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Diagnosis of Pulmonary Embolism by Use of Urinary TNFα and its Soluble TNF Receptor I

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ABSTRACT

The purpose of this clinical study was to determine the concentration of soluble tumor necrosis factor in urine of patients with pulmonary embolism (PE), verses voluntary control individuals. Sixteen patients (ages 24 to 74 years) with diagnosis of PE, documented by ventilation perfusion scan or pulmonary angiogram, were the subjects of this study. Ten cc of urine was obtained from each patient and subjected to a solid-phase enzyme-linked immunosorbent assay thus determining the soluble tumor necrosis factor (TNF) receptor I (R I) and TNFα levels in these samples. In this pilot study of PE cases, a statistically significant elevation in urinary levels of TNFα and soluble TNF R I was demonstrated in PE patients. The average urinary soluble TNF R I in normal subjects was 1,029 pg/mL and in PE patients the average TNF R I was 3,734.4 pg/mL.

The clinical diagnosis of PE is a challenging problem for the physician. Late diagnosis and delayed management of this condition could be associated with massive PE. Although pulmonary angiography is the gold standard for diagnosis of PE, it requires expensive equipment, trained radiologists, and the patient could be at risk of sensitivity to contrast agents.

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Introduction

Pulmonary emboli represent a common disorder and are associated with high mortality and morbidity rates. The clinical diagnosis of pulmonary embolism is often extremely difficult. Chest pain and shortness of breath are not specific for this condition and may represent other pulmonary conditions.[1] Moreover, further diagnostic tests such as ultrasound, ventilation-perfusion ratio ($\dot{V}/\dot{Q}$), and spiral pulmonary computed axial tomography (CAT) scans are technician dependent, are nonspecific, and require expensive equipment. Although the pulmonary angiogram is specific, it requires highly technical personnel and risks possible dye sensitivity. We recently presented a preliminary report on diagnosis of deep venous thrombosis (DVT) by use of plasma-soluble tumor necrosis factor (TNF) receptor I.[1] The positive result of this clinical study prompted us to evaluate soluble TNF receptor I in the urine of patients with pulmonary embolism. The pathogenesis of deep venous thrombosis has been attributed to stasis, hypercoagulability, and inflammation. However, a recent publication[2] shows the interaction of inflammatory procoagulant cytokines, inflammatory cells, and venous endothelium to be the first step in activating the clotting cascade.

Procoagulant cytokines such as TNF and P-selectin up-regulate tissue factor. These cytokines also down-regulate protein-C and thrombomodulin, which ultimately leads to clot formation.

Materials and Methods

After approval by the hospital's Institutional Review Board, 16 patients, 8 men and 8 women whose ages ranged from 24 to 74 years, were the subjects of this clinical study. Twenty healthy volunteers, 12 men and eight women whose ages ranged from 20 to 75 years, served as control subjects. Symptoms among the patients included leg pain (12 patients), chest pain (14 patients), shortness of breath (11 patients), swollen leg (five patients), and hemoptysis (one patient). The work-up included complete blood count, blood chemistry, sedimentation rate, and rheumatoid factors. Ultrasound imaging of the lower extremities, ventilation perfusion ($\dot{V}/\dot{Q}$) scans, and pulmonary angiography in selected cases were used for diagnosis of DVT and pulmonary embolism (PE).

After proper consent, 10 cc of urine was obtained from each subject. These samples were subjected to a solid-phase enzyme-linked immunosorbent assay (ELISA) analysis for TNF alpha as well as soluble TNF receptor I (R I).[3,4] This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for TNFα is precoated onto a microplate.

Table I

TNF R I and TNFα Urine Levels in PE Patients

PE Patient Number	TNF R I (pg/mL)	TNFα (pg/mL)
1	1,474.2	3.1
2	240	0.5
3	5,000	7.1
4	3,973.4	0.7
5	4,514.9	0.5
6	5,000	3.6
7	774.9	2.1
8	1,253.4	0.2
9	657	0.3
10	5,000	0.1
11	598.3	0
12	1,576.9	0.1
13	3,219.1	0
14	5,000	0
15		0.2
16		7.5
Average	3,734.4	1.63
Standard error	521.7	0.62

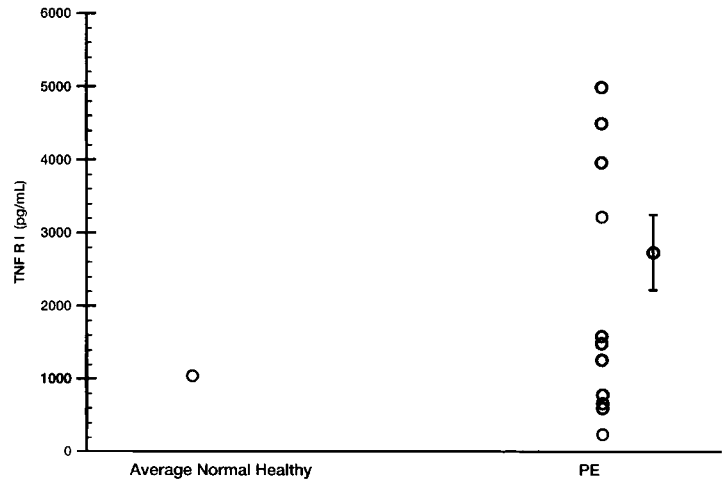

Figure 1.
TNF R I urine levels in PE patients.

Average normal healthy value for TNF R I = 1,029 pg/mL.

Average PE patient value for TNF R I = 2,734.4 pg/mL ±521.7 (std error).

Elevation of TNF R I in PE is 265.7%.

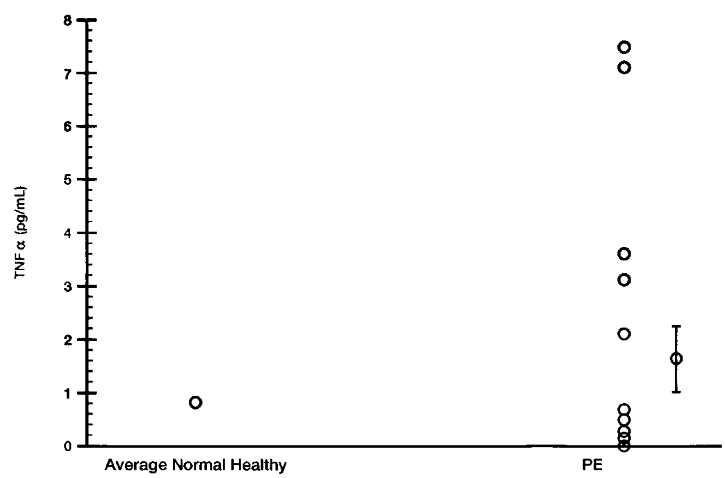

Figure 2.
TNFα urine levels in PE patients.

Average normal healthy value for TNFα = 0.82 pg/mL.

Average PE patient value for TNFα = 1.63 pg/mL ±0.62 (std error).

Elevation of TNFα in PE is 198.7%.

Results

The average normal TNF R I urine level was 1,029 pg/mL and in PE patients the average TNF R I was 3,734.4 pg/mL. The average normal TNFα urine level was 0.82 pg/mL and in PE patients the average TNFα was 1.63 pg/mL (Figures 1, 2). A statistically significant elevation in urinary levels of TNFα ($p < 0.05$) and soluble TNF receptor I ($p < 0.0001$) was demonstrated in PE patients (Table I).

Discussion

Tumor necrosis factors are polypeptide cytokines produced by mononuclear cells. Their activation may cause a severe inflammatory response. TNF receptors have high affinity to TNF[5] and play a key role in the initiation and control of the cellular response to these cytokines. Soluble forms of TNF receptors bind to TNF[6] and can interfere with its binding to the membrane-bound receptors. It has been stated that soluble tumor necrosis factors function as physiological inhibitors of the TNF activity at the cellular membrane.

In venous thrombosis, the venous wall is infiltrated by activated nuetrophils, monocytes,[7] and other inflammatory cells, producing inflammatory and procoagulant cytokines. These cytokines and inflammatory cells in the venous wall have prompted efforts to determine whether local production of these cytokines may play an important role in the genesis and evolution of DVT and pulmonary embolism. These cytokines often induce themselves, activate a cascade of other cytokines, and synergize or antagonize each other's effects.

TNF is a potent polypeptide that could induce other cytokines. It mediates its effects by binding to either the P55 or P75 TNF receptors to transmit signals to the cytoplasm. These two TNF receptors are abundant in cells that were producing TNF, suggesting that autocrine or paracrine stimulation is a constant ongoing process.

The soluble TNF receptors are generated through elastase proteolytic cleavage of the P75 receptors. Since soluble TNF receptors are shed forms of the transmembrane TNF receptor, soluble TNF receptors may act as a regulator of TNF activity at the cellular membrane.

The rapid clearance of TNF from the circulation and binding of TNF to receptors allow urine-soluble TNF receptors' determination to be an alternative marker for diagnosis of DVT and pulmonary embolism. Because of the long half-life of soluble TNF receptors, we chose to use these cytokines as a marker for the diagnosis and evolution of DVT and pulmonary emboli. It is possible that the level of urine-soluble TNF receptor could be used as a quick noninvasive test when the diagnoses of DVT and PE are being entertained.

Conclusion

Although this pilot study has revealed a feasible correlation of urine-soluble TNF receptor and pulmonary embolism, further studies are needed to verify these preliminary data. Urine-soluble TNF receptors could provide a simple, cost effective, and noninvasive screening test for the presence of pulmonary embolism.

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References

1. Taheri SA, Lazar L, Haddad G, et al: Diagnosis of deep venous thrombosis by use of soluble necrosis factor receptor. Angiology 49:537-548, 1998.
2. Miller J, Thevenaus J, Pascal M: A new experimental model of venous thrombosis in rats involving partial stasis and slight endothelial alterations. Thromb Res 45:123-133, 1987.
3. Oliff A: The role of tumor necrosis factor (cachectin) in cachexia. Cell 54:141-142, 1998.
4. Porteu F, Nathan C: Shedding of tumor necrosis factor receptor by activated human Neutrophils. J Exp Med 172:599-607, 1990.
5. Roux-Lombard P, Punzi L, Hasler F, et al: Soluble tumor necrosis factor receptors in human inflammatory synovial fluids. Arthritis Rheum 36:485-489, 1993.
6. Zangerle R, Gallati H, Sarcletti M, et al: Tumor necrosis factor alpha and soluble tumor necrosis factor receptors in individuals with human immunodeficiency virus infection. Immunol Lett 41:229-234, 1994.
7. Wakefield TW, Strieter RM, Downing LJ, et al: P-selectin and inhibition reduce venous thrombosis inflammation. J Surg Res 640:26-31, 1996.