# Carotid imaging in cardiovascular risk assessment

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# CHAPTER 6

## The role of inflammation on atherosclerosis, intermediate and clinical cardiovascular endpoints in type 2 diabetes mellitus

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## ABSTRACT

### Background

Type 2 diabetes mellitus (T2DM) is associated with increased cardiovascular morbidity and mortality. Sub-clinical systemic inflammation is often present in T2DM patients. Systemic inflammation has also been implicated in the pathophysiology of atherosclerosis.

This review investigates the direct evidence present in literature for the effect of inflammation on atherosclerosis, specifically in the setting of T2DM. Special emphasis is given to the pathogenesis of atherosclerosis as well as intermediate and clinical cardiovascular endpoints. The important role of deteriorated endothelial function in T2DM was excluded from the analysis.

### Methods

Extensive literature searches were performed using the PubMed and Web of Science databases. Articles were identified, retrieved and accepted or excluded based on predefined criteria.

### Results

Substantial evidence was found for an important inflammatory component in the pathogenesis of atherosclerosis in T2DM, demonstrated by inflammatory changes in plaque characteristics and macrophage infiltration. Most epidemiologic studies found a correlation between inflammation markers and intermediate cardiovascular endpoints, especially intima-media thickness. Several, but not all clinical trials in T2DM found that reducing sub-clinical inflammation had a beneficial effect on intermediate endpoints. When regarding cardiovascular events however, current literature consistently indicates a strong relationship between inflammation and clinical endpoints in subjects with T2DM.

### Conclusion

Current literature provides direct evidence for a contribution of inflammatory responses to the pathogenesis of atherosclerosis in T2DM. The most consistent relation was observed between inflammation and clinical endpoints.

## INTRODUCTION

Type 2 diabetes mellitus (T2DM) is an independent risk factor for cardiovascular morbidity and mortality. Relative risk of cardiovascular disease is increased 2-3 times in diabetic men and 3-4 times in diabetic women compared to non-diabetic controls.[1-5] Atherosclerosis is the major causal factor for these cardiovascular events. Atherosclerotic plaque formation precedes the clinical signs and symptoms of cardiovascular disease. When the plaque ruptures, thrombus-formation causes rapid vascular occlusion and subsequent myocardial infarction, stroke or death.

Atherosclerosis is now considered an inflammation-driven process. In non-diabetic subjects, the presence of atherosclerosis is linked to a state of chronic systemic sub-clinical inflammation and local inflammatory mediators play a key role in the formation and eventual rupture of plaques.[6-8] The presence or development of T2DM is also associated with sub-clinical systemic inflammation. [9] In this review several examples of inflammatory pathways in T2DM are used to illustrate this association. Therefore it is likely that inflammation is an important component in the development of atherosclerosis specifically in the setting of T2DM. This paradigm seems to be generally accepted. However, due to potential differences in the pathogenesis of atherosclerosis between T2DM and non-diabetic patients, it may not be prudent to extrapolate the contribution of inflammation to atherosclerosis in T2DM from studies in non-diabetic models or patients. The strength of the evidence originating from original research directly supporting the abovementioned theory has to date not been reviewed.

Therefore the aim of the current review was to investigate the evidence present in original research publications regarding the role inflammation on atherosclerosis and its sequelae in T2DM. Special emphasis was given to the pathogenesis of atherosclerosis in diabetic patients and preclinical models, using the pathways of advanced glycation end products and the ubiquitin-proteasome system as illustrative examples. The effects of inflammation on intermediate and clinical cardiovascular endpoints in T2DM are reviewed in the final two paragraphs. The important role of endothelial dysfunction and the nitrous oxide system in the pro-inflammatory profile of diabetes have been reviewed elsewhere.[10]

## METHODS

Literature search was performed in the PubMed and Web of Science databases. Table 1 summarizes the keywords that were used for each of the three paragraphs, the number of references found (both original papers and reviews) and the number of original papers deemed relevant for citation in this review. Articles were identified, retrieved and accepted or excluded using predefined criteria: original articles had to be primarily designed to address the effect of inflammation on atherosclerosis or cardiovascular endpoints, in the setting of diabetes mellitus. If this criterion was not fulfilled, papers were eligible if inflammation was included the article as a predefined parameter of contrast in the study design, or if the role of inflammation on study endpoints has been emphasized in the publication. Several articles were included in which the effects of inflammation on atherosclerosis were evaluated in both diabetic and non-diabetic subjects. However, if the results in diabetic subgroups were not mentioned separately the articles were rejected. Review articles were excluded from the current work, although they have occasionally been used to obtain additional relevant references.

**Table 1 |** Keywords used and number of references found per topic of review.

| section | Keywords used for search |
|---|---|
| I | atherosclerosis; inflammation; diabetes mellitus |
| II | diabetes mellitus; inflammation; CRP OR c-reactive protein; fibrinogen; interleukin-6; IMT OR intima-media thickness; FMD OR flow mediated dilatation OR endothelial function OR endothelial dysfunction; PWV OR pulse wave velocity OR arterial stiffness; cardiovascular magnetic resonance; cac OR coronary artery calcification |
| III | diabetes mellitus; inflammation; CRP OF c-reactive protein; cardiovascular events; cardiovascular disease; cardiovascular mortality; ARIC; Rotterdam; Procam; Monica |

| section | Total search | Review | Original | Included |
|---|---|---|---|---|
| **I** | 428 | 219 | 209 | 12 |
| **II** | 400 | 142 | 258 | 14 |
| **III** | 2045 | 620 | 1425 | 11 |

Original articles were included if data were given on inflammation and atherosclerosis in the setting of type II diabetes mellitus. Roman numerals indicate the following topics: I) pathophysiology; II) intermediate cardiovascular endpoints; III) cardiovascular events

## RESULTS

### The Pathogenesis of Atherosclerosis in Diabetes Mellitus

Twelve papers were retrieved that could be used to review the inflammatory processes involved in the pathogenesis of atherosclerosis in the setting of T2DM. Atherosclerotic coronary plaques of diabetic subjects were found to show different characteristics with more inflammatory cell infiltration and significantly larger necrotic core size compared to non-diabetic subjects.[11;12] Macrophage plaque area, T-cell infiltration and HLA-DR expression were significantly increased in diabetic patients. The pro-inflammatory characteristics of the plaque in T2DM were also illustrated by an increase of TNF-α expression.[13] Furthermore, an increased expression of the receptor for advanced glycation end products (RAGE), which will be discussed in more detail later, was observed in diabetic plaques. The inflammatory changes observed in atherosclerotic plaques in human diabetic subjects are paralleled by very similar plaque characteristics observed in diabetic animal models. In murine models, an increase in plaque area was found in the aorta of diabetic versus non-diabetic mice. In addition, these larger plaques had qualitatively changed in an inflammatory fashion as was shown by increased expression of platelet-derived growth factor B, platelet-derived growth factor receptor, vascular cell adhesion molecule-1 and marked macrophage infiltration.[14;15] In the atherosclerotic plaques of mice with induced diabetes higher numbers of inflammatory cells and pro-atherogenic proteins were found.[15;16]



Thus, several observations point to the presence of an exaggerated inflammatory component in the diabetic atherosclerotic plaque. In contrast, no inflammatory changes but more fibrosis and thrombotic complications were observed by Sommeijer et al., in 11 carotid atherectomy specimens from patients with diabetes compared to 12 specimens from a matched patient group without T2DM.[17] In this study no differences were observed in the amount of SMCs, macrophages, T-cells and AGEs. Furthermore, no differences were found in the presence of tissue factor, endothelial protein C receptor, nuclear factor kappa B (NF-$_{\kappa}$B), and carboxymethyl-lysine-staining between lesions from T2DM patients and controls. Several explanations for this lack of relationship were suggested by the authors. The lesions were all obtained from symptomatic plaques and the

population in this study had a mean age of 70 years. AGE accumulation in tissue is more pronounced in end-stage plaques and advanced age, both in diabetics and non-diabetics.[18] Therefore, differences in inflammatory characteristics of atherosclerotic plaques between diabetics and non-diabetic controls may be more evident in younger subjects with early-stage plaques.

Further exploration revealed several pathways linking inflammation to atherosclerosis in T2DM . It is beyond the scope of the current work to review all these pathways. However, we will discuss two important mechanisms currently under active investigation and showing promise as future therapeutic targets. The receptor for advanced glycation end products (RAGE)-pathway and the ubiquitin-proteasome pathway are related to altered intimal inflammatory responses, especially in the setting of T2DM. Although the current review focuses mainly on the clinical cardiovascular implications of inflammation in T2DM the following paragraph includes several pre-clinical animal studies to clarify and illustrate the cellular mechanisms linking inflammation, atherosclerosis and T2DM.

***RAGE***

Advanced Glycation End products (AGEs) are formed intra- and extracellularly by non-enzymatic reduction of glucose, lipids and amino acids on proteins and nucleic acids. This reaction is partly driven by hyperglycemia, resulting in higher circulating- and tissue-levels of AGE in diabetic subjects. (20-30% higher in patients with uncomplicated T2DM[19] and 40-100% higher in patients with coronary[20] and renal[21] complications associated with T2DM). In T2DM, higher levels of AGEs are associated with overexpression of receptors for advanced glycation end products (e.g. AGE-R1 and RAGE). The positive role of these receptors (especially AGE-R1) is to clear AGEs from the circulation and mitigate their deleterious oxidative and inflammatory effects[22;23]. In contrast, RAGE appears to trigger a pro-inflammatory stress response, leading to cellular dysfunction[24]. RAGE is expressed in several tissues, but also in atherosclerotic plaques, more specifically in macrophages and around necrotic cores[25-27]. Non-diabetic subjects also express RAGE, but to a lesser extent and at a later age. The expression of RAGE has implications for plaque biology including influx of inflammatory cells. This may be due to the induction of numerous cytokines and adhesion molecules such as IL-6, TNF-a, MCP-1, ICAM-1 and VCAM-1. Furthermore, RAGE induced activation of cyclo-oxygenase 2 (COX-2), prostaglandine E

synthase-1 (mPGES-1), matrix metalloproteinase 2 (MMP-2) and MMP-9[28-32] may adversely affect plaque biology. In addition, it has been shown that RAGE is associated with enhanced NF- κB activity,[31-33] which directly links RAGE to the main regulator of the cellular inflammatory response. Moreover several studies have demonstrated that blocking RAGE action leads to attenuation of inflammation and plaque stabilisation.[33;34] Thus, increased RAGE-mediated cellular responses may link the biochemical consequences of hyperglycemia in T2DM to inflammatory atherogenesis and vulnerable plaque formation, by different pathways. Blockade of RAGE action is an important potential target for plaque stabilization in diabetic patients.

***Ubiquitin-Proteasome system***

There is emerging evidence that the ubiquitin-proteasome system, a major protein degradation pathway in eukaryotic cells, induces inflammation during the initiation and progression of atherosclerosis.[35] The pathway is required for activation of NF-κB, by degradation of its inhibitory IκB proteins.[36] Marfella et al.[13] studied the role of the ubiquitin-proteasome system in human carotid endarterectomy samples, and compared diabetic subjects with non-diabetic controls. Notably, ubiquitin-proteasome activity was found to be enhanced in diabetic atherosclerotic lesions, especially in the inflammatory cells therein. Activation of this system was associated with higher NF-κB and MMP activity, thereby leading to an increase in inflammation and potential destabilization of the plaques. Administration of the PPAR gamma agonist rosiglitazone inhibited the ubiquitin-proteasome pathway and partially attenuated the inflammatory changes in plaque composition. This effect was seen in vivo when rosiglitazone was given orally to subjects before endarterectomy and in vitro when rosiglitazone was added to incubated monocytes from the carotid specimens. Upregulation of the ubiquitin-proteasome pathway could be one of the mechanisms contributing to the increased inflammatory response in diabetic atherosclerotic plaques. Inhibition of this system may be a useful therapeutic target in the treatment of vulnerable diabetic plaques.

To summarize, original research articles show that the diabetic atherosclerotic plaque has prominent inflammatory characteristics, including high macrophage and T-cell content. These pro-inflammatory aspects are observed in human

subjects and experimental models. The observations in human subjects are not unequivocal and could not be demonstrated in carotid endarterectomy specimens in one study. Among the proposed mechanisms involved at the level of the intima, RAGE and the ubiquitin-protease system seem to be potential links between diabetes and the inflammatory processes of atherosclerosis.

## Intermediate Endpoints

Intermediate endpoints are surrogate markers which reflect the presence and the progression of a disease. Examples of intermediate endpoints for cardiovascular disease are Intima-Media thickness (IMT), Pulse Wave Velocity (PWV) and Flow Mediated Dilatation (FMD). There are ample epidemiological and trial data to support the use of these intermediate endpoints to provide us with valuable information regarding atherosclerosis-related processes and clinical outcomes. [37;38]

### *Epidemiological studies*

Major studies in which intermediate endpoints were assessed include the Atherosclerosis Risk in Communities (ARIC) study, the Framingham study and The Rotterdam Study. Although no published papers from these studies were set up to directly address the role of inflammation on the endpoints specifically in T2DM patients, they all included a subpopulation of diabetics. Only two large studies assessed the correlation between inflammation and intermediate endpoints in T2DM. Metcalf et al.[39] did a post hoc analysis in a part of the ARIC-population and compared a subpopulation of 921 patients with T2DM to 11.964 non-diabetic controls. Several hemostatic proteins were included in the analysis, but only fibrinogen correlated significantly with IMT in both groups. Correlation coefficients between fibrinogen and IMT are not reported separately but the relationship was significant and independent of other CVD risk factors. As a part of the INVADE-study, associations between CRP and IMT were assessed in 3,534 people, aged >55 years old, of which 882 had T2DM. [35] Multiple regression analysis revealed a positive correlation between CRP and IMT progression in T2DM patients ($\beta$=0.08; p=0.01). In contrast, in the non-diabetics CRP levels were not significantly associated with IMT progression ($\beta$=0.029; p=0.29). These data suggest that low grade systemic inflammation is relevant for the progression of atherosclerotic changes in the vessel walls of

diabetic subjects. Five smaller studies used CRP as a marker for inflammation and showed significant correlations with either IMT [40-42], FMD [43] or arterial stiffness [43;44]. Two studies found a correlation between fibrinogen and IMT [39;42], one study found a correlation between fibrinogen and arterial stiffness [44]. The results of these studies are summarized in table 3. Studies using other inflammation markers, besides the more commonly used inflammatory markers CRP and fibrinogen, have shown similar results. Leukocyte count, amyloid A protein and sialic acid significantly correlated with arterial stiffness. [45] IL-18, which stimulates release of interferon-γ, significantly correlated with IMT(r=0.224; p=0,042) and arterial stiffness (r=0.232, p=0.040) in T2DM.[46] These studies clearly show positive associations between markers for inflammation and intermediate endpoints in T2DM. However, some studies are at variance with these observations. Takebayashi et al. found no correlation between CRP or fibrinogen and IMT in 73 patients with T2DM and poor metabolic control.[47] Leionenen et al. did not find CRP as a determinant of IMT in 239 T2DM patients with cardiovascular disease.[48]. Sigurdardotter et al. concluded that there is no independent relationship between CRP and IMT in Caucasian men with either newly diagnosed or established T2DM [49]. Dullaart et al. found no correlation between CRP and IMT in 84 T2DM patients nor in the 85 controls included in their population[50]. The T2DM patients were very well controlled with use of blood glucose lowering and antihypertensive medication.

Thus, although the majority of data point toward a positive association between low grade systemic inflammation and surrogate cardiovascular endpoints in T2DM, these observations are not unequivocal. Further clarification may come from prospective studies, including clinical trials.

***Clinical trials***

Several clinical trials have been carried out to study the effect of pharmacological interventions on subclinical inflammation in T2DM. In four different studies IMT or FMD were used as intermediate markers. Medication in these different studies included metformin, atorvastatin, pioglitazone and rosiglitazone. In all studies, CRP levels were measured and related to IMT or FMD.

Rosiglitazone, compared to metformin, induced a prompt and marked reduction in CRP levels in diabetics.[51] This change was associated with regression of carotid IMT, independent of the blood glucose lowering effect of

the medication. This suggests that there is a direct correlation between CRP and IMT in T2DM. Another study found significant beneficial effects of rosiglitazone on both subclinical inflammation markers (CRP, MMP-9 and fibrinogen) and IMT in diabetics. However, after secondary analyses no correlation was found between the changes in inflammation markers and IMT regression.[52] Atorvastatin, compared with placebo, significantly decreased CRP levels and improved FMD.[53] Notably, there was no correlation between the percentage change in LDL-C and improvement of FMD, whereas the reduction of CRP levels was significantly associated with improvement of FMD. Pioglitazone treatment resulted a significant decrease in CRP concentrations and improvement of FMD irrespective of metabolic changes[54]. However, a correlation between CRP en FMD was not found, possibly due to the small sample size of the study.

To summarize, most data found about the effect of inflammation on intermediate cardiovascular endpoints support the concept of a positive relation between inflammation and atherosclerosis in the setting of T2DM. However, these observations are not unequivocal and some studies and clinical trials raise questions by demonstrating a lack of association between inflammatory parameters and surrogate markers of atherosclerosis. Moreover, the accuracy of these intermediate endpoints to predict future cardiovascular events is not undisputed, especially in T2DM populations. This point was recently illustrated by the discussion about the effects of rosiglitazone. Despite encouraging results from studies using intermediate endpoints, a recent meta-analysis questions the beneficial effect of rosiglitazone on the occurrence clinical cardiovascular events. Therefore the final paragraph will review the evidence in T2DM for associations between inflammatory markers and clinical cardiovascular endpoints.

## Clinical Endpoints

The aim of this section is to review evidence linking CRP and fibrinogen as inflammatory markers to clinical cardiovascular outcomes in T2DM. Cardiovascular events that were included in our research are coronary heart disease (CHD: coronary heart disease related death, myocardial infarction, coronary revascularization), peripheral arterial disease (PAD: leg revascularisation, leg amputation, intermittent claudication) and stroke.

***Population-based studies***

Many large population-based studies have assessed the importance of different cardiovascular risk factors, including inflammation on clinical outcomes. We evaluated these epidemiological studies for evidence of inflammation being a risk factor for the development of cardiovascular events in T2DM (sub)populations. The following studies were included: the Atherosclerosis Risk In Communities study (ARIC), the Framingham heart study, the Munster heart study (PROCAM), the Rotterdam study, the Cardiovascular Health study, the Multinational Monitoring of trends and determinants in Cardiovascular disease study (MONICA) and the Hoorn study. All studies observed a significant positive correlation between CRP levels and incidence of cardiovascular events[55-62]. However, a separate analysis of the diabetic population was made only in the Cardiovascular Health study and the Hoorn study (table 2). Relative risk for the diabetics with CRP >3 mg/L versus those with CRP level <1 mg/L was 1.49 (95%CI 1.02-2.18) whereas in non-diabetics the relative risk was found to be 1.74 (95% CI 1.30-2.32).[57] The Hoorn study provided long-term follow-up data on the effect of cardiovascular risk factors on mortality in a general population (n=2484) in The Netherlands. In a sub-analysis [63] (n=631) it was demonstrated that approximately 43% of the excess cardiovascular mortality in T2DM patients was explained by endothelial dysfunction and low-grade inflammation. The negative impact of endothelial dysfunction on survival was greater in diabetic patients than in non-diabetic patients (hazard ratio for cardiovascular mortality 1.87 [1.43-2.54] in diabetics vs. 1.23 [0.86-1.75] in non-diabetics; p=0.06). However, the contribution of low-grade inflammation to cardiovascular mortality was not significantly different in diabetic and non-diabetic subjects (hazard ratio for cardiovascular mortality 1.43 [1.17-1.77], not mentioned separately for diabetics). These findings suggest that, in the Hoorn study, the presence of endothelial dysfunction (with or without low-grade inflammation) was the main determinant of excess cardiovascular risk in diabetics compared with non-diabetic subjects. The impact of low-grade inflammation alone on cardiovascular mortality, although significant, was not different in diabetic subjects compared to their non-diabetic counterparts.

**Table 2 |** Summary of studies evaluating the effects of inflammatory markers on surrogate cardiovascular markers in T2DM patients.

| Intermediate endpoint studies | | | | | |
|---|---|---|---|---|---|
| **Authors** | **n** | **% T2DM** | **Inflammatory marker** | **CVD marker** | **Correlation (coefficient)** |
| Metcalf PA et al.[39] | 12.876 | 7.2 | Fibrinogen | IMT | + (NR) |
| Mita T et al.[40] | 75 | 100 | CRP | IMT | + (r=0.484, p<0.0001) |
| Sander D et al.[69] | 3534 | 25.0 | CRP | IMT | + (B=0.08, p=0.01) |
| Corrado E et al.[70] | 200 | 50 | CRP<br>Fibrinogen | IMT | + (r=0.591, p<0.0001) |
| Hedblad B et al.[52] | 555 | 50 | CRP | IMT | + (NR) |
| Wakabayashi I et al.[45] | 97 | 100 | Fibrinogen | PWV | + (r=0.216, p<0.050) |
| Nakamura A et al.[46] | 82 | 100 | IL-18 | IMT<br>PWV | + (r=0.225, p=0.042)<br>+ (r=0.232, p=0.040) |
| Nystrom T et al.[43] | 45 | 30.8 | CRP | FMD | None (r=-0.4, p=NS) |
| Leionen ES et al.[48] | 239 | 100 | CRP | IMT | None (NR) |
| Sigurdardottir V et al.[49] | 271 | 27.3 | CRP | IMT | None (r=0.20, p=NS) |
| Dullaart RP et al.[50] | 169 | 49.7 | CRP<br>TNF-alpha | IMT | None (NR)<br>None (NR) |

Most authors report a positive association. In several series no association could be demonstrated. T2DM=type 2 Diabetes Mellitus; CVD=cardiovascular disease; IMT=intima-media thickness; PWV=pulse wave velocity; FMD=flow mediated dilatation; CRP= C-reactive protein; IL=Interleukin; TNF=tumour necrosis factor; NR= not reported

### *Prospective follow-up studies*

In several prospective cohorts as well as cross sectional studies, populations were analyzed in order to determine whether inflammation and its markers played an important role in cardiovascular risk assessment. Only studies specifically addressing T2DM patients and studies with large diabetic subpopulations were included. In a study performed by Matsumoto et al. 350 Japanese T2DM patients were followed for a period of 1-7 years. When patients were subdivided in tertiles according to baseline CRP levels, the relative risk for cardiovascular events in patients in the highest tertile of CRP was 2.00 (95% CI 1.03-3.85) compared to patients in the lowest tertile.[64] High plasma levels of CRP were also associated with an increased risk of incident cardiovascular events among 746 T2DM men in a study performed by Schulze et al. A relative risk of 2.62 (95% CI 1.29-5.32) for cardiovascular events was observed in T2DM patients in the highest quartile of CRP compared to those in the lowest quartile was during a five year follow-up period.[65] Wattanakit et al. followed 1651 diabetic subjects for 10 years using

**Table 3 |** Summary of studies evaluating the effects of inflammatory markers on cardiovascular events in T2DM patients.

| **Large population-based studies** | | | | |
|---|---|---|---|---|
| **Study (authors)** | **n** | **% T2DM** | **Correlation CRP-CVE** | **Correlation assessed in diabetes specifically** |
| ARIC *(Ballantyne et al.[55])* | 12819 | 21% | + | no |
| ARIC *(Folsom et al.[58])* | 15792 | 6,8% | + | no |
| Framingham *(Rost et al.[61])* | 1462 | 9,4% | + | no |
| PROCAM *(Heinrich et al.[59])* | 2116 | unclear | + | no |
| Rotterdam *(Bos et al.[56])* | 6340 | 10,7% | - | no |
| MONICA *(Koenig et al.[60])* | 3435 | 5,7% | + | no |
| Cardiovascular Health *(Cushman et al.[57])* | 3971 | 14,4% | + | yes |

| **Clinical cardiovascular end-point studies** | | | | |
|---|---|---|---|---|
| **Authors** | **n** | **% T2DM** | **Follow-up period** | **RR (95% CI)** |
| Matsumoto et al.[64] | 350 | 100 | 1-7 years | 2.00 (1.03-3.85) |
| Schulze et al.[65] | 746 | 100 | 5 years | 2.62 (1.29-5.32) |
| Schillinger et al.[67] | 454 | 39,9 | 21 months | 2.13 (p=0.007) |
| Wattanakit et al.[66] | 1651 | 100 | 8.7 years | + |
| Jager et al.[68] | 610 | 27,7 | 5 years | 1.4 (0.6-3.5) |

In the large-scale epidemiological studies most authors did not specifically report associations in the T2DM sub-population. The studies that assessed diabetics demonstrated significant elevation of relative risk of a cardiovascular event of around twofold in subjects with high inflammatory markers, compared with low levels. *T2DM=type 2 diabetes mellitus; CRP=C-reactive protein; CVE=cardiovascular events; RR=relative risk*



fibrinogen levels as marker for inflammation. Risk for peripheral artery disease was found to be higher in subjects with high fibrinogen levels. The relative risk for cardiovascular events of patients in the highest quartile of fibrinogen compared to those in the lowest was 2.52 (95% CI 1.43-3.24)[66]. Schillinger et al. followed 454 patients, of which 181 had T2DM, for a median of 21 months. HbA1c and CRP were measured at baseline. Levels of CRP correlated positively with incident cardiovascular events. Hazard ratio for subjects in the highest quartile of CRP compared to those in the lowest was 2.13 (95% CI 1.22-3.70). The deleterious effect of CRP on event risk was more evident in subjects with high HbA1c levels [67].

In contrast to these studies, Jager et al. did not observe a significant elevation of relative cardiovascular mortality risk in patients in the highest tertile of CRP compared to those in the lowest (RR= 1.34; 95%CI: 0.41-4.43). The study was

performed in a sample of 169 T2DM subjects selected from a larger population of 610 subjects and followed for 5 years.[68]

***Cross-sectional studies***

In 202 diabetic patients with or without macrovascular disease mean CRP levels did not significantly differ between the groups (4.2mg/L and 5.5 mg/L respectively). Fibrinogen levels however, were significantly higher in subjects with prevalent macrovascular disease (420 mg/L versus 382 mg/L).[62]

To summarize, several large epidemiological studies showed a positive correlation between levels of inflammation markers and incidence of cardiovascular events. However, whether this can also be found in diabetic subjects was not specifically answered in most of these studies. The one study that did specifically analyse the diabetic population showed that CRP was of added value in the risk assessment in diabetes[57]. All but one follow-up study revealed that higher levels of CRP and fibrinogen were associated with higher risk for cardiovascular events[64-68]. This observation could not be extended to cardiovascular mortality[68]. Based on the current literature it seems that levels of inflammation markers predict incident cardiovascular disease, also in the setting of T2DM.

## CONCLUSIONS

At the level of cellular pathogenesis of atherosclerosis, significant differences are seen between diabetic subjects and non-diabetics. Atherosclerotic plaques show higher expression of inflammatory receptors and proteins, marked infiltration of inflammatory cells and larger necrotic-core size in the setting of T2DM. It is likely, but not unequivocally proven, that increased inflammation markers in atherosclerotic plaques are related to systemic inflammation.

Regarding intermediate endpoints there was a positive correlation with inflammation in most but not all studies in T2DM. The impact of inflammation seems to be more outspoken when related to clinical endpoints compared to intermediate endpoints. High inflammation markers are linked with more cardiovascular events, of which most are to be expected to have a negative

influence on life expectancy. This may support the hypothesis that increased cardiovascular risk in diabetes in not only due to increased progression of atherosclerosis (as measured by most intermediate endpoints) but also to more severe atherothrombosis.

Whether levels of CRP and fibrinogen can be seen as causal factors for developing cardiovascular events in type 2 diabetes patients has still to be proven. To date, most studies used them as markers of a state of low grade systemic inflammation. However, based on the current review linking systemic inflammation to the pathophysiology and outcome of atherosclerosis in T2DM, it seems warranted to further unravel the components of the systemic and local inflammatory components in prospective research. Hopefully this will result in new therapeutic strategies to treat the major cause of mortality in patients with T2DM.



**Learning Points**

- Current literature indicates that atherosclerotic plaques in the setting of type 2 diabetes mellitus exhibit more inflammatory properties than non-diabetic plaques
- Most published data suggest that elevated inflammatory markers are associated with detrimental outcome of intermediate cardiovascular endpoints in patients with type 2 diabetes mellitus. This association, however, is not unequivocally established
- The association between inflammation and unfavourable cardiovascular outcome in type 2 diabetes patients is most consistent when regarding clinical endpoints

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