Blood Viscosity Abnormalities in Large and Small Vessel Diseases: Future Directions for Plasma Medicine

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ABSTRACT: This review contrasts the effect of blood viscosity abnormalities in large and small vessels. Blood viscosity is an underutilized biophysical parameter and a critical determinant of endothelial shear stress. Epidemiologic studies have shown that blood viscosity may play a prognostic role in monitoring the development of cardiovascular diseases. For microcirculatory disorders, observational studies have demonstrated an association between blood viscosity and complications arising from type 2 diabetes, such as diabetic retinopathy. A new therapeutic modality for treating blood using nonequilibrium plasma (nonthermal ionized gas) is reviewed as an emerging technology in the treatment of hemodynamic and rheologic syndromes in large and small vessel diseases.

KEY WORDS: blood viscosity, endothelial shear stress, coronary artery disease, carotid artery disease, peripheral arterial disease, diabetic retinopathy, nonequilibrium plasma.

I. INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of the death both in the United States and worldwide.1 Over 700,000 people in the United States and 13.5 million individuals worldwide die each year from CVD. In the United States alone, CVD is responsible for >$400 billion in medical expenditures and lost productivity each year.2 Because of numerous technological innovations, improved management of risk factors, and advances in treatment, overall CVD mortality is decreasing in the United States.3 Paradoxically, however, as the rate of CVD-related mortality falls, the overall prevalence of CVD will increase unless the incidence of CVD also decreases—leading to a vicious cycle of ever-increasing economic costs associated with this disease state.4 As such, the containment of CVD-related healthcare costs through technological innovation is likely to become a self-subverting effort, in which medical innovation and reduced CVD mortality rates actually worsen the fiscal solvency of industrial societies. The high prevalence of CVD underscores the fact that the etiology of the disease remains elusive, in spite of steadily improving clinical management practices. Significant etiologic and epidemiologic advances are needed in addition to new paradigms for CVD diagnosis and treatment.

The blood is the largest organ in the body: It is 3–4 times larger than the brain by volume and 2–3 times larger than the liver. Efficient blood flow is necessary for life-sustaining perfusion and influences the health of every other organ system in the body. CVDs due to atherosclerosis and microvascular circulatory disorders are two
contrasting manifestations of pathophysiologic blood flow. In the case of the former, the site specificity of atherosclerotic lesions has for decades provided pathological support for the direct involvement of hemodynamic forces in the development of plaque. Among the hemodynamic forces that have been studied extensively, one in particular—endothelial shear stress (ESS)—has emerged as a key link in the localization of atherosclerotic plaques.

ESS is the frictional force per unit area (N/m² or Pa) applied by blood flow tangentially upon the endothelial wall and has been shown to act as a critical factor in the development of atherosclerosis and the evolution of a rupture-prone lesion.⁵⁻⁹ To preserve endothelial function, blood flow should be maintained at a steady laminar ESS within a physiologic range reported to be between 10 and 70 dyne/cm².⁵ When ESS falls outside of the physiologic range, as is the case with turbulent oscillatory ESS or low ESS <4 dyne/cm² at arterial bifurcations or distal segments of plaques, endothelial cell integrity is compromised and the apoptosis rate of endothelial cells increases as much as 7-fold.¹⁰ Mathematically, ESS is defined as the product of blood viscosity and shear rate, in which the shear rate is a function of the ratio of flow velocity to lumen diameter (4V/d). As such, ESS (τₚₖ) can be determined as the product of blood viscosity (μ) and shear rate (4V/d), as follows in Eq. 1.

\[
\tau_w = \mu \cdot \left[ \frac{4V}{d} \right]
\] (1)

where V is the maximum velocity at the center and d is the lumen diameter. Changes in viscosity directly modulate ESS (i.e., friction at the vascular wall).

In the large arteries, blood flows at relatively high velocities of 10–70 cm/s at peak systole.¹¹⁻¹⁴ Because both flow velocity and lumen diameter vary greatly throughout the systemic circulation, a dimensionless number called the Reynolds number can be used to characterize the regime of vascular blood flow. The Reynolds number represents the ratio of inertial forces (i.e., kinetic forces) to the viscous forces in flow. It is defined as ρVd/μ, where ρ and μ are the density and viscosity of blood, respectively, V is the flow velocity, and d is the lumen diameter. The Reynolds number corresponding to large-arterial flow is in the range of 100–850.¹⁵ This range of values indicates that because of higher flow velocities, the inertial forces are approximately 100–850 times greater than the viscous forces in the large arteries.

The microvasculature is far more intricate than the large arteries and is crowded with a number of branches and anastomoses in a relatively small volume of space. In arteriole flow, the corresponding Reynolds number is on the order of 0.0006 due to lower flow velocities and smaller diameters, indicating that the viscous forces are approximately 1600 times greater than the inertial forces in the arteriole regimes.¹⁵ For capillary flow, the corresponding Reynolds number is approximately 0.001–0.0005 with a flow velocity in the range of 0.05–0.1 cm/s.¹¹ At capillaries, hematocrit levels have been reported to be as much as 21% lower than systemic hematocrit levels (33% ± 7% versus 42% ± 6%, respectively).¹³ Not only is hematocrit reduced at the capillaries, but blood viscosity levels are also lower at capillaries relative to systemic viscosity because
of the Fähraeus-Lindqvist wall effect at the capillary.\textsuperscript{11,16} Despite these factors, the Reynolds number still holds an order of magnitude of 0.001 at the capillaries, indicating that viscous forces are about 1000 times greater than inertial forces.

In the context of this fluid mechanical framework and the Reynolds numbers typically found in large and small vessels, it is valid to postulate that blood flow in the microvasculature is essentially determined by viscous forces (i.e., the viscosity of blood). This review describes and contrasts the impact of blood viscosity abnormalities in large and small vessels. In addition, an overview of the determinants of blood viscosity is provided together with epidemiologic reports on the prognostic role of blood viscosity in CVD. For microvascular disorders, an overview of observational clinical studies linking blood viscosity to microcirculatory complications of diabetes is provided with an emphasis on diabetic retinopathy. In addition, a mechanistic approach is taken to describe how blood hyperviscosity effects blood flow through capillaries. Finally, a new therapeutic modality for treating blood with nonequilibrium plasma (nonthermal ionized gas) is reviewed as an emerging technology in the treatment of hemodynamic and rheologic syndromes.

II. DETERMINANTS OF BLOOD VISCOSITY

Blood viscosity is defined as the inherent resistance of blood to flow and can be thought of as the thickness and stickiness of blood. Blood is said to behave as a non-Newtonian fluid insofar as its viscosity is not a constant value but is shear dependent.\textsuperscript{17} Shear rate is a function of the ratio between flow velocity and vessel diameter and can be visualized as the rate at which layers of fluid slide over one another (see Fig. 1). Blood flow in the human body is not continuous but pulsatile. As shear rate increases, blood viscosity tends to fall. For example, as blood flow velocity increases to a maximum value during peak systole, erythrocytes disaggregate, disperse, and deform, causing viscosity to decrease. By contrast, as flow velocity decreases to a minimum during end diastole, blood viscosity increases due to interactive aggregation of erythrocytes and formation of rouleaux.

The principal determinants of blood viscosity are hematocrit, erythrocyte deformability (i.e., the structural response of red blood cells to applied forces), plasma viscosity and the concentrations of plasma proteins, and finally, temperature. Hematocrit is the volume fraction of erythrocytes in whole blood and has the greatest influence over blood viscosity. As hematocrit increases, flow resistance necessarily increases between the moving blood and the arterial wall, eventually resulting in hyperviscosity syndrome as often observed in untreated polycythemia vera, in which hematocrit can be 60\%–65\% or greater.\textsuperscript{18} At such high levels of cellular content, blood viscosity increases exponentially with hematocrit. A one-unit increase in hematocrit has been reported to account for as much as a 4\% increase in blood viscosity.\textsuperscript{19} This relationship between hematocrit and viscosity has been described as linear but generally does not hold at high or low hematocrit concentrations in which cases the relationship is exponential.\textsuperscript{20}

Erythrocyte deformability also has an important effect on blood viscosity, particularly in the microvasculature. As cells move from large to small vessels, the shape and
velocity of red blood cells change substantially, and their deformability plays a major role in their microvascular penetration. Increases in erythrocyte membrane surface viscosity and whole blood viscosity were demonstrated in a hypertonic environment in contrast with hypotonic solutions. At high shear rate flow regimes, reduced erythrocyte deformability is associated with elevated blood viscosity, but at low shear flow, impaired deformability can lead to lower blood viscosity levels. At low shear rates, healthy erythrocytes naturally stack and form aggregates (rouleaux), but when erythrocyte deformability is impaired and cells become stiff and rigid, as can occur in infections, diabetes, and sickle cell anemia, the red blood cells do not aggregate easily and rouleaux do not form. Thus, at low shear rates, viscosity can actually be diminished in cases in which the blood contains higher proportions of rigid cells.

A third major determinant of blood viscosity is the viscosity of plasma. Plasma, like water, is a Newtonian fluid, the viscosity of which is independent of shear rate. However, plasma viscosity is affected by the concentrations of fibrinogen and other plasma proteins such as immunoglobulins and lipoproteins. Larger lipoprotein particles, such as chylomicrons and very low-density lipoproteins (VLDLs), have greater effects on increasing plasma or serum viscosity than smaller size lipoproteins such as low-density lipoproteins (LDLs). Trained athletes were previously reported to have lower plasma viscosity values than sedentary subjects, and cardiovascular risk factors such as hypertension have been linked to elevated plasma viscosity levels. Separately, earlier observational studies reported plasma viscosity to be statistically independent of age and sex.
Viscosity is also dependent upon temperature. Generally, blood viscosity increases with lower temperatures, and each 1°C reduction in temperature causes a 2% increase in blood viscosity. A prior benchmark *in vitro* study funded by the National Institutes of Health and the Maine Heart Association observed that when the temperature of blood is decreased from 37°C to 22°C, blood viscosity increases as a direct result by 50%–300%. In addition, viscosity is reported to have positive correlations with total cholesterol, triglycerides, LDL cholesterol, and apolipoprotein B concentrations, whereas negative correlations with blood viscosity is attributed to higher levels of high-density lipoprotein (HDL) cholesterol concentrations.

A selection of blood viscosity determinants at high and low blood shear rates is illustrated in Fig. 2. In areas of the circulation with high shear rate, blood viscosity is principally determined by hematocrit, red blood cell deformability, and plasma viscosity. In low shear rate areas, the molecules and cells interact such that aggregates or rouleaux are formed and platelet aggregation and other intermolecular reactions occur.

### III. BLOOD VISCOSITY IN CARDIOVASCULAR EPIDEMIOLOGY

Data from a number of clinical studies support a link between hyperviscosity and CVD events. The largest prospective study performed using blood viscosity was the Edinburgh Artery Study, which followed 1592 randomly selected men and women aged between 45 and 59 years for 5 years. The Edinburgh Artery Study showed that 55% of the major CVD events occurred in patients in the highest blood viscosity quintile, whereas only 4% of those in the lowest viscosity quintile had a significant CVD event. In addition, the study demonstrated that after adjustment for age and sex, blood viscosity, hematocrit, hematocrit-corrected blood viscosity, plasma viscosity, and fibrinogen were all significantly higher in individuals who experienced CVD events than in those who did.

**FIG. 2:** Blood viscosity profile as a function of shear rate. Viscosity unit: 10 mP = 1 cP = 1 mPa·s. RBC, red blood cell; WBV, whole blood viscosity.
not \( (P = 0.0003) \).\(^{37}\) As a signal for CVD events (acute coronary syndrome or ischemic stroke), blood viscosity was at least as strong a predictor as diastolic blood pressure and LDL cholesterol and was a stronger predictor than cigarette smoking.

In a prospective study of 331 middle-aged hypertensive men followed for up to 12 years, patients were divided into three groups according to low-shear blood viscosity measured at baseline. Those in the highest tertile for blood viscosity had a \( >3 \)-fold higher risk of CVD events than those in the lowest blood viscosity tertile (hazard ratio, 3.42; 95% confidence interval, 1.4–8.4; \( P = 0.006 \)).\(^{38}\)

A separate analysis within the Edinburgh Artery Study, involving 1581 adults assessed for peripheral artery disease, showed that blood viscosity and fibrinogen were independently associated with peripheral artery narrowing.\(^{39}\) The authors implicated a critical role for blood viscosity in the pathogenesis of lower-limb ischemia in the general population. An earlier study compared 120 patients having intermittent claudication with normal age-matched controls and found that blood viscosity was significantly higher among claudicants \( (P < 0.001) \) with the greatest difference in blood viscosity observed at lowest shears. At high shear rates, patients with blood viscosity \( >4.5 \, \text{cP} \) had a mean claudication distance of 126 m compared to 289 m for patients with high-shear viscosity below that threshold. Hyperviscosity among claudicants was not attributable to differences in hematocrit but rather to plasma fibrinogen. The researchers sought to examine whether the principal cause of circulatory insufficiency in patients with symptoms of intermittent claudication was an abnormally high blood viscosity rather than narrowing of the arteries. They found that many patients with abnormally high blood viscosity have normal arteriograms despite severe symptoms of claudication and suggested the use of the term *rheological claudication* to describe approximately 25% of moderate to severe claudicants with hyperviscosity of blood having significantly worse prognoses.\(^{40}\)

A cross-sectional clinical study of 430 patients found that those who had suffered an ischemic stroke within the prior 7 d had mean low-shear blood viscosity levels 27% higher than those of age and sex-matched healthy individuals. Among the 430 individuals evaluated for low-shear blood viscosity were 135 patients who had suffered a stroke within the prior 7 d, 89 patients who had experienced a transient ischemic attack (TIA) within the prior 14 d, 115 patients with stroke risk factors but who had not experienced a stroke or TIA in the prior 6 mo, and 91 healthy controls. Differences in average blood viscosity between patients who had experienced an ischemic event were significant \( (P < 0.003 \) for stroke and TIA compared with healthy controls).\(^{41}\)

Hyperviscosity has been correlated with a number of conventional CVD risk factors, and it has been suggested that increased blood viscosity may be an integrated mechanism by which each of the traditional CVD risk factors promotes atherosclerosis and the evolution of atheroma into a rupture-prone lesion.\(^{42,43}\) The development of atherosclerotic lesions is a process that is site and region specific. Atherosclerotic plaques form not only in large arteries close to the heart that feed the heart and brain but also in the lower extremities. However, atherosclerosis rarely occurs in the arteries of the upper extremities or in a woman’s mammary arteries. Mechanistically, proximity to the force-
fully contracting left ventricle aggravates exposure of endothelial cells in the coronary and carotid arteries to mechanical injury. In peripheral disease, in which occlusions form in the arteries of the lower extremities, the pull of gravity causes blood to accelerate when a person is standing upright, increasing turbulence and creating flow regimes of oscillatory or vulnerable low ESS.

The localization of atherosclerotic plaques at arterial segments subjected to disturbed blood flow and shear stress has been reported for several decades. Several researchers recently reconstructed coronary and carotid arteries in 3D using magnetic resonance angiography and intravascular ultrasonography to produce blood flow simulations and demonstrated linkages between vulnerable low ESS and plaque progression as well as new plaque formation. Through a process called mechanotransduction, endothelial cells exposed to low shear stress lose their elongated phalanx structure, adopting a rounder profile associated with augmented expression of inflammatory genes responsible for increased synthesis of endothelial-localized adhesion molecules, transmigration of mononuclear cells into subendothelial space, and intrusion and incorporation of lipoproteins into tissue macrophages. Using coronary intravascular ultrasonography to characterize atherosclerotic plaque volume in humans, low ESS predicted histopathological features of inflammation, thin fibrous cap, and development of other vulnerable plaque attributes associated with ischemic events.

IV. BLOOD VISCOSITY IN MICROCIRCULATORY COMPLICATIONS OF DIABETES

Diabetes has long been recognized as a risk factor for the development of large artery disease and increased CVD morbidity and mortality. However, type 2 diabetes is also responsible for an increased risk of microcirculatory complications. Blood viscosity plays a direct role in modulating peripheral vascular resistance and ESS in large arteries with diameters that are orders of magnitude larger than those of individual erythrocytes. However, blood cannot be viewed to flow as a uniform liquid in capillary vessels with lumen diameters that are of the same order of magnitude as the size of erythrocytes. In the microvasculature, the determinants of blood viscosity (e.g., hematocrit, erythrocyte deformability, and plasma protein concentrations) must be reconsidered individually. For example, in the microvasculature, hematocrit is generally lower than in large vessels.

A number of clinical studies provide evidence that elevated blood viscosity is a pathogenetic factor of diabetic microangiopathy, altering microcirculation and leading to insufficient tissue perfusion. In addition, increased blood viscosity has been implicated in the etiology of diabetic retinopathy, manifesting with dilated veins, microaneurysms, hemorrhages, and vessel proliferation. A previous study of blood viscosity in patients with diabetic microangiopathy linked hyperviscosity directly with microcirculatory impairments leading to prolonged reductions in capillary perfusion vessels. In a study of 64 patients with diabetes and 61 matched control subjects without diabetes, low shear blood viscosity was demonstrated to be significantly higher in patients with diabetes than in controls. The difference in blood viscosity was greatest in
patients with either proliferative retinopathy or nephropathy, although blood viscosity differences were also present to a lesser extent in patients with diabetes with evidence of myocardial or peripheral ischemia. In this study, erythrocyte deformability was reported to be lower (i.e., red blood cells were more rigid) in the 14 patients with diabetes with the most extensive microangiopathy than in 22 patients with diabetes with little or no microcirculatory complications or in controls. The researchers implicated both hyperviscosity and impaired erythrocyte deformability as factors in the progression of microcirculatory complications of diabetes.

Another cross-sectional study of 38 male patients with diabetes compared with 38 age matched controls showed that patients with diabetes both with and without retinopathy had significantly higher blood viscosity when measured at a high shear rate of 100 s⁻¹ (7.53 ± 0.17 and 7.07 ± 0.14 cP, respectively) compared with controls (6.75 ± 0.08 cP) \((P < 0.01\) for both comparisons). After blood viscosity levels were adjusted for hematocrit, the differences persisted and remained significant \((P < 0.01)\). Blood viscosity was also measured at a low shear rate of 0.94 s⁻¹, demonstrating a similar step-wise trend for healthy controls (18.7 ± 0.6 cP), patients with diabetes without retinopathy (21.2 ± 0.8 cP; \(P < 0.025\)), and patients with diabetes with retinopathy (24.3 ± 1.1 cP; \(P < 0.01\)). These differences in low-shear blood viscosity also remained significant after viscosity was adjusted for hematocrit. The researchers attributed the increased whole blood viscosity to relative elevations in fibrinogen and the viscosity of plasma.

Hyperviscosity of the blood increases peripheral vascular resistance, and slower flow promotes hemostasis due to stagnation of blood in the capillaries and postcapillary venules in patients with diabetes. Microcirculatory hemostasis in turn plays a direct etiological role in capillary ischemia and failure, which represents a major contributor to diabetic retinopathy leading to blindness. Increases in blood viscosity reduce retinal blood flow, impairing microcirculatory perfusion and leading to localized hypoxia, lactic acidosis, and ischemia. Several clinical researchers previously demonstrated associations between hyperviscosity and nondiabetic retinopathies, which were then successfully treated by lowering blood viscosity.

Erythrocyte deformability is a critical determinant of blood viscosity in the microvasculature because of the size of erythrocytes relative to the capillary lumen. In their widely read physiology text, Guyton and Hall state the minimum diameter of capillary lumen to be approximately 4–9 μm. Elsewhere, the minimum capillary lumen size is reported to be 4–6 μm or 4–8 μm. Assuming that the average erythrocyte has a diameter of approximately 8 μm, the ability of the red blood cell to deform is pivotal for microcirculatory perfusion insofar as the erythrocyte must change shape in order to pass through these vessels.

V. FUTURE THERAPEUTIC DIRECTIONS FOR HYPERVISCOSITY SYNDROMES: NONEQUILIBRIUM PLASMA

Controlled discharge of nonthermal, nonequilibrium atmospheric plasma (ionized gas) is an emerging technology for a wide range of biomedical applications. This methodol-
ogy is being developed and evaluated for sterilization applications, as well as for blood coagulation, treatment of nonhealing ulcers, and stimulation of apoptosis in malignant tissues.\textsuperscript{71–74}

Recent studies reported on the feasibility of applying nonequilibrium plasma to blood \textit{ex vivo} for the purpose of selectively coagulating viscogenic plasma proteins. In an \textit{in vitro} study of the effect of pulsed corona-type plasma discharges on blood viscosity, plasma viscosity, and LDL cholesterol, whole blood drawn in tubes containing K2 ethylenediaminetetraacetic acid anticoagulant was centrifuged to separate blood plasma, and the blood plasma was treated with a pulsed corona discharge. Blood was treated with a range of corona-type plasma pulses (15, 30, 60, and 90 pulses). Application of the pulsed corona to blood plasma caused observable precipitation and particulate formation. Plasma viscosity, as well as blood viscosity and LDL cholesterol—after resuspension of erythrocytes in treated blood plasma—were evaluated with and without 0.2-μm filtration of precipitated particulates. Plasma viscosity was reported to increase by 14.2\% with 90 pulses of corona treatment; however, plasma viscosity was shown to decrease by 25.8\% after filtration. Blood viscosity was also shown to increase with corona treatment, but blood viscosity was reduced in the cases with filtration prior to resuspension (see Fig. 3). In this feasibility study, LDL cholesterol was observed to decrease approximately 31\% after 60 pulses of corona treatment, followed by 0.2-μm filtration, and resuspension of native erythrocytes.\textsuperscript{75} Similar results on the effect of nonequilibrium plasma (ionized gas) on blood viscosity were reported with dielectric barrier-type plasma treatment.\textsuperscript{76}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Fig3.png}
\caption{Changes in blood viscosity after treatment of blood plasma by pulsed corona discharge and filtration.\textsuperscript{75}}
\end{figure}
The application of nonequilibrium plasma as an *ex vivo* lipid-lowering medical device therapy represents a significant opportunity for future research in CVD and microcirculatory disease. Lipoproteins and triglycerides in the blood can have profound effects on blood flow. Isolated chylomicrons, VLDLs, and LDLs added to plasma or serum *in vitro* cause a dose-dependent and exponential rise in viscosity. VLDL was accompanied by a greater viscosity change than LDL, thereby supporting the influence of plasma protein size on viscosity. Separately, a negative relationship with blood viscosity was reported for HDL cholesterol at both high and low shear rates, and the negative association persisted after blood viscosity was adjusted for hematocrit. In an observational study of 27 healthy men and women aged 10–25 years having baseline LDL cholesterol concentrations ranging from 88 to 258 mg/dL, blood viscosity was shown to be positively correlated with LDL ($r = 0.443; P = 0.021$) and inversely correlated with LDL size ($r = -0.429; P = 0.029$) at a high shear rate of 1000 s$^{-1}$. In summary, all sizes of VLDL and only the small subclasses of LDL and HDL increase the risk of the development of coronary disease, whereas large HDL does the opposite.

At low shear rates, lipoproteins have been convincingly established to modulate blood viscosity. LDL particles are large enough to simultaneously bind two erythrocytes, augment erythrocyte aggregation, and increase low-shear blood viscosity. HDL particles are too small to simultaneously bind two erythrocytes, and by competing with LDL for erythrocyte binding sites, they antagonize erythrocyte aggregation and decrease viscosity.

VI. CONCLUSIONS

Blood viscosity is directly associated with the classic risk factors for atherosclerosis, including LDL cholesterol, and inversely with HDL cholesterol. Although half of myocardial infarctions occur in patients with no overt hyperlipidemia and 20% occur in the absence of cardiovascular risk factors, lipid-lowering therapies such as statins remain a stalwart therapeutic treatment modality for managing and preventing CVD events. *Ex vivo* treatment of blood using nonequilibrium plasma (ionized gas) is an emerging area that was shown in feasibility studies to lower not only LDL cholesterol but also blood viscosity. Blood viscosity is the only biomarker that explains observations of the site specificity of atherosclerotic lesions and is a critical determinant of ESS. Because viscosity is inversely proportional to flow, hyperviscosity predisposes to vulnerable low ESS, hemostasis, arterial occlusion, and atherothrombotic events. Additional research is needed to develop and validate nonequilibrium plasma technologies, such as pulsed corona-type plasma discharge and dielectric barrier plasma discharge, as a therapeutic approach to treat CVD and to prevent microcirculatory disorders, especially those that arise as complications of type 2 diabetes such as diabetic retinopathy.

REFERENCES


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