Leukocytes and Acute Myocardial Infarction

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Epidemiologic studies have demonstrated correlations between the white blood cell count and the risk of acute myocardial infarction and stroke. The risk of AMI is approximately four times greater in persons with WBC counts high in the normal range (>9,000/μl) than in persons with WBC counts low in the normal range (<6,000/μl). A high WBC count also predicts a greater risk of re-infarction and of in-hospital death. In the last decade it has become evident that the pathogenesis of atherosclerosis involves several complex mechanisms; these include the immune system, the inflammatory response, and the infectious etiologies.

- **The immune system:** it has been demonstrated that there is a high prevalence of anti-beta2 glycoprotein I antibodies in patients with ischemic heart disease [1], as well as active participation of T lymphocytes in stable and unstable angina pectoris [2].
- **The inflammatory system:** highly sensitive C-reactive protein has been shown to predict cardiovascular disease. The 20 year follow-up of healthy young men in the Honolulu Heart Program has shown that the odds of myocardial infarction rose with increasing levels of C-reactive protein as early as 5 years into follow-up, and these associations appeared to persist even beyond this period [3]; and another study demonstrated a possible combined additive effect of these two systems: patients whose serum amyloid levels were increased by >100% in the first 24 hours after coronary angioplasty and developed a positive antibody result (antinuclear factor or antiphospholipid antibodies) had a relative risk of 10.6 to develop restenosis in the first year after angioplasty [4].
- **The infectious theory:** *Chlamydia pneumoniae* has been demonstrated in atherosclerotic plaques, and there is a high incidence of immunoglobulin G antibodies to *C. pneumoniae* in AMI patients [5].

However, in this review we would like to focus on the role of the white blood cell in atherogenesis, atherothrombosis, and the risk of developing AMI and its complications.

Epidemiologic observations

In 1974, Friedman et al. reviewed 464 patients who had suffered a first AMI and whose WBC count had been measured in the preceding 2 years. These patients were compared with two control groups: one was matched for age, gender and race, and the other for these variables and for conventionally recognized risk factors for infarction. It was found that the WBC count was a strong predictor of infarction. The predictive value of the WBC count was similar to that of a serum total cholesterol measurement or a single determination of blood pressure. The height of the WBC count correlated positively with tobacco smoking, but only about two-thirds of the predictive value of the WBC count could be explained on the basis of this observation. In another study 7,000 males were followed for an average of 6.5 years. Among smokers, the WBC count correlated strongly with the risk of AMI. Smokers with WBC counts exceeding 9,000/μl had an incidence of AMI four times higher than in smokers with a leukocyte count below 6,000/μl, a difference that was statistically significant [6]. In the Hiroshima/Nagasaki survivors' surveillance, the WBC count correlated significantly with the incidence of coronary heart disease. That is, a total WBC count in excess of 10,000/μl was associated with a risk that was approximately twice that seen when the WBC count was at or below 4,000/μl. This excess risk was independent of gender, smoking history, blood pressure, and cholesterol level. Examination of differential cell counts showed the strongest association to be with the neutrophil count. It has been shown (also in the Hiroshima/Nagasaki survivors' surveillance) that total WBC count was correlated with the risk of thrombotic cerebral infarction; the 469 patients suffering thrombotic strokes had a statistically significantly higher antecedent WBC count than did members of the cohort not experiencing ischemic events [7]. Again, the examination of differential WBC counts showed a statistically significant predictive power only for neutrophils. A strong independent correlation was found in the Multiple Risk Factor Intervention Trial (MRFIT) between total WBC count and the risk of coronary heart disease [8]. Even when tobacco smoking was controlled/corrected for, the WBC count was found to predict coronary heart disease prevalence, risk of non-fatal MI, and risk of sudden cardiac death. Moreover, if the WBC count declined during the period of surveillance, so did the CHD risk: a decrement of 1,000 WBC/μl was associated with a 14% decrement in risk of cardiac death, unexplainable by changes in other cardiac risk factors. In the PARIS-1 study, 2,026 patients were examined 2–60 months after suffering a first AMI; the total WBC count obtained at that time was found to correlate strongly with risk of re-infarction. Men with WBC counts exceeding 9,000/μl had a relative risk of re-infarction of 3.5, when 1.0 was set equal to the risk for men with WBC counts at or below 5,000/μl.

AMI = acute myocardial infarction
WBC = white blood cells

CHD = coronary heart disease

As early as 1954, Cole et al. [9] reported that AMI patients with WBC counts higher than 15,000/μl who were admitted to the hospital had a risk of death within 2 months that was four times as great as the risk of patients with normal WBC counts (<10,000/μl). Lowe and colleagues [10] confirmed that this observation could be extended to patients with stroke. In their study, stroke patients were stratified according to their WBC counts on admission to the hospital: 51 of 144 patients (35.4%) with normal counts (<10,000/μl) died, while the other 93 lived to be discharged. In contrast, 69 of the 126 patients with elevated counts died (54.8%), and only 57 lived to be discharged (P = 0.002). This observation was confirmed in a different patient population in the National Survey of Stroke [11]. Maisel et al. [12] reported that the WBC count on admission to the hospital was an independent predictor of early ventricular fibrillation: 3% of patients with normal or modestly elevated WBC counts (>15,000/μl) suffered ventricular fibrillation, while 15% of patients with more elevated counts experienced this complication. Furman and co-workers [13] examined the relationship between WBC and short-term prognosis following AMI, and found that patients in the uppermost quintiles of WBC were more likely to have a complicated hospital course and more extensive acute myocardial necrosis. Patients in the highest WBC quintiles were least likely to receive therapies (anticoagulants, antiplatelet agents, beta blockers, calcium antagonists, or thrombolysis) associated with a favorable in-hospital survival following AMI. This analysis confirmed the independent association of an elevated WBC with in-hospital case fatality following AMI. The results of this study suggest that presenting WBC is associated with short-term mortality following AMI. This finding is complementary to, and in agreement with, previous epidemiologic data associating WBC with development of cardiovascular events.

Potential mechanisms for WBC predictive power

There are several possible explanations: a) the WBC count might serve as a marker for one or more disease processes that lead to vascular injury and ultimately to ischemia, and b) WBCs might play a pathogenetic role in vascular injury, with the WBC count providing a rough measure of the intensity of that process.

An elevated WBC count may be a marker for chronic inflammation secondary to tobacco smoking as well as to other factors, and this inflammation could contribute to ischemic risk. Alternatively, a high WBC count might be seen as a manifestation of a "hematological stress syndrome" [14]; this syndrome has been described to include a variety of other hematologic abnormalities and has been argued to represent a non-specific response to (or marker for) atherosclerosis. "Stress" is accepted by many as a genuine cardiovascular risk factor, associated with increased catecholamine levels in the blood and with an elevated WBC count. Recent advances in the understanding of microvascular injury, leukocyte activation, and hemorrheology strengthen the hypothesis that WBCs are major contributors to microvascular injury and atherogenesis and that chronic leukocytosis reflects ischemic risk in a direct rather than an indirect manner.

There are at least three mechanisms whereby leukocytes may contribute to microvascular injury: a) pressure-dependent plugging of microvessels by leukocytes, b) rheologic abnormalities of leukocytes, and c) endothelial cell injury caused by leukocytes and their release products – toxic oxygen compounds, proteolytic enzymes, and long-acting oxidants.

The diameters of erythrocytes and leukocytes are larger than the internal diameters of most nutritive capillaries, and the rheologic properties of blood cells are major determinants of microvascular perfusion. WBCs exert an influence on blood flow disproportionate to their number for two reasons: First, they are larger than red blood cells and are spherical in shape. Second, they are much stiffer than red blood cells, with an average cytoplasmic viscosity 1,000-fold that of red blood cells [15]. Thus, the capillary transit of WBCs is frequently associated with slowing, or even momentary stoppage, of blood flow. This transient capillary plugging by leukocytes occurs frequently even under normal pressure/flow circumstances, when it seems not to interfere with adequacy of nutrient delivery. Under certain pathologic circumstances, however, this phenomenon may be far less benign. Since normal WBCs face difficulties in traversing capillaries, it follows that alterations in the rheologic properties of the leukocytes might have a major impact on microvascular flow and thus might provide another pathophysiologic link between these cells and tissue ischemia. The prognostic significance of altered leukocyte rheology in patients with AMI suggests a possible vicious circle: ischemic injury begets leukocytosis and decreased WBC deformability; these in turn beget further ischemia, contributing to infarct extension and a variety of clinical complications [16]. Another contributor to a leukocytic role in ischemia is increased leukocyte adhesiveness, which may be provoked by a variety of stimuli. Craddock et al. [17] found that granulocytes shared with platelets the ability to aggregate when stimulated and thus – at least theoretically – to embolize to microvascular sites. Such aggregation has been shown to occur *in vitro* in response not only to complement activation (with the activation of C5a), but also to bacterial oligopeptide chemotaxins, immune complexes, and certain complex lipids such as leukotriene B4 [18]. Enhanced granulocyte adhesion to endothelial cells and granulocyte aggregation have both been demonstrated to occur *in vivo*, by use of fluorescent intravital microscopy [19], infusing complement-activated plasma or complement activators into animals harboring fluorescein-tagged neutrophils. Once the granulocyte has arrived at a microvascular site in an activated state – either by embolization in an aggregate or by an increase in its tendency to adhere to endothelial cells – it can deliver a variety of insults to the vessel lining [20]. These injurious substances include proteolytic enzymes and oxidants, which may be mutually amplifying. Actual cytotoxic injury to endothelial cells appears to result largely from oxidative assault. Early studies by Sacks et al. [21] showed that cultured endothelial cells were injured on exposure to activated granulocytes and that such injury could be prevented by antioxidant enzymes, superoxide dismutase and catalase. Endothelial dysfunction, however, can be induced by an injury that is insufficient to be detected as cytotoxicity. Thus, Harlan and colleagues [22] have shown that granulocyte neutral proteases can promote endothelial cell detachment from substrata. In addition to causing acute microvascular leak, such an event *in vivo* would expose suben-

dothelial collagen and fibronectin, allowing platelet adherence and activation. These processes may be synergistic in several ways. First, the simultaneous activation of platelets augments granulocyte adhesiveness to foreign surfaces, granulocyte aggregation, and granulocyte-mediated endothelial cell injury. Second, the enzymatic alteration of endothelial and subendothelial fibronectin renders the endothelium more adhesive and thus more susceptible to subsequent attack by granulocytes [23]. Third, the oxidants produced by activated granulocytes may damage the methionine-rich active sites of such anti-proteases as α1-proteinase inhibitor [24]. Activated leukocytes damage the surrounding tissue by releasing reactive oxygen species and proteolytic enzymes before self-necrosis. Leukocyte necrosis further exacerbates inflammation and promotes chemotaxis and leukocyte recruitment. It has been demonstrated that leukocytes from patients with essential hypertension released superoxide anion faster than those of normotensives cells. Essential hypertension is accompanied by a primed state of the leukocytes that does not correlate with the levels of blood pressure. Leukocyte priming in hypertensive patients reflects an *in vivo* exposure to a constant stimulus, ending in oxidative stress, increased self-necrosis, and cell recruitment. Oxidative stress and inflammation will result in endothelial damage and atherosclerosis in the long run [25]. Another study determined the extent to which peripheral leukocytes contribute to oxidative stress and inflammation in type 2 diabetic patients. It has been shown that leukocytes from diabetic patients stimulated by PMA released superoxide significantly faster, and plasma-reduced glutathione was lower in diabetic patients than in normal control subjects. Besides, the *in vitro* survival of normal control leukocytes was reduced when incubated with diabetic serum, whereas normal control sera promoted the survival of diabetic leukocytes. Peripheral leukocyte counts were higher in diabetic patients than in normal control patients. This study has demonstrated that type 2 diabetes is accompanied by a priming of leukocytes, resulting in oxidative stress and increased self-necrosis. Necrosis starts a chain of inflammatory reactions that result in cell recruitment, and in the long term, with the oxidative stress, may result in endothelial dysfunction [26].

Elevation in WBC count was a strong predictor of the subsequent development of congestive heart failure independent of epicardial or microvascular coronary blood flow. In this study an elevated WBC count was associated with reduced epicardial patency and greater thrombus formation at the site of the ruptured plaque, suggesting that an elevated WBC count may be a marker of a hypercoagulable or thromboresistant state [27]. Several studies have documented that a systemic inflammatory response occurs in patients with AMI and that plasma from patients with AMI stimulates the expression of interleukin-1β and IL-8 in leukocytes. The induction of monocyte procoagulant activity with either IL-6 or IL-8 has been proposed as a possible link between the inflammation and thrombosis in patients with coronary artery disease. Neuman et al. [28] investigated the effects of both of these cytokines on monocyte tissue factor expression, because the assembly of tissue factor with factor VIIa initiates the extrinsic pathway of the coagulation cascade. IL-6 and IL-8 caused an increase in tissue factor expression on the surface of monocytes, as well as a time- and dose-dependent increase in procoagulant activity. Furthermore, this increase in procoagulant activity was induced at concentrations found in peripheral blood of patients with AMI.

It has been hypothesized that the procoagulant activity of circulating leukocytes could be increased via another mechanism. Mac-1 (CD11b/CD18), a β2-integrin that is involved in leukocyte adhesion, also catalyzes the conversion of factor X to factor Xa and binds fibrinogen [29]. It has been demonstrated that there was an increase in procoagulant activity in patients who underwent successful primary angioplasty for AMI and that this increase in procoagulant activity was associated with an increase in Mac-1 expression on circulating leukocytes. Finally, the adherence of activated platelets to polymorphonuclear leukocytes via Mac-1 may also play a role in thrombus formation. Patients with an elevated WBC count had reduced patency and greater thrombus burden and also had a poorer downstream microvascular perfusion as assessed with TIMI perfusion grade. One option is that this impaired myocardial perfusion reflects leukocyte-mediated endothelial dysfunction and microvascular plugging, as described in animal models of ischemia reperfusion [30].

Cytokines, T lymphocytes, cell adhesion molecules, and the acute coronary syndromes

In AMI early recanalization of the occluded artery can salvage myocardium and improve clinical outcome. Despite these beneficial effects of timely restoration of coronary blood flow, local inflammatory responses of the ischemic and reperfused heart exert deleterious effects on the myocardium [31]. Several experimental studies have shown that cytokines are important regulators of inflammatory reactions during reperfusion. Among those, the predominantly leukocyte-derived cytokines, tumor necrosis factor alpha and IL-1β appear to play a key role due to their ability to induce secondary cytokines, such as IL-1, IL-6 and IL-8. IL-8 has potent actions in the recruitment and local activation of neutrophils, whereas IL-6, in concert with IL-1β and TNFα, induces predominantly systemic inflammatory responses. Blood samples of 10 patients with an AMI were obtained simultaneously from the coronary sinus and the aorta before and 5 minutes after recanalization of the coronary occlusion. Ten patients with elective percutaneous transluminal coronary angioplasty served as a control group. Leukocytes from healthy donors were incubated with plasma samples and analyzed mRNA expression of IL-1β, IL-6, IL-8 and TNFα by Northern blot analysis. In patients with AMI, plasma obtained from the coronary sinus after recanalization increased the mRNA expression of IL-1β and IL-8 compared with that of plasma before recanalization. No induction of IL-6 and TNFα expression could be observed. No changes found in the study patients were

IL = interleukin

TNF = tumor necrosis factor

detectable in the control group [32]. A study that investigated the role of IL-10 (an anti-inflammatory cytokine capable of modulating extracellular matrix biosynthesis) found that in post-MI reperfusion there was a significant up-regulation of IL-10 mRNA and protein in the ischemic and reperfused myocardium. *In vitro* experiments demonstrated late post-ischemic cardiac lymph-induced tissue inhibitor of metalloproteinases-1 mRNA expression in isolated canine mononuclear cells. This effect was inhibited when the incubation contained a neutralizing antibody to IL-10. These findings suggest that lymphocytes infiltrating the ischemic and reperfused myocardium express IL-10 and may play a significant role in healing by modulating mononuclear cell phenotype and inducing TIMP-1 expression [33].

Another study characterized the T lymphocyte profile during AMI and explored whether these cells might play a detrimental role in the extent of myocardial insult. Levels of T lymphocyte subpopulations, free soluble IL-2 receptor, and IL-1β, were measured during the first week of AMI. This study demonstrated that low CD4/CD8 ratio and low CD4 cell count on the first day of AMI were strongly correlated with low left ventricular ejection fraction and high myocardial mass destruction as reflected by creatine phosphokinase levels. Patients with the lowest CD4 count on admission and those whose CD4 counts did not rise had re-infarction or died. Significantly higher levels of sIL-2R and IL-1β were found in the AMI patients compared with the healthy control group. Patients who suffered re-infarction had increased cytokine levels toward the 7th day: the higher the level, the lower the left ventricular ejection fraction and the greater the probability of death [34].

Another study, mentioned earlier [2], demonstrated that T lymphocytes are activated in stable angina pectoris, and that the level of sIL-2R can be a reliable laboratory marker for follow-up of patients after coronary angioplasty, especially in those who had high sIL-2R levels before the procedure [2].

Cell adhesion molecules and the ischemic heart

Several studies have examined the association and the role of cell adhesion molecules in reperfusion injury and in ischemic heart disease. Their findings are as follows:

- Myocardial ischemia/reperfusion induces ventricular reperfusion arrhythmias. A significant part of the reperfusion arrhythmias are evoked by oxygen free radicals. It is known that activated leukocytes release oxygen free radicals, and that intercellular adhesion molecule-1 plays a major role in leukocyte activation and infiltration. A prospective study compared two groups of AMI patients, all of whom underwent percutaneous balloon angioplasty (with or without reperfusion arrhythmias). The plasma soluble ICAM-1 levels at admission were significantly higher in patients with reperfusion arrhythmias ($P < 0.05$). Plasma soluble ICAM-1 levels were followed for 3 weeks and were found to be consistently higher in the group of patients with reperfusion arrhythmias. Simple regression analysis showed no significant relationship between plasma ICAM-1 levels and age, systolic and diastolic blood pressures, or serum creatine kinase activity. The increase in the plasma levels of ICAM-1 was observed in patients manifesting ventricular reperfusion arrhythmias. This increase in ICAM-1 levels was observed as early as at admission. The increased plasma ICAM-1 levels may be a useful biochemical marker for predicting myocardial reperfusion injury such as reperfusion arrhythmias in AMI [35].
- Vascular endothelial growth factor is a potent endothelial cell-specific mitogen and could affect the outcome of AMI. It was found that serum VEGF levels elevated gradually after the onset of AMI and reached a peak on day 14. VEGF levels in a culture medium of peripheral blood mononuclear cells after incubation for 24 hours were maximally elevated 7 days after the onset. It was found that patients showing improvement in left ventricular systolic function during the course of AMI showed significantly higher PBMC-VEGF levels than patients without improvement. It is possible that VEGF produced by PBMC may play an important role in the improvement of left ventricular function by promoting angiogenesis and re-endothelialization after AMI [36].
- Neutrophil and monocyte counts increase within days of onset of AMI. Because leukocytes are recruited to the involved myocardial region, it was postulated that these activated cells would display an increased expression of adhesion molecules necessary for effective endothelial transmigration. The expression of neutrophil and monocyte lymphocyte function-associated antigen-1, Mac-1, very late after activation antigen-4 and ICAM-1 were measured by flow cytometry throughout the acute phase of AMI in 25 patients with AMI and in 10 age-matched control subjects. It was found that the expression of Mac-1 on neutrophils increased significantly, whereas no expression of VLA-4 and ICAM-1 was detected. The expression of LFA-1, Mac-1, VLA-4 and ICAM-1 on the monocyte cell membrane in patients with an AMI was increased compared with that in control subjects by 22% (on day 7), 67%, 13% and 44% (all on day 4), respectively (all $P < 0.001$). The conclusions of the study were that an increased expression of neutrophil and monocyte adhesion molecules may contribute to their adhesion to the endothelium in the ischemic territory. This adhesion could feasibly precipitate vasoconstriction or add a local thrombotic effect due to tissue factor expression secondary to Mac-1 engagement. In addition, the manifestations of increased density of LFA-1 and Mac-1 by activated leukocytes with monocytes also expressing ICAM-1 suggests that leukocytes may form microaggregates that could cause microvascular plugging. This mechanism may facilitate the occurrence of the "no-reflow" phenomenon or slow coronary filling after AMI [37].
- In another study, sICAM-1 and E-selectin levels were measured in patients with AMI and following balloon angioplasty. It was found that in patients with AMI the plasma levels of sICAM-1

TIMP = tissue inhibitor of metalloproteinases
sIL-2R = free soluble IL-2 receptor
ICAM = intercellular adhesion molecule
VEGF = vascular endothelial growth factor
PBMC = peripheral blood mononuclear cells
VLA = very late after activation antigen
LFA = lymphocyte function-associated antigen

exceeded those observed in age- and gender-matched healthy subjects. E-selectin levels were normal on admission, increased at 6 hours and at day 1, before decreasing to normal levels on the fifth day. After brief myocardial ischemia occurring during PTCA, an increased level of sICAM-1 was observed following balloon deflation in the coronary sinus as compared to the subjects undergoing coronary angiography [38]. Thus, soluble adhesion molecules expressed by activated endothelial cells are released into peripheral blood during both AMI and brief myocardial ischemia, and measurements of such molecules may prove useful for monitoring vascular endothelium activation following myocardial ischemia/necrosis.

What is the significance of soluble cell adhesion molecules? First it was considered as a way of peptide excursion, but recent studies have demonstrated that cardiovascular death or myocardial infarction was associated with elevated ICAM-1 and vascular adhesion molecule-1, and E-selectin levels tended to be higher, indicating increased inflammatory activity [39]. An animal study has shown that shedding the blood with recombinant ICAM-1 reduced leukocyte adherence to mesenteric venules in post-ischemic reperfusion injury dose dependently [40].

Conclusions

Patients with elevated WBC counts have been shown to have a higher risk of developing an AMI and to be at higher risk for adverse events during the acute setting. In this review we reviewed the clinical data on the association between WBC count of AMI patients (on admission) and the prognostic outcome of these patients; we discussed possible mechanisms and the possible correlation between high WBC count and the development of myocardial damage. It is possible that measuring WBC count, WBC subpopulations, cell adhesion molecule and cytokine levels should be performed in order to seek an improved method for risk stratification of patients admitted with AMI.

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PTCA = percutaneous transluminal coronary angioplasty

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Research Project

Expression of basic fibroblast growth factor is associated with poor outcome in non-Hodgkin's lymphoma

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Background: It is now clear that angiogenesis and angiogenesis factors are important in the pathogenesis of hematologic malignancies. High pretreatment levels of serum basic fibroblast growth factor (bFGF) have been shown to be associated with poor prognosis in patients with non-Hodgkin's lymphoma (NHL) [1].

Objectives: To evaluate whether NHL cells express bFGF and/or its receptor (bFGFR-1) and whether bFGF expression correlates with bFGF serum levels, intratumoral microvessel density, and patient outcome.

Methods: We measured bFGF by enzyme-linked immunosorbent assay in sera taken from 58 patients with NHL before treatment and in 19 of them also after treatment. Pathologic specimens at diagnosis were evaluated by immunohistochemistry staining using polyclonal antibody against factor-VIII-related antigen, bFGF and bFGFR-1 to determine the expression of the microvessel count and bFGF and bFGFR-1.

Results: The lymphoma specimens demonstrated positive staining for bFGF (in 23%) and bFGFR-1 (in 58.5%). The patients who expressed bFGF had a significantly worse progression-free and overall survival than those who did not ($P = 0.003$ and $P = 0.03$ respectively, see figures below), while patients expressing bFGFR-1 were less likely to achieve complete remission than those lacking the receptor (33% vs. 65%, $P = 0.047$). There was no correlation of bFGF staining with either serum bFGF levels or microvessel count. bFGF serum levels did not change significantly after treatment.

Conclusions: NHL specimens express bFGF and its receptor (bFGFR-1) and this expression is associated with poor patient outcome.

References

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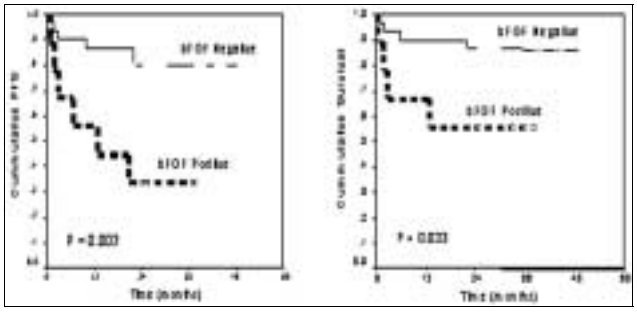

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