Is Measurement of Serum Vascular Endothelial Growth Factor Reliable in Patients With Active Pulmonary Tuberculosis?

To the Editor:

In a recent issue of the CHEST (June 2004), Alatas et al measured serum vascular endothelial growth factor (VEGF) concentrations in subjects with active pulmonary tuberculosis, and compared the changes in concentration before and after therapy. We would like to bring to your attention some methodological aspects of this study that might have caused relevant bias in the conclusion.

It is well known that VEGF resides in the α-granules of platelets and is released during blood clotting. Therefore, serum VEGF level is not a reliable indicator of the circulating extracellular VEGF levels. The authors reported that serum VEGF levels are increased in patients with active pulmonary tuberculosis when compared with those of subjects with inactive tuberculosis and those of healthy subjects. Thrombocytosis is common in subjects with active pulmonary tuberculosis, and it has been shown that, in these patients, platelets aggregate excessively in vitro. As a consequence, we think that the higher serum VEGF levels observed by the authors in the patients with active pulmonary tuberculosis were caused by increased platelet counts and increased platelet degranulation in vitro rather than by the fact that the “inflammatory mass stimulates the production of VEGF in order to supplement the needed blood supply by the formation of new blood vessels,” as suggested by the authors. Our hypothesis is supported by the authors’ observation that VEGF levels decrease after treatment when platelet count typically declines. Considering that a direct correlation between platelet count and serum VEGF level has been described, it is advisable to correct VEGF serum levels for variations in platelet count.

Furthermore, it must be pointed out that the authors did not report the length of time between venipuncture and the separation of serum from blood cells. This interval should always be standardized and declared because serum VEGF concentrations increase in a time-dependent manner. In particular, allowing the whole-blood sample to clot for between 2 and 6 h before serum is collected may reduce the time-dependent, nonuniform release of VEGF.

In plasma, platelet degranulation is minimized by adding anticoagulants to blood samples, and, as a consequence, plasma VEGF concentrations are up to 20 times lower than the matched serum VEGF concentrations. Citrate/theophylline/adenosine/dipryridamole plasma should be used when circulating extracellular VEGF levels are measured.

In conclusion, we think that the increase in serum VEGF levels observed by the authors in subjects with active pulmonary tuberculosis was caused by higher platelet counts and enhanced platelet degranulation in vitro, and does not reflect increased circulating extracellular VEGF levels in vivo. A meticulous standardization of blood sampling is mandatory when serum VEGF levels are measured. Serum VEGF concentrations should always be corrected for the variations in platelet count. Finally, it would be interesting to confirm the results reported by the authors with citrate/theophylline/adenosine/dipryridamole plasma samples.

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To the Editor:

We read with interest Dr. Ferrero’s comments regarding our recently published work (June 2004) about vascular endothelial growth factor (VEGF) levels in tuberculosis patients. We disagree, however, with his conclusion that for the accurate measurement of circulating VEGF levels, measuring serum levels is completely unsuitable. Indeed, VEGF expression has been found not only in platelets, but also in activated macrophages, neutrophils, smooth muscle cells, monocytes, and T lymphocytes. However, the extent to which these cells contribute to the circulating VEGF levels in vivo, and its physiologic and pathologic relevance is not yet clear. In a recent article, Mittermayer et al showed that systemic plasma concentrations of VEGF are increased by inflammation that is independent from thrombin formation but is associated with neutrophil degranulation. They proposed that the activation of leukocytes rather than platelets plays a role in the marked increase in VEGF levels. In another view, activated platelets have been found to release VEGF together with β-thromboglobulin, suggesting that VEGF resides in the α-granules of the platelets. This finding leaves open the possibility that at least part of the VEGF in platelets has come from plasma by endocytosis.

Dr. Ferrero incorrectly stated that thrombocytosis is common in patients with active pulmonary tuberculosis. Thrombocytosis is not common in patients with active pulmonary tuberculosis. Recently, Olaniyi and Aken Ova observed thrombocytosis only in 12.9% of their patients with active pulmonary tuberculosis. Other articles by Morris and Akintunde et al have observed thrombo-

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cytosis in 33% and 18%, respectively, of their pulmonary tuberculosis patients. We observed thrombocytosis in only 11% of pulmonary tuberculosis patients. Moreover, we found thrombocytosis in only 1 of 10 patients with active pulmonary tuberculosis in whom we were able to examine serum VEGF levels at the end of tuberculosi treatment. Thrombocytosis is a consequence of the general inflammatory state of the body against the infection.

Also, VEGF is known to be involved in inflammation and wound healing. Therefore, in our view it is hard to explain the increase of serum VEGF levels in patients with active pulmonary tuberculosis by using thrombocytosis alone.

The second important issue raised by Dr. Ferrero is the standardization of the sampling process. In our study setting, all samples were drawn and processed by the same biochemist. The separation of sera from blood cells was performed in 30 to 45 min. Few studies in the literature concerning VEGF levels in tuberculosis patients have determined serum VEGF levels. Moreover, intense angiogenesis was shown ultrastructurally in active pulmonary tuberculosis lesions. The expression of VEGF in alveolar macrophages around active tuberculosis lesions was shown by immunohistochemistry.

Finally, the percentage of thrombocytosis present in our active pulmonary tuberculosis patients is not high, and the sample preparation procedures are well-designed and standardized. Although the type of sample that should be used in VEGF measurements is still a matter of debate, all studies regarding VEGF levels in pulmonary tuberculosis patients were performed using serum samples. However, further research is needed to compare the relationship of VEGF levels in serum and plasma, and different anticoagulant agents, since the existing studies comparing serum and plasma VEGF levels were performed with cancer patients.

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Troponin in Septic and Critically Ill Patients

To the Editor:

With great interest, we read the recent review by Roongsritong et al (May 2004) on the causes of elevations in troponin level in patients who had not experienced myocardial infarction, particularly the section about troponin levels in septic and critically ill patients. As the authors state, the exact mechanism of increased cardiac troponin levels in patients with sepsis remains unknown.

The authors suggest several mechanisms for troponin release in septic patients. One of their suggestions is that cytokines or endotoxin might cause myocardial injury. Circulating mediators such as tumor necrosis factor (TNF)-α and interleukin-1β have been shown to cause myocardial depression in patients with sepsis. Cytokines might increase the permeability of the myocyte membrane with the leakage of free cardiac troponin I (cTnI) from the cytoplasm while the myocyte-contraction complex remains intact. We could not confirm this using a human endotoxin model. Our volunteers showed a septic profile after being injected with endotoxin. Troponin levels were not measurable, although TNF-α levels were high enough to cause myocardial depression. In these volunteers, we also measured increased levels of death hormones, TNF, and tumor necrosis factor-related apoptosis-inducing ligand treatment, suggesting active apoptosis. Therefore, we think that it is unlikely that cTnI levels are elevated due to the leakage of free cytoplasmic cTnI induced by cytokines or apoptosis during the early phase of sepsis. Another proposed mechanism of troponin release is the dysfunction of microcirculation. However, it is questionable whether dysfunctional microcirculation during sepsis also occurs in the heart.

Finally, Roongsritong et al conclude that troponin measurements in critically ill septic patients provide valuable prognostic information. We would like to point out that, although several studies have shown that troponin could be used as a prognostic marker in critically ill patients, Kollef et al have shown with a multivariate analysis that left ventricular function, not troponin level, was a predictor for mortality. We conclude that the mechanism and meaning of the elevation of troponin levels in patients with sepsis still has to be elucidated, but that the confusion exists only with marginally elevated troponin levels. Higher levels of troponin (i.e., > 10 ng/mL),