Prognostic Impact of Fibrinogen in Carotid Atherosclerosis
Nonspecific Indicator of Inflammation or Independent Predictor of Disease Progression?

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Background—Fibrinogen is a key factor in the coagulation cascade, it exhibits proinflammatory properties, and it is suggested to play a pivotal role in atherogenesis. We investigated whether fibrinogen predicts future progression of carotid atherosclerosis, analyzing whether fibrinogen levels add to the prognostic information of other inflammatory parameters.

Methods—We prospectively studied 1268 consecutive patients without recent (12 months) symptoms from cerebrovascular disease. Patients underwent serial ultrasound investigations in 6- to 9-month intervals, categorizing carotid arteries as 0% to 29%, 30% to 49%, 50% to 69%, 70% to 89%, or 90% to 99% stenosed, or occluded. Fibrinogen levels were determined at baseline and follow-up. The risk for progressive carotid atherosclerosis according to fibrinogen levels was calculated, adjusting for traditional risk factors and other inflammatory parameters (C-reactive protein and serum amyloid A).

Results—Progression of carotid atherosclerosis was found in 117 of 1268 patients (9.2%) after a median of 8 months (range 6 to 18). Adjusted hazard ratios for atherosclerosis progression with increasing quartiles of baseline fibrinogen were 1.83 (\(P=0.037\)), 2.09 (\(P=0.008\)), and 2.45 (\(P=0.002\)), respectively, compared with the lowest quartile. Fibrinogen at follow-up also was associated with progressive disease (\(P=0.004\)). However, additionally adjusting for other inflammatory parameters diminished these associations to a nonsignificant level.

Conclusion—Elevated fibrinogen, reflecting the level of inflammatory activity, is associated with progression of carotid atherosclerosis, as it was demonstrated previously for other inflammatory parameters. However, this association seems to be nonspecifically related to the extent of the inflammatory process in atherosclerotic disease rather than to specific properties of fibrinogen. (Stroke. 2005;36:1400-1404.)

Key Words: carotid stenosis ■ ultrasonography ■ inflammation

Fibrinogen plays a pivotal role in the initial phase and the advanced stages of atherosclerosis. In addition to its essential properties as a cofactor of platelet aggregation and as the main substrate for thrombin in the plasmatic coagulation, fibrinogen is suggested to trigger the formation of progressive atherosclerotic plaques.1 A significant inverse relationship between plasma levels of fibrinogen and thickness of the fibrous cap of atheroma has been observed,2 which results in a greater incidence of plaque rupture and thrombosis in subjects with increased fibrinogen levels. Moreover, plaque composition of patients with elevated fibrinogen levels is characterized by the presence of a high number of inflammatory cells localized mainly in the shoulder and in the cap of the plaque.2

Progressive or so-called “vulnerable” atherosclerotic plaques are associated with a markedly increased risk for clinical complications in virtually any segment of the arterial circulation.3-5 Vulnerable lesions are histologically heterogeneous and may be characterized by an inflammatory necrotic lipid core, fibrocalcified nodules, a thin fibrous cap of the atheroma, superficial erosion, thrombus apposition, or intraplaque hemorrhage.3-5 Acute-phase reactants such as C-reactive protein (CRP), serum amyloid A (SAA), and fibrinogen provide an indirect measure of the cytokine-dependent inflammatory process in the arterial wall, which is a key feature particularly of the inflammatory activity surrounding the necrotic lipid core.6 In this context, we demonstrated previously that levels of the inflammatory parameters CRP and SAA predict the progression of atherosclerotic lesions in the carotid arteries.7 The aim of the present analyses was to assess whether fibrinogen levels are similarly associated with future progression of carotid atherosclerosis.
and to analyze whether fibrinogen levels add to the prognostic information of other inflammatory parameters (ie, whether fibrinogen is merely a nonspecific indicator of the level of inflammation or it exerts specific properties promoting disease progression). Furthermore, addressing a potential clinical impact, we investigated the predictive value of progressive carotid disease and fibrinogen levels on the occurrence of future neurological adverse events.

Methods

Study Designs and Patients

We prospectively enrolled 1268 of 1363 consecutive white patients in the Inflammation in the Carotid Arteries Risk for Atherosclerosis Study (ICARAS) protocol. Study design, patient characteristics, and the association between CRP and SAA with progression of carotid atherosclerosis have been reported. Briefly, patients without recent symptoms from carotid artery disease, assessed by a neurologist and defined as the absence of transient ischemic attacks, amaurosis fugax, or stroke 12 months before inclusion, underwent serial carotid duplex ultrasound investigations in 6- to 9-month intervals. Primary study end point was unilateral or bilateral progression of carotid atherosclerosis in the extracranial internal carotid arteries (ICAs) measured by duplex ultrasound. In the current analysis, we extended the follow-up period from initially only 1 follow-up duplex investigation at median 7 months (range 6 to 9 months) to currently up to 3 follow-up investigations after median 8 months (range 6 to 18 months) and followed patients for the occurrence of ischemic neurological events during this time as a secondary end point. The study complied with the Declaration of Helsinki and was approved by the institutional ethics committee. All patients provided written informed consent.

Carotid Ultrasound

We used the following categories to quantify the degree of ICA stenosis at baseline and follow-up: 0% to 29% (carotid plaques), 30% to 49% (advanced plaques), 50% to 69% (moderate stenosis), 70% to 89% (high-grade stenosis), 90% to 99% (subocclusive stenosis), and occlusion. The agreement of duplex ultrasound with respect to North American Symptomatic Carotid Endarterectomy Trial (NASCET) angiographic criteria was assessed previously in our duplex laboratory in an independent cohort including 1006 carotid arteries. Positive and negative predictive values ranged from 70% to 98%, and interobserver agreement with respect to the degree of stenosis was excellent ($\kappa$, 0.83; 95% CI, 0.79 to 0.88). Progression of carotid disease was defined as an increase of the degree of stenosis by at least 1 category. Progression of stenosis in either I or both ICAs was considered indicative of progressive disease. Duplex recordings were done by experienced medical technicians under supervision by one of the authors. Two independent investigators ($\kappa$, 0.85, 95% CI, 0.80 to 0.89) determined progression of carotid atherosclerosis on the basis of the recordings from baseline and follow-up duplex investigations. We censored duplex follow-up for ischemic neurological events and carotid revascularization.

Clinical and Laboratory Data

Completeness and accuracy of clinical and laboratory data were ascertained by 2 independent observers. Fibrinogen at baseline and follow-up examinations was measured according to Claus using STA Fibrinogen (Diagnostica Stago; detection level 20 mg/dL; coefficient of variation 3.0%). Neurological examinations at all follow-up visits were performed according to standard questionnaires. Patients with clinical suspicion of neurological events during the follow-up time were further evaluated by a neurologist and mandatory cranial computed tomography. Events resulting in permanent neurological deficits (ie, minor or major strokes) were considered a secondary study end point.

Statistical Methods

Data are presented as the median and the interquartile range (IQR; range from the 25th to the 75th percentile) or counts and percentages. We used $\chi^2$ tests, Mann–Whitney $U$ tests, and Spearman correlation coefficients for univariate analyses as appropriate. Event-free survival rates are presented as Kaplan–Meier curves and compared by means of the log rank test. For multivariate analysis, we used Cox proportional hazards models adjusting for potential confounders in a hierarchical fashion, giving hazard ratios (HRs) and 95% CIs. We tested for interactions between baseline variables by multiplicative interaction terms and log likelihood ratio $\chi^2$ tests. Analyses including both carotid arteries were adjusted for clustering by patient using the Huber–White robust estimator of variance for the Cox model. We assessed the overall model fit using Cox–Snell residuals and tested the proportional hazards assumption for all covariates using Schoenfeld residuals (overall test) and the scaled Schoenfeld residuals
(variable-by-variable testing). According to the tests, the proportional hazards assumption was not violated. A 2-sided \( P \) value <0.05 was considered statistically significant. Calculations were performed with Stata (release 8.0; Stata) and SPSS for Windows (Version 10.0; SPSS Inc).

Results

Patients

We included 1268 patients with complete baseline and follow-up data in the final analysis. The median age was 69 years (IQR, 60 to 76) and 793 patients (63%) were male (Table). Median fibrinogen levels at baseline were 376 mg/dL (IQR, 333 to 431). Fibrinogen was significantly correlated to high-sensitivity CRP (hs-CRP; \( r = 0.35; P < 0.001 \)) and SAA (\( r = 0.24; P < 0.001 \)). Body mass index (\( r = 0.084; P = 0.003 \)), glycohemoglobin (\( r = 0.11; P < 0.001 \)), high-density lipoprotein cholesterol (\( r = -0.11; P < 0.001 \)) were also significantly but weakly correlated with fibrinogen. Furthermore, smokers (\( P = 0.067 \)) and patients with positive family history of cardiovascular disease (\( P = 0.010 \)) had higher fibrinogen levels.

Progression of Carotid Atherosclerosis

During the median follow-up period of 8 months (range 6 to 18) 1013 patients (80%) underwent 1 follow-up duplex ultrasound investigation of the carotid arteries, 225 (18%) had 2 serial follow-up ultrasound investigations, and 30 (2%) had 3 follow-up ultrasound investigations. Progression of carotid lesions was found in 117 of 1268 patients (9.2%). Of these, 10 patients (9%) had progressive lesions in both carotid arteries, and 30 (26%) had a progression to a degree of stenosis >60%. Progression by 1 category of the degree of stenosis was found in 94 of 117 patients (81%), progression by 2 categories in 19 patients (16%) and by 3 categories in 4 patients (3%). Eight patients (0.6%) developed a de novo occlusion of a carotid artery; all of these patients had an ipsilateral subocclusive stenosis (90% to 99%) at baseline, and 2 experienced an ipsilateral neurological event. Overall, 21 patients underwent elective carotid stenting during follow-up, and 3 were scheduled for carotid endarterectomy.

Fibrinogen and Progressive Disease

Baseline levels of fibrinogen (quartiles) were significantly associated with progression of carotid atherosclerosis during follow-up (Figure 1). Adjusted HRs for atherosclerosis progression with increasing quartiles of baseline fibrinogen were 1.83 (\( P = 0.037 \)), 2.09 (\( P = 0.008 \)), and 2.45 (\( P = 0.002 \)), respectively, compared with the lowest quartile (Figure 2).

Alternatively, we included fibrinogen as a continuous variable into the adjusted multivariable model, also revealing a significant association with disease progression (adjusted HR per mg/dL fibrinogen increase 1.003; 95% CI, 1.001 to 1.005; \( P = 0.019 \)).

Additionally adjusting for other inflammatory parameters such as hs-CRP diminished the association between fibrinogen and atherosclerosis progression to a nonsignificant level, although the adjusted HRs of 1.05, 1.47, and 1.5 still indicated an increasing risk with increasing quartiles of fibrinogen (Figure 2). Virtually identical results were obtained when we adjusted for SAA instead of hs-CRP.

Fibrinogen Levels at Follow-Up

Median fibrinogen levels at follow-up were 391 mg/dL (IQR, 340 to 446). Patients with progressive carotid atherosclerosis had significantly higher levels of fibrinogen at follow-up compared with patients with stable atherosclerosis (median 385 mg/dL and IQR, 342 to 440 versus median 375 mg/dL and IQR, 332 to 430; \( P = 0.004 \)). However, adjusting this association for the respective level of hs-CRP diminished this association to a nonsignificant level (\( P = 0.49 \)). Similarly, the change of fibrinogen from baseline to follow-up (median 6
mg/dL; IQR, −44 to 60) only showed a significant association with progressive disease in univariate analysis (P = 0.045) but not when adjusting for the change in hs-CRP (P = 0.65).

**Neurological Events**

Ischemic neurological events (all unrelated to carotid revascularization) were observed in 19 patients (1.5%) during the follow-up period. Six of 117 patients (5.1%) with ipsilateral lesion progression had a neurological event compared with 13 of 1151 patients (1.1%) with stable disease (P = 0.011; Figure 3). A progressive ICA lesion was associated with a 2.89-fold increased adjusted risk for ipsilateral neurological events (95% CI, 1.045 to 7.99; P = 0.041), as indicated by multivariate Cox proportional hazards analysis entering neurological events as a time-dependent variable and adjusting for age, sex, cardiovascular risk factors, and comorbidities. Adjusted HRs for occurrence of neurological events with increasing quartiles of fibrinogen were 1.98 (P = 0.017), 2.35 (P = 0.002), and 2.74 (P < 0.001) compared with the lowest quartile, which, again, were diminished by accounting for hs-CRP levels to 1.69 (P = 0.95 to 3.02), 1.96 (P = 0.023), and 1.99 (P = 0.014), respectively.

**Discussion**

We found that fibrinogen was significantly associated with progression of atherosclerotic lesions in the carotid arteries with a consistent temporal correlation between progressive disease and elevation of fibrinogen at baseline and follow-up. However, adjusting for other inflammatory parameters such as hs-CRP or SAA substantially diminished these associations, suggesting that fibrinogen is mainly an indicator of the level of inflammatory activity rather than exerting specific properties in promoting progression of the disease. Identifying patients with progressive carotid stenosis seems important because the risk for future stroke may be substantially higher in patients with stable disease. Fibrinogen and other inflammatory parameters might be useful prognostic biomarkers in this context.

Accumulating epidemiological data evolved, confirming that elevation of fibrinogen is associated with clinically silent atherosclerosis and carotid intima-media thickness as well as with the presence of carotid plaques in subjects free of overt cardiovascular disease. But already a decade ago, Sanguigni et al showed in 61 patients that individuals with progressive lesions had higher fibrinogen levels compared with subjects without lesion progression at 6 months (400 mg/dL versus 330 mg/dL, respectively). Large population-based studies such as the Copenhagen city study (n = 8755) and the Gothenburg Study (n = 792) unequivocally demonstrated an increasing risk for future stroke with increasing levels of fibrinogen, suggesting that fibrinogen may be worth investigating further with respect to its role in cerebrovascular disease.

Despite major advances in treatment of stroke, early identification and prevention of incident events remain crucial because more than two thirds of all strokes occur without previous symptoms. In this context, the prediction of carotid plaque progression seems of major importance because progressive carotid plaques are associated with a markedly increased risk for stroke. Bertges et al reported recently an alarming incidence of neurological events >10% at 1 year even in initially asymptomatic patients with rapidly progressive carotid stenosis. Our current observation confirms these findings. Attempting to identify predictors of progressive lesions, we found previously that the acute-phase parameters hs-CRP and SAA, surrogate markers for the level of inflammatory activity, were associated closely with progression of carotid stenosis in the current series of 1268 patients. However, fibrinogen was described not merely as marker of inflammation and atherosclerosis risk but also to directly promote endothelial cell activation, adhesion molecule expression, and resultant endothelial dysfunction, suggesting a potential causal involvement in promoting disease progression. Furthermore, carotid bifurcation plaque composition was histologically different in patients with higher fibrinogen levels, predisposing these plaques to progression, rupture, and thrombosis. Our observation suggests that patients with progressive atherosclerotic lesions exhibit an enhanced inflammatory activity that can be quantified by measuring fibrinogen. Furthermore, this association seems to be stable over time because follow-up fibrinogen levels were consistently higher in patients with progressive disease. However, we did not find a significant independent association of fibrinogen and progression of atherosclerotic lesions when additionally adjusting the HRs for other parameters of inflammation such as hs-CRP and SAA. This suggests that fibrinogen in the context of progressive carotid atherosclerosis serves primarily as a nonspecific indicator of inflammation, which may be replaced by other inflammatory parameters such as CRP rather than exerting specific properties. Experimental studies are needed to confirm whether this observation indicates the absence of a causal relationship between fibrinogen and atherosclerosis progression. Nevertheless, although fibrinogen cannot be referred to as an independent risk predictor, it still may be worth considering an elevation of fibrinogen >432 mg/dL (highest quartile) as an indicator for progressive carotid atherosclerosis and potentially for occurrence of future neurological complications.
particularly because fibrinogen in contrast to hs-CRP or SAA is a widespread available biomarker in clinical routine.

Limitations

We are aware of some methodological limitations of the present study. Particularly, the number of strokes during follow-up was rather low, resulting in wide CIs of the respective multivariate analyses. Therefore, these data have to be interpreted cautiously. Nevertheless, even with the currently low number of neurological end points, we observed a clearly significant association between progressive carotid disease, fibrinogen, and the occurrence of strokes, indicating a potential immediate clinical impact and warranting further investigations.

Conclusion

These findings suggest that elevated fibrinogen, indicating the level of inflammation, is associated with progression of carotid atherosclerosis because it was demonstrated previously for other inflammatory parameters. However, this association seems to be nonspecifically related to the inflammatory process in atherosclerotic disease rather than to specific properties of fibrinogen. Thus, fibrinogen seems not an independent predictor of atherosclerosis progression.

References

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