Value of peripheral vascular endothelial function in the detection of relative myocardial ischemia in asymptomatic type 2 diabetic patients who underwent myocardial perfusion imaging

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Background. **Endothelial dysfunction precedes overt atherosclerosis and is present in patients with type 2 diabetes mellitus (T2DM). Myocardial perfusion imaging (MPI) is an effective method of detection of coronary artery disease (CAD); however, the relationship between endothelial function and MPI in asymptomatic patients with T2DM has not been examined.**

Methods and Results. **This study used a subset of the population from the Detection of Ischemia in Asymptomatic Diabetics (DIAD) study. Endothelium-dependent vasodilation (EDV) and endothelium-independent vasodilation (EIV) were measured by use of brachial artery ultrasonography in 75 asymptomatic patients with T2DM (56 men; mean age, 58.6 ± 6.4 years; mean duration of diabetes, 8.4 ± 7.5 years) who underwent adenosine MPI. Of the patients, 15 (20%) had evidence of relative ischemia (MPI^+) whereas 60 (80%) had a normal study (MPI^-). Both EDV (3.5% ± 3.7% vs 4.5% ± 6.6%, *P* = not significant) and EIV (15.1% ± 7.5% vs 16.8% ± 8.4%, *P* = not significant) were similar in the 2 groups. On the basis of a receiver-operator analysis, an EDV response of 8% was selected as a cut point, with a negative predictive value of 93% (13/14 subjects with EDV ≥8% were MPI^-).**

Conclusions. **Endothelial function in asymptomatic patients with T2DM is not associated with the presence of relative myocardial ischemia by MPI; however, an EDV of 8% or greater has a high negative predictive value for the exclusion of CAD. (J Nucl Cardiol 2006;13:362-8.)**

Key Words: Endothelial function • myocardial perfusion imaging • type 2 diabetes mellitus

Coronary artery disease (CAD) is a major cause of morbidity and death among patients with type 2 diabetes mellitus (T2DM).[1] Myocardial ischemia is often asymptomatic in patients with T2DM and frequently manifests at an advanced stage.[1] Thus identification of CAD in an asymptomatic population is of considerable interest and importance. Dysfunction of the coronary vascular endothelium occurs early in the atherosclerotic process[2] and has been shown to predict cardiovascular morbidity.[3] The association between coronary and peripheral vascular endothelial function is modest to good.[4,5] T2DM has been associated with impaired peripheral endothelial function.[6] Over the past decade, a noninvasive method has evolved by which to evaluate both endothelium-dependent vasodilation (EDV) and endothelium-independent vasodilation (EIV) of the brachial artery, reflecting peripheral

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endothelial and smooth muscle function, respectively.[7] Myocardial perfusion imaging (MPI) is an effective method of detection of CAD with a sensitivity of approximately 90%.[8] A previous study found no correlation between the presence of abnormal MPI and brachial artery reactivity (BAR) in patients with known or suspected CAD and with multiple CAD risk factors (46% with diabetes) who were referred for clinically indicated MPI.[9] However, the relationship between peripheral endothelial function and MPI in asymptomatic patients with T2DM has not been examined. Within the framework of the Detection of Ischemia in Asymptomatic Diabetics (DIAD) study,[10] we examined whether BAR can be used to predict or exclude silent relative myocardial ischemia as detected by positive MPI findings in patients with T2DM.

METHODS

Patient Population and Study Design

The patient cohort consisted of subjects enrolled in the DIAD study, who underwent assessment of BAR (DIAD-BAR substudy). The DIAD study is a prospective, multicenter, randomized trial evaluating the prevalence of adenosine technetium 99m sestamibi single photon emission computed tomography (SPECT) imaging abnormalities in asymptomatic patients with T2DM and its association with adverse clinical outcomes. The inclusion and exclusion criteria have been published elsewhere.[10] In brief, eligible individuals were asymptomatic patients with T2DM aged between 50 and 75 years without known CAD as assessed by history, electrocardiogram, and prior noninvasive or invasive evaluation. Those enrolled underwent an extensive clinical and laboratory evaluation and were randomized to undergo either adenosine SPECT imaging or no imaging (control group) with 5 years of follow-up. Of the 143 patients with BAR studies who were enrolled in the DIAD-BAR substudy,[11] 78 subjects underwent MPI and 75 were included in the final analysis (3 subjects were excluded—2 with unacceptable BARs and 1 with nonischemic cardiomyopathy). Baseline history, according to the standard DIAD questionnaire, physical examination, and laboratory tests were obtained at patient centers. Subjects were subsequently categorized as those with positive MPI findings (MPI^+, n = 15) and those with normal MPI findings (MPI^-, n = 60). Each center's institutional review committee approved the study, and all patients gave informed consent.

Clinical and Laboratory Measurements

The following clinical characteristics were collected for each patient: age, sex, smoking history, duration of diabetes mellitus, type of diabetes therapy, history of hypertension and therapy, history of hyperlipidemia and treatment, and body mass index. Systolic and diastolic blood pressure was defined as the mean of 3 measurements taken with the patient in the supine position. Blood and urine samples were obtained during morning hours after an overnight fast. This method is preferred because of the known diurnal variation in albumin excretion.[12] Both blood and urine specimens were sent for analyses to a central laboratory (LabCorp, Raritan, NJ), with the exception of homocysteine and high-sensitivity C-reactive protein (CRP) measurements, which were performed elsewhere (LipoScience, Raleigh, NC). The DPC Immulite 2000 Homocysteine assay and the DPC high-sensitivity CRP assay (Diagnostic Products Corporation, Los Angeles) were used to determine total plasma homocysteine and CRP levels, respectively. Patients with elevated (>10 mg/L) CRP levels had repeated measurements and were excluded if there was evidence of infection or systemic inflammation. Urinary albumin and creatinine concentrations were determined by immunoturbidimetric and kinetic methods, respectively. With these methods, the coefficients of variation for albumin and creatinine were 2.7% and 3.5%, respectively.

Measurement of Flow- and Nitroglycerin-Mediated Vasodilation

All measurements of BAR were obtained in the morning after an overnight fast, with medications withheld the morning of the study. Patients were also instructed to avoid caffeine-containing products, smoking, and exercise for at least 12 hours before the test. Images were obtained with an Acuson 10.0-MHz linear array transducer and an Aspen cardiac ultrasound system (Acuson Corp, Elmwood Park, NY) via a standard technique at all participating centers. After initial baseline brachial artery diameter measurement, a blood pressure cuff was placed around the forearm, distal to the segment of the artery scanned, and was inflated 60 mm Hg above the patient's systolic blood pressure for 5 minutes. After deflation, the brachial artery diameter was recorded at 1 minute and 3 minutes after occlusion. After a 15-minute break and once the brachial artery diameter was back to baseline, 0.4 mg of sublingual nitroglycerin was administered. The brachial artery diameter response was recorded at 3 and 5 minutes after administration of nitroglycerin. Ten cardiac cycles were analyzed for each scan, and measurements were averaged. The brachial artery diameter was measured at a fixed distance from an anatomic marker as the distance between the near and far intima. EDV and EIV were calculated as the percentage maximal increase in arterial diameter 1 and 3 minutes after occlusion and 3 and 5 minutes after nitroglycerin administration, respectively. To be consistent with the literature, the EDV response at 1 minute after occlusion was used for further analysis. BAR examinations from Hartford Hospital (Hartford, Conn) and Yale University (New Haven, Conn) were recorded by use of CVI Acquisition Software (Data Translation Inc, Marlboro, Mass). Images from the other 3 participating centers were recorded on tapes. Two different interpreters at Hartford Hospital analyzed all scans independently using CVI Analysis Software. The intraobserver and interobserver variability in our laboratory was 1.1% and 2.1%, respectively.

MPI Protocol

Electrocardiography (ECG)–gated adenosine Tc-99m sestamibi SPECT imaging was performed in accordance with the standards of the American Society of Nuclear Cardiology.[13] Rest imaging and stress imaging were performed on the same day if body mass index was less than 30 kg/m^2; otherwise, stress imaging was performed on a separate day. Vasodilator stress was performed by intravenous infusion of adenosine (140 $\mu g \cdot kg^{-1} \cdot min^{-1}$), with simultaneous treadmill exercise at a very low level (Bruce stage 1) when feasible.[13] This approach was used because many patients with T2DM may be unable to complete a symptom-limited exercise test because of obesity, peripheral vascular disease, and peripheral neuropathy. Vasodilator stress can be applied to almost all patients and ensures a reproducible intervention. A 12-lead electrocardiogram was recorded each minute during the procedure.

Image Analysis

Unprocessed ECG-gated SPECT image data were sent to the Yale University radionuclide core laboratory for quantitative analysis.[14] Myocardial perfusion defects were quantified as a percentage of the left ventricle in comparison to a normal reference database. The left ventricular ejection fraction was derived from the ECG-gated images.[15]

After the completion of patient enrollment, a panel of 3 expert readers (F.J.Th.W., A.E.I., and G.V.H.), blinded to the patient's identity, ECG responses, and symptoms during adenosine infusion, interpreted all perfusion images by consensus and confirmed the quantitative analysis. Images were presented in random order and mixed with an unknown number of non-DIAD images to prevent interpretation bias. Stress and rest myocardial perfusion abnormalities were described as reversible (relative ischemia), fixed (scar), or mixed (scar and relative ischemia). For the purpose of our study, any combination of ECG or relative scan ischemia was considered a positive MPI study (MPI$^+$). SPECT images revealing increased radiotracer lung uptake, left ventricular dilation after stress, and resting left ventricular dysfunction (left ventricular ejection fraction <0.45) were also categorized as abnormal.

Statistical Analysis

Results are presented as mean $\pm$ SD, unless otherwise stated. The χ^2 or Fisher exact test was used to compare categoric data. For continuous variables, differences between patients with positive and negative MPI findings were compared by use of the 2-sample independent t test or with the Mann-Whitney U test. A receiver operator characteristic curve (carried out to the higher level of numerical precision) was generated to determine the predictive power of EDV for CAD as detected by a positive MPI study. Results were considered statistically significant at $P < .05$. Analysis was performed with the statistical package SPSS 10.1 (SPSS Inc, Chicago, Ill).

RESULTS

Subject Population and Characteristics

The baseline clinical and biochemical characteristics of the entire study population are shown in Table 1. All 15 patients had positive studies either by ECG or by scan (or both), and there was no case of scar. There were no significant differences between the MPI$^+$ and MPI$^-$ groups, with the exception of a trend toward a higher urine albumin-to-creatinine ratio in the MPI$^+$ group. There were also no significant differences regarding use of medications (Table 2), with the exception of angiotensin receptor blockers being used more frequently in patients with an MPI$^+$ study.

Relationship Between BAR and MPI

Baseline brachial artery diameter was not different between MPI$^+$ and MPI$^-$ subjects. In addition, both EDV (3.5% $\pm$ 3.7% vs 4.5% $\pm$ 6.6%, P = not significant) and EIV (15.1% $\pm$ 7.5% vs 16.8% $\pm$ 8.4%, P = not significant) were also not significantly different between MPI$^+$ and MPI$^-$ patients, respectively (Table 3).

The exact value of EDV that represents a normal endothelium-dependent response has not been established.[7] However, a cutoff value of 8% or greater has been proposed to represent a normal EDV response.[16,17] Sensitivity and specificity, along with positive and negative predictive values, of brachial artery ultrasound for detection of CAD (MPI$^+$ patients) were analyzed at various EDV cut points. For an EDV cut point of 6%, the sensitivity was 80% and the specificity was 38%, with a negative predictive value of 88%. For an EDV cut point of 14%, the sensitivity improved significantly, but the specificity decreased (100% vs 8%, respectively), whereas the negative predictive value improved to 100%. On the basis of a receiver-operator analysis, an EDV response of 8% was chosen as a cut point for further analysis, as this maximized the negative predictive value and had the least impact on sensitivity (Figure 1). Sixty-one subjects had an EDV of less than 8%, whereas fourteen had an EDV of 8% or greater. Fourteen of fifteen subjects with MPI$^+$ studies had an EDV of less than 8% (sensitivity, 93%), whereas thirteen of fourteen subjects with an EDV of 8% or greater were MPI$^-$ (negative predictive value, 93%), as illustrated in Figure 2. Mean EDV and EIV values in this subset were not different compared with the whole DIAD-BAR cohort.[11]

DISCUSSION

The purpose of this study was to investigate the relationship between vascular reactivity and MPI in

Table 1. Baseline clinical and biochemical characteristics of patients

Characteristic	MPI^+ (n = 15)	MPI^- (n = 60)	All subjects (n = 75)	*P* value
Sex				
Men	8 (53)	34 (57)	42 (56)	.81
Women	7 (47)	26 (43)	33 (44)	
Age (y)	58.3 ± 6.0	59.9 ± 6.6	59.6 ± 6.4	.39
Race				
White	10 (67)	47 (78)	57 (76)	.29
Black	3 (20)	11 (18)	14 (19)	
Other	2 (13)	2 (4)	4 (5)	
Hypertension	9 (60)	28 (47)	37 (49)	.36
Hyperlipidemia	9 (60)	35 (58)	44 (59)	.91
Current smoking	1 (7)	5 (8)	6 (8)	.83
Family history of CAD	3 (20)	10 (14)	13 (17)	.76
Peripheral neuropathy	0 (0)	5 (8)	5 (7)	.25
Retinopathy	1 (7)	6 (10)	7 (9)	.53
Duration of T2DM (y)	9.4 ± 7.9	8.2 ± 7.5	8.4 ± 7.5	.66
Systolic blood pressure (mm Hg)	130 ± 19	127 ± 23	127 ± 23	.59
Diastolic blood pressure (mm Hg)	77 ± 8	77 ± 14	77 ± 13	.62
Body mass index (kg/m^2)	33.7 ± 7.6	31.2 ± 7.1	31.7 ± 7.2	.22
Total cholesterol (mg/dL)	182 ± 38	191 ± 38	189 ± 38	.43
Low-density lipoprotein (mg/dL)	108 ± 33	100 ± 39	102 ± 38	.48
High-density lipoprotein (mg/dL)	49 ± 10	52 ± 14	52 ± 14	.62
Triglycerides (mg/dL)	123 ± 47	151 ± 115	146 ± 106	.98
HbA_{1c} (%)	6.7 ± 1.3	6.9 ± 1.3	6.9 ± 1.3	.43
Serum creatinine (mg/dL)	0.9 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	.49
Urine albumin-creatinine ratio (μg/mg creatinine)	47.9 ± 61.2	17.6 ± 16.1	23.6 ± 32.6	.07
CRP* (mg/L)	4.2 ± 0.5	4.7 ± 1.3	4.3 ± 1.5	.81
Homocysteine (μmol/L)	6.7 ± 3.0	6.8 ± 2.1	6.8 ± 2.3	.85

Categoric variables are presented as absolute numbers (%) or mean ± SD.
HbA_{1c}, Hemoglobin A_{1c}.
*Values for CRP are given as mean ± SEM.

asymptomatic patients with T2DM. Our results demonstrated that brachial artery endothelial function is not associated with the presence of silent relative myocardial ischemia. However, an EDV of 8% or greater carried a high negative predictive value for the exclusion of CAD. These data indicate that "preserved" EDV (≥8%) might be useful in identifying patients with T2DM at low risk for obstructive CAD.

Endothelial dysfunction precedes overt atherosclerosis, and the noninvasive assessment of BAR by high-resolution ultrasound has the potential to be a preclinical marker of cardiovascular disease[2,3,7] and relates to coronary risk factors including T2DM.[6,16] Previous reports have associated abnormalities in EDV of the brachial artery with the presence of CAD as demonstrated by long-term cardiovascular events,[2,17] coronary angiography,[18,19] or MPI.[20] In our study both MPI^+ and MPI^- patients had similar EDV responses, probably indicating that diabetes by itself affects endothelial function before the appearance of atherosclerotic obstructive disease.

The exact values of normal brachial artery EDV and EIV have not been established primarily because of differences in age, sex, and methods.[21] In a study correlating clinically indicated exercise MPI along with BAR, an EDV of less than 10% was used as a cut point to optimize the negative predictive value of brachial artery ultrasound to predict the absence of CAD, while preserving the sensitivity of the test.[20] A retrospective study in patients undergoing cardiac catheterization demonstrated that a brachial artery EDV of less than 10% was associated with an increased likelihood of myocardial infarction, coronary angioplasty, or coronary bypass surgery during a 5-year follow-up period.[22] In patients with chest pain syndrome undergoing coronary angiography and BAR testing, an optimal EDV cutoff value of 8.8% or less was used with a sensitivity and negative

Table 2. Baseline medications of patients

Medication	MPI^+ (n = 15)	MPI^- (n = 60)	All subjects (n = 75)	*P* value
Angiotensin-converting enzyme inhibitors	6 (40)	22 (37)	28 (37)	.81
β-Blockers	1 (7)	5 (8)	6 (8)	.83
Calcim channel blockers	2 (13)	5 (8)	7 (9)	.55
Angiotensin receptor blockers	2 (13)	0 (0)	2 (3)	.04
Diuretics	3 (20)	5 (8)	8 (11)	.19
Sulfonylureas	3 (20)	22 (37)	25 (33)	.22
Metformin	6 (40)	25 (42)	31 (41)	.90
Thiazolidinediones	5 (33)	14 (23)	19 (25)	.43
Insulin	4 (27)	18 (30)	22 (29)	.47
Aspirin	10 (67)	25 (42)	35 (47)	.08
Statins	9 (60)	32 (53)	42 (56)	.64
Hormone replacement therapy*	3 (37)	10 (42)	13 (41)	.83

Categoric variables are presented as absolute numbers (%).
*Hormone replacement therapy data refer to 32 postmenopausal women (8 in MPI^+ group and 24 in MPI^- group).

Table 3. EDV and EIV of brachial artery in patients with positive MPI findings compared with those with negative MPI findings

	MPI^+ (n = 15)	MPI^- (n = 60)	All subjects (n = 75)	*P* value
Baseline diameter (mm)	3.8 ± 0.6	3.9 ± 0.6	3.9 ± 0.6	.45
EDV at 1 min (%)	3.5 ± 3.7	4.5 ± 6.6	4.3 ± 6.1	.57
EDV at 3 min (%)	3.3 ± 3.5	2.1 ± 5.3	2.4 ± 5.0	.43
Maximal EDV (%)	4.3 ± 3.2	5.2 ± 6.0	5.0 ± 5.5	.58
EIV at 3 min (%)	10.3 ± 6.1	13.0 ± 8.6	12.5 ± 8.2	.25
EIV at 5 min (%)	15.1 ± 7.5	16.8 ± 8.4	16.5 ± 8.2	.49
Maximal EIV (%)	15.1 ± 7.5	17.0 ± 8.6	16.6 ± 8.4	.44

Data are given as mean ± SD.

predictive value of 90%,[23] whereas in patients with peripheral vascular disease, an EDV of less than 8.1% was associated with 9-fold increases in rates of morbidity and mortality compared with those with an EDV of 8.1% or greater.[17] In healthy nonsmokers the mean EDV at 1 minute has been reported to be 7.7%,[24] and in our laboratory an EDV of 8% or greater at 1 minute after lower-arm occlusion is considered a normal response in healthy subjects of a similar age and body size. With this cutoff point, both the sensitivity and negative predictive value of EDV of less than 8% were 93%, thus underscoring the concept that BAR might be an effective test for excluding CAD in asymptomatic T2DM diabetic patients. The relatively poor positive predictive value (22%) of BAR probably results from the fact that impaired endothelial function far precedes the development of atherosclerotic stenoses that would be detected by MPI. Thus impaired brachial EDV response (<8%) in asymptomatic patients with T2DM does not necessarily indicate that CAD has developed, whereas a normal EDV of 8% or greater indicates a low likelihood of advanced CAD.

Coronary risk factors and their treatment may potentially have an impact on the endothelium-mediated response in the brachial artery.[16] In our study both MPI^+ and MPI^- patients had no significant baseline differences, besides a trend toward a higher urine albumin-to-creatinine ratio in the MPI^+ group. We have already demonstrated in the DIAD-BAR substudy, from which our study population was derived, that the presence of microalbuminuria is an independent predictor of both EDV and EIV.[11] Finally, active treatments, including hormone replacement therapy, that may potentially favor endothelial function were also not different between MPI^+ and MPI^- patients, with the only exception being that angiotensin receptor blockers were used more fre-

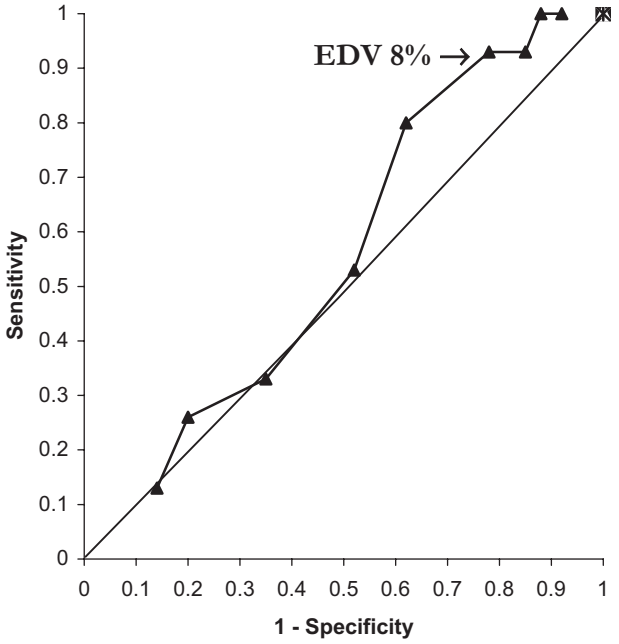

Figure 1. Receiver operator characteristic curve examining predictive power of EDV for relative myocardial ischemia defined by MPI for various cut points (−2% to 14%). An EDV of 8% was chosen (*arrow*) as a cut point for further analysis based on the high sensitivity (93%) and negative predictive value (93%). The area under the curve was 0.595.

quently in subjects with MPI^+ studies. However, this difference was attributed to only 2 patients with positive studies taking an angiotensin receptor blocker compared with none in the MPI^- group.

Study Limitations

Our study is limited by its cross-sectional design and the small sample size, especially in the arm of MPI^+ patients. The small sample size precluded a meaningful multivariate statistical analysis of the incremental value of BAR to the strongest clinical predictors, including traditional CAD risk factors. Future studies in larger populations may be needed to determine this value. In addition, the sensitivity of MPI for the detection of CAD is approximately 90%. Thus, although some patients with obstructive CAD may have been missed, this is likely a small number and is unlikely to have significantly altered the findings. A technical limitation of our study is that flow velocity measurements by Doppler were not included. Finally, our EDV cutoff of 8% may not be applicable to other laboratories performing brachial artery ultrasound because of the lack of standardization of this method.[21] However, after a receiver operator characteristic curve analysis was performed, this cutoff value maximized the negative predictive value with the least impact on sensitivity.

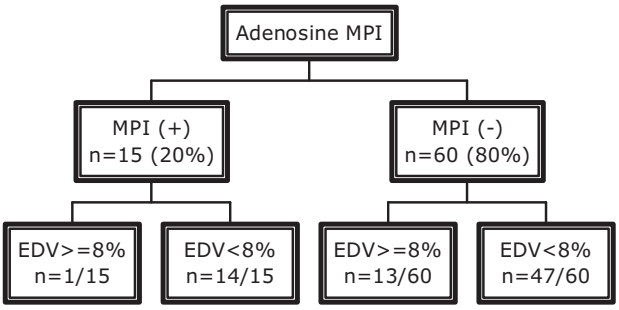

Figure 2. Flow diagram detailing 75 patients who underwent MPI. Subjects were initially divided based on the MPI result and subsequently based on the EDV cut point of 8%. Of the 15 MPI^+ subjects, 14 had an EDV of less than 8% (sensitivity, 93%), whereas 13 of 14 subjects with an EDV of 8% or greater were MPI^- (negative predictive value, 93%).

Potential Clinical Implications

Assessment of BAR has the potential to be a surrogate marker of subclinical cardiovascular disease.[2,3,7] Ongoing studies in several large populations, including the Framingham Heart Study and the Cardiovascular Health Study, may determine whether BAR testing identifies patients at risk for the development of CAD and whether it is a practical clinical tool. Our study suggests that "normal" vascular reactivity, as defined by an EDV of 8% or greater, may potentially be useful in predicting the absence of advanced CAD in high-risk patients, such as those with T2DM. If verified by larger-scale trials, determination of peripheral endothelial function may confirm a low probability of CAD and, thus, may preclude the need for more elaborate testing.

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