C} \text{ vs } \Delta T(R) = 0.89 \pm 0.48 \, ^\circ\mathrm{C} (P = 0.15) \text{ and } \Delta T(L) = 0.72 \pm 0.44 \, ^\circ\mathrm{C} \text{ vs } 0.76 \pm 0.46 \, ^\circ\mathrm{C} (P = 0.24)] \).

In patients with unilateral carotid atherosclerotic plaques \( (n = 67) \), there was also a correlation, albeit weaker, between left and right \( \Delta T \) in both the DM and non-DM groups \( (r = 0.44, P = 0.04 \text{ and } r = 0.35, P = 0.02, \text{ respectively}) \).

Ten patients in the DM group and 10 patients in the non-DM group underwent right humeral artery measurements. There was no significant difference between the two groups \( (0.31 \pm 0.12 \, ^\circ\mathrm{C} \text{ vs } 0.29 \pm 0.12 \, ^\circ\mathrm{C}; P = 0.76) \).

4. Discussion

Our present study showed that in patients with CAD and non-significant carotid artery stenosis:

- DM is an independent predictor of increased local carotid plaque inflammation both unilaterally and bilaterally;
- inflammation is more frequently bilateral in the carotids of patients with than without DM;
- morphological characteristics were similar between patients with and without DM.

The association of DM with stroke has been well established from previous studies [2]. Carotid disease is a significant cause of stroke, and inflammation plays a crucial role in the process of carotid plaque destabilization [4]. Yet, the identification and quantification of carotid plaque inflammatory activation cannot be performed by the currently available conventional imaging methods, although recent advances in novel imaging methods, such as assessing \( ^{18}\text{FDG} \) uptake by PET, can provide quantifiable measures of inflammation. However, conflicting results have been demonstrated by these new methods regarding the estimation of inflammation and its prognostic role in this high-risk population [12,15,16,27–29]. Moreover, the widespread application of this new method is currently not feasible.

MWR is a non-invasive technique that allows quantification of inflammation in internal tissues [18,19]. Previous ex
vivo [30] and in vivo experimental and human studies with MWR [18,19] have demonstrated a correlation between inflammatory activation and increases in temperature. Nevertheless, a causal relationship between heat generation and local or systemic inflammation has not been confirmed in the process of atherosclerosis. MWR has also been applied for the detection of local in vivo carotid plaque temperatures in patients with CAD [20,31]. Our present study aimed to investigate whether DM is associated with an increased carotid plaque temperature. In fact, the presence of DM was an independent predictor of plaque inflammation in both patient- and vessel-based analyses. This finding is in agreement with studies using PET scans in which type 2 DM was also associated with increased carotid wall FDG uptake in patients with known or suspected cardiovascular disease [12]. It is noteworthy that, in our present study, the two study groups with CAD did not differ in the extent of CAD or in the use of medications with potential anti-inflammatory properties, such as statins, angiotensin receptor blockers and angiotensin-converting enzyme inhibitors. Moreover, lipid levels and especially low-density lipoprotein appeared to be lower in patients with DM.

In our study population, which included intermediate lesions as shown by mean plaque thickness, bilateral inflammation was more frequent in the carotids of patients with than without DM. Previous studies had already suggested that atheromatosis of the human carotid arteries is generally a bilateral disease [15,32]. Indeed, several surrogate markers for atheromatosis, such as total atheroma wall volume or normal wall volume, have very high correlation coefficients for left vs right carotid artery volumes based on magnetic resonance imaging (MRI) [32]. Furthermore, strong associations between FDG uptake in left vs right carotid arteries were found in a recent study [15], implying the bilateral nature of the inflammatory process in carotid atherosclerotic plaques. This finding supports the concept of diffuse vascular inflammatory activation in patients with DM [33–36]. Thus, although interventional treatment is required for carotid artery disease, including stents and endarterectomy, the constraint or inhibition of systemic inflammation by pharmaceutical agents such as statins and aspirin seems to have a pathophysiological substrate. Interestingly, the association between left and right carotid ΔT was statistically stronger in patients with DM. This finding may explain the conflicting results for an additive role of CRP in our present risk scores and more accurate prediction of symptomatic carotid artery disease, as the association may be weaker in large-scale studies that include mostly patients without DM [8,37,38]. As inflammatory activation is widespread in patients with CAD and especially in the presence of DM [15,33], the correlation of ΔT between left and right carotids is reasonable. Also, local factors, such as plaque characteristics and shear stress, may explain the possible differences between left and right carotid local inflammatory activation. Thus, the value of close monitoring of systemic inflammation in patients with DM needs to be reassessed in dedicated studies.

In the present study, the two study groups did not differ in the morphological characteristics of their carotid arteries, as evaluated by ultrasound. Indeed, their cCIMT and plaque thickness values, as well as ultrasound plaque characteristics (texture, surface and echogenicity), were similar between the two patient groups with CAD. On the other hand, patients with DM exhibited higher atherosclerotic plaque temperatures on both vessel- and patient-based analyses. It appears that, in patients with non-significant carotid plaque stenosis and DM, functional abnormalities may be more profound than structural changes. The impact of such functional alterations on prognosis still remains to be elucidated.

Moreover, these functional abnormalities cannot be attributed to the microvascular dysfunction found in DM. After performing MWR measurements in the right humeral arteries of 10 patients from both study groups, there were no significant differences, thereby suggesting that carotid atherosclerotic plaque inflammation plays an important role in heat generation in DM.

4.1. Clinical implications

The results of the present study have demonstrated that DM is an independent prognostic factor for carotid plaque inflammation. The identification of inflamed plaques in intermediate carotid lesions may have important clinical implications, first because the management of this population may then be more aggressive and, second, monitoring in primary prevention protocols may also be more effective in preventing possible events.

In addition, the identification of bilateral inflammation by MWR may be a marker of patient vulnerability, whereas the presence of multiple carotid lesions with increased inflammatory activity may be a marker of plaque instability across the arterial bed. Especially in the study population with concomitant CAD, such a marker, which is so easily applied, would have significant value.

4.2. Study limitations

Our study identified carotid segments with the greatest plaque thickness for MWR measurements. However, in cases of diffuse inflammatory activation of the vessel wall, our measurements may have been underestimated, as the temperature of the reference segment with the lowest temperature may be increased. Also, the potential impact of high body surface area (BSA) on the reliability of measurements due to increased distance of the carotid arteries from the body surface cannot be excluded, although in such rare cases, slight pressure on the MWR probe was applied.

In addition, the evaluation of CAD was based on the angiographic presence of stenosis (≥ 50%), whereas implementation of invasive imaging modalities, such as intravascular ultrasound, may have increased the number of patients with extensive CAD. Thus, the sensitivity of MWR measurements may have been underestimated based on the angiographic criteria.

Although our two study groups did not differ in baseline characteristics, a proportion of patients in the non-DM group could have potentially met criteria for the metabolic syndrome, which is also associated with high arterial wall inflammation. As these patients were not excluded from the analysis, their impact may also have undermined our results.
In the present study, there was no difference in ΔT_{max} measurements between patients with ACS and SA. However, such an analysis was not the primary aim of our study and the baseline characteristics of the two specific populations were different. Furthermore, patients with carotid plaques were asymptomatic. Evaluation of carotid plaque temperatures in patients with recent stroke could have led to different results.

Systemic inflammatory markers such as CRP, tumor necrosis factor and interleukin-6 were not measured in our study population. However, the prognostic role of these inflammatory factors has been challenged by recent studies [8,39].

5. Conclusion

In patients with CAD, DM is an independent predictor of local carotid plaque inflammation, as evaluated by MWR. Further studies are now warranted to investigate whether the assessment of functional plaque characteristics by MWR independently of structural features could serve as an additional prognostic tool.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

Appendix A. Supplementary data

Supplementary data (French abstract) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.diabet.2014.05.005.

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