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Tumor Necrosis Factor Alpha (TNF α) and Mortality in Heart Failure: a community study

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Abstract

Background—Tumor necrosis factor α (TNF α), an inflammatory cytokine, was reported to be elevated in trials of heart failure (HF) with reduced ejection fraction (EF) and associated with mortality. Whether this is true for HF with preserved EF is unknown and community data are lacking. We evaluated the distribution of TNF α , its association with baseline characteristics and mortality, and its benefit to assess risk in community HF patients.

Methods and Results—Olmsted County residents with active HF from July 2004 to March 2007 (n=486, mean age 76.7 years, 55% EF \geq 50%) were prospectively recruited. Clinical characteristics and TNF α were measured. Elevated TNF α ($>$ assay limit of normal of 2.8pg/mL) was present in 143 (29%). Higher TNF α was associated with decreased creatinine clearance, non-smoking status, anemia, and greater comorbidity ($p_{\text{trend}} < 0.05$ for all). Mortality increased with increasing TNF α ($p = 0.016$), with 1-year mortality estimates of 16%, 18%, 23%, and 32% from lowest to highest quartile, respectively. After adjustment for age, sex, and EF, the hazard ratios for death were 1.24, 1.37, and 1.90 from second to highest TNF α quartile, respectively ($p_{\text{trend}} = 0.007$). TNF α contributed to risk assessment as indicated by the increases in the area under the receiver operating characteristics curves in all models examined ($p < 0.05$ for all). Results did not differ by EF ($p = 0.60$ interaction term of TNF α and EF).

Conclusions—TNF α was elevated in a large portion of community HF patients, was associated with a large decrease in survival, and provided a significant incremental increase in risk assessment above established indicators. TNF α is useful for risk assessment in HF patients with preserved and reduced EF.

Keywords

heart failure; inflammation; epidemiology; risk factors

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Disclosures- none

Introduction

Inflammation plays a key role in the pathogenesis of heart failure (HF)¹. Tumor necrosis factor alpha (TNF α), an inflammatory cytokine, has been implicated in HF progression as a mediator of myocardial dysfunction and adverse remodeling². Studies to date have shown that some patients with HF have elevated circulating levels of TNF α compared with controls^{3,4} and have suggested that increased circulating levels are associated with increased mortality^{5,6}. However, attempts to utilize TNF α as a therapeutic target in patients with severe systolic dysfunction has resulted in an increase in all-cause mortality⁷⁻⁹. To date, the role that TNF α plays in HF has not been fully elucidated and warrants further investigation.

Most published studies on TNF α are not generalizable to the entire HF population for several reasons. The vast majority of reports pertained to small number of patients (<150) and focused on clinical trial populations. In addition, they have only included patients with severe systolic dysfunction (ejection fraction (EF)<30-35%), although heart failure with preserved EF accounts for approximately half of all HF cases¹⁰. The substantial selection biases hinder the use of TNF α for risk prediction among all patients with HF.

In the present study, we aim to address these gaps in knowledge by examining the distribution of TNF α and its association with baseline characteristics among all HF patients in a population-based setting. Next, we determined whether an association between serum TNF α level and mortality exists and whether this association differs by EF. Finally, we evaluated whether TNF α confers an incremental benefit in predicting risk above recognized predictors in community HF patients.

Methods

Study design

This study is a population-based study conducted in Olmsted County which is located in Southeastern Minnesota and has a population of 124,277 (90% Caucasian, 51% female)¹¹. This type of research is feasible in Olmsted County because nearly all medical care in virtually every specialty is provided by relatively few providers including Mayo Clinic, Olmsted Medical Center, and a few private practitioners. The records from each institution are easily retrievable because Mayo Clinic maintains extensive indices which, through the Rochester Epidemiology Project, are extended to the records of other care providers to county residents. The result is the linkage of all medical records from all sources of care through a centralized system¹².

Identification of patients

To identify potential HF cases, natural language processing of the text of the electronic medical record is utilized¹³. The vast majority of patient visits to the system are transcribed and appear in the record within 24 hours, making prompt ascertainment of newly diagnosed HF cases possible. Next, the complete records of potential cases are reviewed by trained abstractors to collect additional data and verify HF cases using Framingham criteria¹⁴. Patients are then contacted directly to obtain consent for participation in the study, which involves Doppler echocardiography and obtaining venous blood samples to measure biomarkers. Recruitment for the cohort is ongoing, allowing assessment of multiple factors in community HF patients over time. Hospitalized patients are contacted while in the hospital, and patients recruited from a clinical setting are contacted at their next clinic visit for consent, enrollment and collection of data. All patients provided written authorization to participate in the study, which was approved by the Mayo Clinic Institutional Review Board.

Data Collection

Echocardiography—All echocardiograms were obtained and analyzed at Mayo Clinic Echocardiography laboratory according to the guidelines of the American Society of Echocardiography¹⁵. Left ventricular ejection fraction was measured using M-mode, quantitative, and semi-quantitative methods as previously described and validated¹⁶ with excellent correlation between the methods. Ejection fraction values were averaged when multiple measurements were performed. Though EF was dichotomized (<50%, ≥50%)¹⁰ for descriptive purposes, it was examined as a continuous variable in all analyses. Diastolic function was assessed by an approach which integrates Doppler measurements of the mitral inflow and Doppler tissue imaging of the mitral annulus using the medial annulus velocity, a method which has been previously validated¹⁷. Diastolic function was then classified based on these results into four categories including normal diastolic function, mild diastolic dysfunction (impaired relaxation without increased filling pressures), moderate diastolic dysfunction (impaired relaxation or pseudonormal with moderate elevation of filling pressures) and severe diastolic dysfunction (severely reduced compliance). In the case of severe mitral stenosis, mitral valve prosthesis, or missing data, diastolic function was classified as indeterminate.

TNF α measurement—Venous samples were obtained as soon as possible after recruitment of patients in both the inpatient and outpatient setting. All venous samples were stored in EDTA tubes, processed and stored at -70°C until TNF α measurement took place (samples were not thawed in the interim). TNF α level was quantified by enzyme-linked immunoassay (TNFSF1A Immunoassay, RND systems, Minneapolis, MN). Samples were incubated in microtitre plate wells with a monoclonal antibody specific for TNF α . Following a wash, an enzyme linked polyclonal antibody specific for TNF α was added. After incubation and second wash, an amplifier solution was added and the color developed in proportion to the amount of TNF α bound in the first step. The optical density of each well was measured to determine the concentration of TNF α present in each sample. The reference range for the assay is 0.550 pg/mL to 2.816 pg/mL. All of the assays were performed in the Mayo Clinic Immunochemical Core Laboratory and technicians were blinded to the clinical characteristics and outcomes of the patients.

Additional patient data—Data pertaining to baseline patient characteristics were obtained by review of the medical record by trained nurse abstractors. Prior myocardial infarction (MI) occurring in Olmsted County was defined through use of standardized MI criteria, which have been previously described and validated¹⁸. Physician's diagnosis was used to document history of coronary artery disease (CAD), history of chronic obstructive pulmonary disease (COPD), and history of atrial fibrillation/flutter. Hypertension was defined as systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg or the use of anti-hypertensive medications. Smoking status was classified as 'current', 'former' (quit at least 6 months ago), or 'never' based on documentation. Hyperlipidemia was defined using National Cholesterol Education Program guidelines¹⁹ or use of hyperlipidemia medications. Diabetes mellitus was defined using the American Diabetes Association criteria²⁰. The height and weight documented at the time of HF diagnosis were utilized to calculate body mass index (BMI). Creatinine clearance was calculated based on the last outpatient creatinine value using the Cockcroft-Gault equation²¹. Severe renal disease was defined as a Creatinine clearance <30ml/min. The hemoglobin value nearest the date of HF diagnosis was used to diagnose anemia using World Health Organization criteria (hemoglobin <13 mg/dL in men or 12 mg/dL in women)²². Charlson Index, a comorbidity score reflecting the cumulative increased likelihood of one-year mortality, was also collected²³. New York Heart Association functional class was also collected.

Mortality Follow-up—Follow-up took place through passive surveillance of the community medical records. The ascertainment of death included death certificates filed in Olmsted County, obituary notices and electronic files of death certificates obtained from the State of Minnesota Department of Vital and Health Statistics¹². All-cause mortality was used as the endpoint in all analyses.

Statistical Analysis

Subjects were divided into quartiles based on TNF α level (<1.5 , $1.5 \leq \text{TNF}\alpha < 2.1$, $2.1 \leq \text{TNF}\alpha < 3.1$, ≥ 3.1 pg/mL). Because the TNF α distribution was skewed, log TNF α was utilized for all analyses of TNF α as a continuous variable. Baseline characteristics are presented as frequencies or means with standard deviations. Trends in baseline characteristics across quartiles were analyzed using generalized linear models for continuous variables and Mantel-Haenszel χ^2 for categorical variables. In addition, log TNF α was analyzed as a continuous variable with all baseline characteristics to ensure that a significant association was not missed by quartile analysis. All patients had TNF α measurements and echocardiography performed. Data was complete in all other areas with the exception of smoking status (n=1 missing), prior history of MI (n=11 missing, also used to calculate Charlson index), hemoglobin (n=1 missing) and creatinine clearance (n=14 missing).

Mortality was assessed using the Kaplan-Meier method with censoring at the time of last follow-up. Cox proportional hazard regression analysis was utilized to estimate both unadjusted and adjusted hazard ratios for mortality by TNF α group using the lowest quartile as the reference. Baseline clinical characteristics were adjusted for in the multivariable models using forced entry. Individual comorbidities were adjusted for rather than Charlson index score. In an ancillary analysis, to investigate whether the association between TNF α and mortality differed among those with incident or prevalent HF, we restricted the sample to those with incident HF and performed Cox proportional hazard regression analysis. Finally, logistic regression analysis was performed to assess the incremental value of TNF α in determining risk of death in HF patients. Models before and after the addition of TNF α were compared using the area under the receiver operating characteristic (ROC) curve with methods previously described²⁴ and were one-tailed. A p value <0.05 was used as the level of significance. Analyses were performed using SAS 9.1 (SAS Institute Inc., Cary, NC) and JMP Version 6.0.0. The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Patient Identification and Baseline Characteristics

Four hundred eighty-six consecutive patients with active HF were enrolled in the study between July 29, 2004 and March 7, 2007 and underwent echocardiography and TNF α assay. The consent rate for the study during this time period was 71%. The mean age of participants was 76.7 ± 13.0 years, 236 (48.6%) were male, and 266 (54.8%) had preserved EF ($\geq 50\%$) (Table 1). The population had a high index of comorbidity with 316 (66.5%) with Charlson Index ≥ 3 and overall poor functional status with 355 (73.0%) having NYHA class III/IV HF symptoms.

TNF α Levels

TNF α levels ranged from 0.3 to 28.0 pg/mL with a mean value of 3.1 ± 3.4 pg/mL and median value of 2.1 pg/mL (25th–75th percentile 1.5–3.1 pg/mL). 143 (29%) of the cohort had TNF α levels above the reference range for the test (>2.8 pg/mL). The distribution of TNF α did not differ significantly between patients recruited in an inpatient and outpatient setting ($p=0.31$). Higher TNF α was associated with current non-smoking status, increased prevalence of anemia, and a higher level of comorbidity (Table 1). In addition, higher TNF α was associated with

decreasing creatinine clearance (Figure 1a) and decreasing hemoglobin (Figure 1b). The graded association is suggestive of a “dose response” effect. There was no association between TNF α and BMI or NYHA functional class. The distribution of TNF α [median (25th–75th percentile)] was 2.2 (1.5–3.2) and 1.9 (1.4–2.9 pg/mL) among those with preserved and reduced EF, respectively. Though a non-significant trend toward increasing age and EF with higher TNF α quartile were noted, both relationships remained non-significant when TNF α was examined as a continuous variable ($p=0.13$ age vs. log TNF α , $p=0.15$ EF vs. log TNF α , data not shown), indicating there was no significant association between age and TNF α or EF and TNF α . No gender-based differences were present. Finally, there was a trend toward increased frequency of CAD among those with higher TNF α , but it was not significant ($p=0.069$).

TNF α Level and Association with Mortality

After a mean follow-up of 17 ± 10 months, 147 (30.2%) patients were dead. The one year Kaplan-Meier mortality estimate was 22% (18%–26%) overall, and increased from lowest to highest quartile [16% (9%–22%), 18% (11%–25%), 23% (15%–29%), and 32% (23%–40%)] respectively $p=0.016$, Figure 2). The unadjusted hazard ratios (HR) (95% CI) for death were 1.34 (0.82–2.21), 1.47 (0.89– 2.44), 2.10 (1.30–3.38) from lowest to highest quartile, respectively, using the lowest quartile as the referent ($p_{\text{trend}}=0.002$, Table 2). After adjustment for age, sex, EF, and comorbidities, this relationship held with a hazard ratio for death of 1.88 (1.09–3.25) in the highest vs. lowest quartile (p_{trend} across quartiles= 0.028). The largest impact on the increase in hazard ratios observed from Model 1 to 2 was due to adjustment for smoking status, while adjustment for creatinine clearance and anemia attenuated the association between TNF α and mortality. Adding C-reactive protein (CRP) to the model did not appreciably change the association between TNF α and mortality. The relationship between TNF α and mortality was similar among those with incident HF compared with the overall population. No interaction between TNF α and EF was noted ($p=0.60$ for interaction term), indicating that associations between TNF α and death did not differ significantly for patients with preserved vs. reduced EF.

Addition of TNF α Level to Mortality Risk Assessment

Inclusion of TNF α in the model resulted in a notable increase in predictive value of mortality in patients with heart failure (Table 3). For instance, the area under the receiver operating characteristic curve increased from 0.67 to 0.70 in a model that included age and sex ($p=0.035$), and from 0.74 to 0.78 in a model that include age, sex, EF, NYHA functional class, and traditional risk factors ($p=0.018$).

Discussion

In the present study, we demonstrated that serum TNF α is elevated in a large proportion of community HF patients with a wide range of EF, and that elevated circulating TNF α was strongly associated with decreased creatinine clearance, anemia, and a high degree of comorbidity. Second, we identified a strong independent association between elevated TNF α and mortality in HF patients, irrespective of EF. Finally, we have demonstrated that TNF α improved risk prediction in HF above traditional risk indicators.

HF is characterized by an inflammatory state with reports of elevation of several inflammatory cytokines, including TNF α in selected patients with HF^{1,25}. TNF α is an inflammatory cytokine produced mainly by monocytes and macrophages with pleiotropic effects. It binds to cell surface receptors preventing the usual rise in intracellular calcium concentrations, resulting in activation of multiple signal transduction pathways, kinases, and transcription factors^{26,27}. TNF α was first recognized as a possible mediator of cardiac dysfunction in sepsis after exposure to endotoxin^{28,29}. Since that time, elevated TNF α has been demonstrated in many

cardiac conditions, including myocarditis, hypertrophic cardiomyopathy³⁰, MI³¹, following cardiopulmonary bypass³², and HF.

TNF α has been implicated in HF disease progression. Animal studies have demonstrated increased cardiac expression of TNF α in failing myocardium³³ and noted that overexpression of myocardial TNF α resulted in left ventricular dysfunction and dilatation^{34–37}. TNF α has also been shown to be a mediator of hypertrophy³⁸ and to contribute to adverse left ventricular remodeling³⁹. Despite its many deleterious effects, TNF α was demonstrated to have some protective effects, namely against acute ischemic injury^{40,41}. Overall, TNF α has a complex role in the inflammatory processes that is still incompletely elucidated.

Human studies of TNF α were performed in specific populations, such as those enrolled in a randomized controlled trial of the inotropic drug vesnarinone⁵, and are thus not generalizable to the majority of HF patients. Specifically, clinical studies evaluating serum TNF α and clinical trials targeting TNF α were restricted to patients with severe systolic dysfunction (EF \leq 30–35%). Thus, although more than half of all HF patients have preserved EF¹⁰, there have been no clinical studies examining the role of TNF α in these patients. Further, while prior studies reported that TNF α was elevated in the setting of systolic dysfunction^{3,4,42}, its distribution and determinants remain incompletely defined. Indeed, there are conflicting reports on the association of TNF α level with HF severity with some studies demonstrating increased levels with worsening NYHA functional class^{6,43}, and other studies demonstrating no association^{5,42}. Similarly, studies noted that higher TNF α may be associated with older age⁵, decreased renal function^{3,6}, and decreased body weight³, while other failed to do so⁴³. Finally, the need for community studies examining the role of TNF α was recognized² but up to now not addressed.

The present study contributes to address these gaps in knowledge as it demonstrates that elevated circulating TNF α is present in approximately one third of community HF patients with a wide range of EF and is associated with decreasing hemoglobin and creatinine clearance, greater comorbidity, and current non-smoking status. While we identified a marginal trend towards higher TNF α levels with older age, we however, found no association between TNF α and NYHA functional class. In addition, the present data challenges the prior notion that TNF α may be a marker of “cardiac cachexia”³ given the lack of association with BMI.

In clinical trials of HF with severe systolic dysfunction, elevated TNF α was associated with increased mortality^{5,6}. However, data from HF patients in the community and with preserved EF were lacking. The present study demonstrated that elevated TNF α is independently associated with mortality in patients with both preserved and reduced EF, thereby underscoring the relevance of this biomarker for risk prediction in all categories of the HF syndrome.

Further, the data presented herein indicated that TNF α makes a substantial contribution to risk prediction. Indeed, as recently underscored, hazard ratios and p values are useful in determining statistical significance, but in order to demonstrate clinical utility in risk prediction, further measures must be reported⁴⁴. One established way of examining incremental changes in predictive ability involves measuring the area under the ROC curve. By using ROC analysis, we demonstrated that TNF α provides an improved risk prediction over established indicators.

Strengths and Limitations

Though Olmsted County is becoming more diverse, the population studied was primarily Caucasian, so these data should be replicated in other racial and ethnic groups. The participation rate for the study was 71%, which is similar to rates reported in other community studies of cardiovascular disease^{45,46}. Further, it should be noted that blood specimens are stored for

future research as a part of the present study, and assessment of other factors, including biomarkers, is likely in future investigations. The present study has several notable strengths. First, we examined TNF α in a large community HF population, which has not been previously reported. Second, patients with the full spectrum of EF were examined, and complete EF ascertainment was present. This enabled evaluating the role of TNF α in patients with preserved EF for the first time.

Conclusions

Elevated levels of the inflammatory cytokine TNF α are present in a large subset of community patients with HF, both with preserved and reduced EF. TNF α is independently associated with increased mortality. Furthermore, TNF α improves risk prediction above other established indicators, and can be useful for risk stratification in patients with HF.

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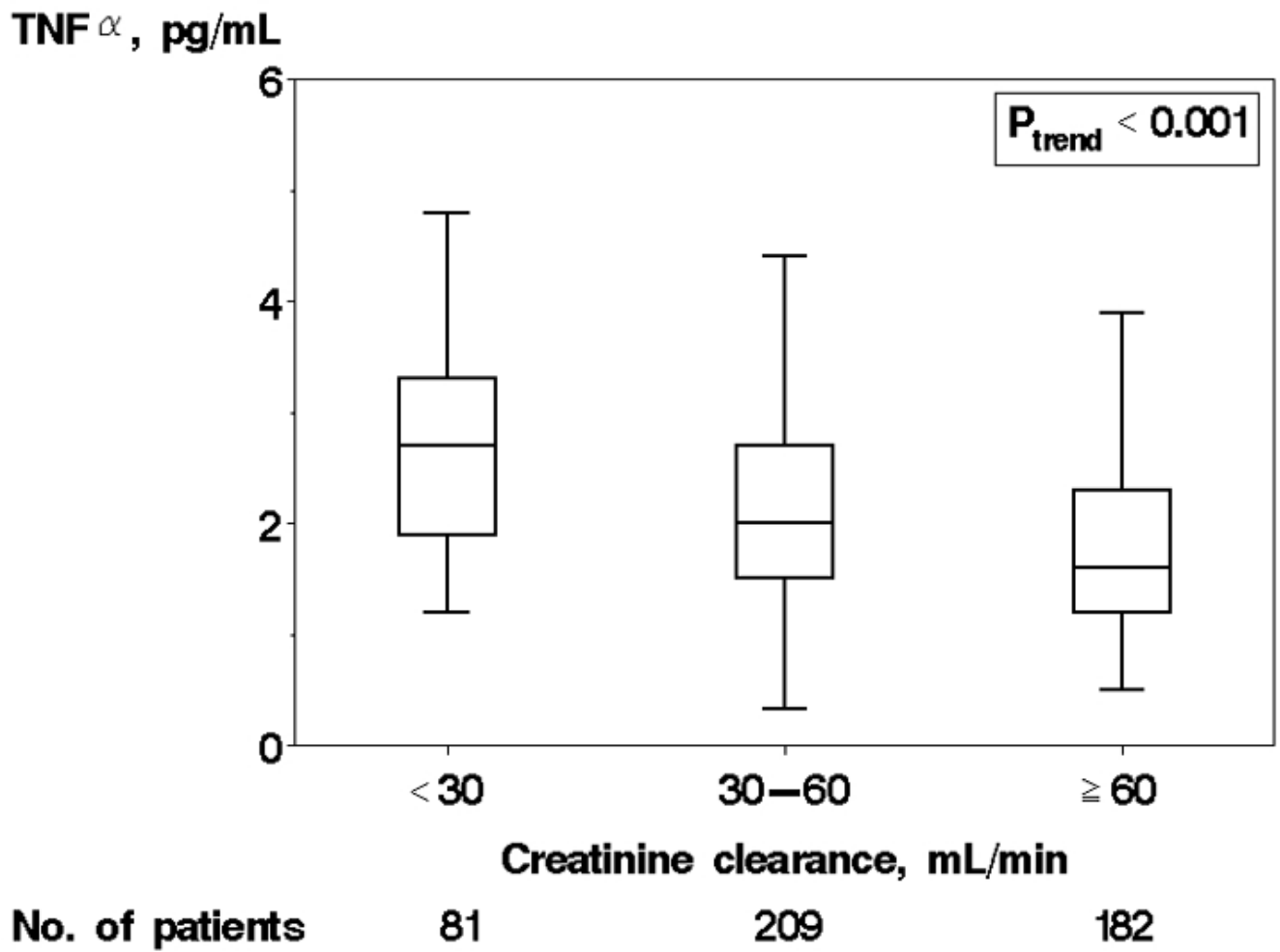
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References

1. Torre-Amione G. Immune activation in chronic heart failure. *American Journal of Cardiology* 2005;95 (suppl):3C–8C.
2. Armstrong EJ, Morrow DA, Sabatine MS. Inflammatory biomarkers in acute coronary syndromes: part I: introduction and cytokines. *Circulation* 2006;113:e72–e75. [PubMed: 16476853]
3. Levine B, Kalman J, Mayer L, Fillit HM, Packer M. Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. *New England Journal of Medicine* 1990;323:236–41. [PubMed: 2195340]
4. Dutka DP, Elbown JS, Delamere F, Shale DJ, Morris GK. Tumour necrosis factor alpha in severe congestive heart failure. *British Heart Journal* 1993;70:141–3. [PubMed: 8038024]
5. Deswal A, Peterson NJ, Feldman AM, Young JB, White BG, Mann DL. Cytokines and cytokine receptors in advanced heart failure: An analysis of the cytokine database from the vesnarinone trial (VEST). *Circulation* 2001;103:2055–9. [PubMed: 11319194]
6. Rodriguez-Reyna TS, Arrieta O, Castillo-Martinez L, Guevara P, Rebollar V, Granados J. Tumour necrosis factor alpha and troponin T as predictors of poor prognosis in patients with stable heart failure. *Clinical Investigation in Medicine* 2005;28:23–9.
7. Chung ES, Packer M, Lo KH, Fasanmade AA, Wilkerson JT. Randomized, double-blind, placebo-controlled, pilot trial of Infliximab, a chimeric monoclonal antibody to tumor necrosis factor- α , in patients with moderate-to-severe heart failure. *Circulation* 2003;107:3133–40. [PubMed: 12796126]
8. Mann DL, McMurray JJ, Packer M, Swedberg K, Borer JS, Colucci WS, Djian J, Drexler H, Feldman A, Kober L, Krum H, Liu P, Nieminen M, Tavazzi L, van Veldhuizen DJ, Waldenstrom A, Warren M, Westheim A, Zannad F, Fleming T. Targeted anticytokine therapy in patients with chronic heart failure: results of the randomized Etanercept worldwide evaluation (RENEWAL). *Circulation* 2004;109:1594–1602. [PubMed: 15023878]
9. Anker SD, Coats AJ. How to RECOVER from RENAISSANCE? The significance of the results from RECOVER, RENAISSANCE, RENEWAL, and ATTACH. *International Journal of Cardiology* 2002;86:123–30. [PubMed: 12419548]
10. Bursi F, Weston SA, Redfield MM, Jacobsen SJ, Pakhomov S, Nkomo VT, Meverden RA, Roger VL. Systolic and diastolic heart failure in the community. *Journal of the American Medical Association* 2006;296:2209–16. [PubMed: 17090767]

11. U.S. Census Bureau State and County QuickFacts. [Accessed September 27, 2007]. Available at: <http://quickfacts.census.gov/qfd/states/27/27109.html>
12. Melton LJ. History of the Rochester Epidemiology Project. *Mayo Clinic Proceedings* 1996;71(3): 266–74. [PubMed: 8594285]
13. Pakhomov SV, Buntrock J, Chute CG. Prospective recruitment of patients with congestive heart failure using an ad-hoc binary classifier. *Journal of Biomedical Informatics* 2005;38:145–53. [PubMed: 15797003]
14. Ho KK, Anderson KM, Kannel WB, Grossman W, Levy D. Survival after the onset of congestive heart failure in Framingham Heart Study subjects. *Circulation* 1993;88:107–15. [PubMed: 8319323]
15. Lang RM, Bierig M, Devereux RB. Recommendations for chamber quantification: A report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Echocardiography. *Journal of the American Society of Echocardiography* 2005;18:1440–63. [PubMed: 16376782]
16. Quinones MA, Waggoner AD, Reduto LA, Nelson JD, Young JB, Winters WL, Ribeiro LG, Miller RR. A new, simplified and accurate method for determining ejection fraction with two-dimensional echocardiography. *Circulation* 1981;64:744–53. [PubMed: 7273375]
17. Ommen SR, Nishimura RA, Appleton CP, Miller FA, Oh JK, Redfield MM, Tajik MM. Clinical utility of doppler echocardiography and tissue doppler imaging in the estimation of left ventricular filling pressures: a comparative simultaneous doppler- catheterization study. *Circulation* 2000;102:1788–1794. [PubMed: 11023933]
18. Roger VL, Killian J, Henkel M, Weston SA, Goraya TY, Yawn BP, Kottke TE, Frye RL, Jacobsen SJ. Coronary disease surveillance in Olmsted County objectives and methodology. *Journal of Clinical Epidemiology* 2002;55:593–601. [PubMed: 12063101]
19. Grundy SM, Becker D, Clark LT, et al. Third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *Circulation* 2002;106:3143–57. [PubMed: 12485966]
20. Genuth S, Albert KG, Bennett P. Follow-up report on the diagnosis of diabetes mellitus. *Diabetes Care* 2003;26:3160–71. [PubMed: 14578255]
21. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976;16:31–41. [PubMed: 1244564]
22. World Health Organization. Nutritional Anemias: Report of a WHO scientific group. WHO Technical Report Series 1968;405:1.
23. Charlson ME, Pompei P, Ales KL, McKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *Journal of Chronic Diseases* 1987;40:373–83. [PubMed: 3558716]
24. Hanley JA, McNeil BJ. A method of comparing the areas under receiver operating characteristic curves derived from the same cases. *Radiology* 1983;148:839–43. [PubMed: 6878708]
25. Mann DL. Targeted anticytokine therapy and the failing heart. *American Journal of Cardiology* 2005;95(suppl):9C–16C.
26. Khanna D, McMahon M, Furst DE. Anti-tumor necrosis factor α therapy and heart failure. *Arthritis & Rheumatism* 2004;50:1040–50. [PubMed: 15077286]
27. Vilcek J, Lee TH. Tumor necrosis factor: new insights into the molecular mechanisms of its multiple actions. *Journal of Biological Chemistry* 1991;266:7313–6. [PubMed: 1850405]
28. Suffredini AF, Fromm RE, Parker MM, Brenner M, Kovacs JA, Wesley RA, Parillo JE. The cardiovascular response of normal humans to the administration of endotoxin. *New England Journal of Medicine* 1989;321:280–7. [PubMed: 2664516]
29. Kapadia S, Lee J, Torre-Amione G, Birdsall HH, Ma TS, Mann DL. Tumor necrosis factor- α gene and protein expression in adult feline myocardium after endotoxin administration. *Journal of Clinical Investigation* 1995;96:1042–52. [PubMed: 7635940]
30. Matsumori A, Yamada T, Suzuki H, Matoba Y, Sasayama S. Increased circulating cytokines in patients with myocarditis and cardiomyopathy. *British Heart Journal* 1994;72:561–6. [PubMed: 7857740]

31. Latini R, Bianchi M, Correale E, Dinarello CA, Fantuzzi G, Fresco C, Maggioni A, Mengozzi M, Romano S, Shapiro L, Zironi M, Tognoni G, Turato R, Ghezzi P. Cytokines in acute myocardial infarction: selective increase in circulating tumor necrosis factor, its soluble receptor and interleukin-1 receptor antagonist. *Journal of Cardiovascular Pharmacology* 1994;23:1–6. [PubMed: 7511719]
32. Hattler BG, Zeevi A, Oddis CV, Finkel MS. Cytokine induction during cardiac surgery: analysis of TNF-alpha expression pre and post-cardiopulmonary bypass. *Journal of Cardiac Surgery* 1995;10:418–22. [PubMed: 7579836]
33. Torre-Amione G, Kapadia S, Lee J, Bias RD, Liebowitz R, Mann DL. Expression and functional significance of tumor necrosis factor receptors in human myocardium. *Circulation* 1995;92:1487–93. [PubMed: 7664431]
34. Bryant D, Becker L, Richardson J, Shelton-I J, Franco F, Peshock R, Thompson M, Biroir B. Cardiac failure in transgenic mice with myocardial expression of tumor necrosis factor- α . *Circulation* 1998;97:1375–81. [PubMed: 9577949]
35. Kubota T, McTiernan CF, Frye CS, Slawson SE, Lemster BH, Koretsky AP, Demetris AJ, Feldman AM. Dilated cardiomyopathy in transgenic mice with cardiac-specific overexpression of tumor necrosis factor- α . *Circulation Research* 1997;81:627–35. [PubMed: 9314845]
36. Sun M, Dawood F, Wen W, Chen M, Dixon I, Krishenbaum LA, Liu PP. Excessive tumor necrosis factor activation after infarction contributes to susceptibility of myocardial rupture and left ventricular dysfunction. *Circulation* 2004;110:3221–8. [PubMed: 15533863]
37. Bozkurt B, Kribbs SB, Clubb FJ, Michael LH, Didenko VV, Hornsby PJ, Seta Y, Oral H, Spinale FG, Mann DL. Pathophysiologically relevant concentrations of tumor necrosis factor- α promote left ventricular dysfunction and remodeling in rats. *Circulation* 1998;97:1382–91. [PubMed: 9577950]
38. Yokoyama T, Nakano M, Bednarczyk JL, McIntyre BW, Entman M, Mann DL. Tumor necrosis factor α provokes a hypertrophic growth response in adult cardiac myocytes. *Circulation* 1997;95:1247–52. [PubMed: 9054856]
39. Sun M, Chen M, Dawood F, Zurawska U, Li JY, Parker T, Kassiri Z, Kirshenbaum LA, Arnold M, Khokha R, Liu PP. Tumor necrosis factor- α mediates cardiac remodeling and ventricular dysfunction after pressure overload state. *Circulation* 2007;115:1398–1407. [PubMed: 17353445]
40. Nakano M, Knowlton AA, Dibbs Z, Mann DL. Tumor necrosis factor α confers resistance to hypoxic injury in the adult mammalian cardiac myocyte. *Circulation* 1998;97:1392–1400. [PubMed: 9577951]
41. Lecour S, Smith RM, Woodward B, Opie LH, Rochette L, Sack MN. Identification of a novel role for sphingolipid signaling in TNF α and ischemic preconditioning mediated cardioprotection. *Journal of Molecular and Cellular Cardiology* 2002;34:509–18.
42. Aukrust P, Ueland T, Lien E, Bendtzen K, Muller F, Andreassen AK, Norday I, Aass H, Espevik T, Simonsen S, Froland SS, Gullestad L. Cytokine network in congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. *American Journal of Cardiology* 1999;83:376–82. [PubMed: 10072227]
43. Torre-Amione G, Kapadia S, Benedict C, Oral H, Young JB, Mann DL. Proinflammatory cytokine levels in patients with depressed left ventricular ejection fraction: A report from the studies of left ventricular dysfunction (SOLVD). *Journal of the American College of Cardiology* 1996;27:1201–6. [PubMed: 8609343]
44. Greenland P. When is a new prediction marker useful? *Arch Intern Med* 2005;165:2454–2456. [PubMed: 16314539]
45. Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families. The Framingham offspring study. *American Journal of Epidemiology* 1979;110:281–90. [PubMed: 474565]
46. Luepker RV, Jacobs DR, Gillum RF, Folsom AR, Prineas RJ, Blackburn H. Population risk of cardiovascular disease: the Minnesota Heart Survey. *Journal of Chronic Disease* 1985;38:671–82.



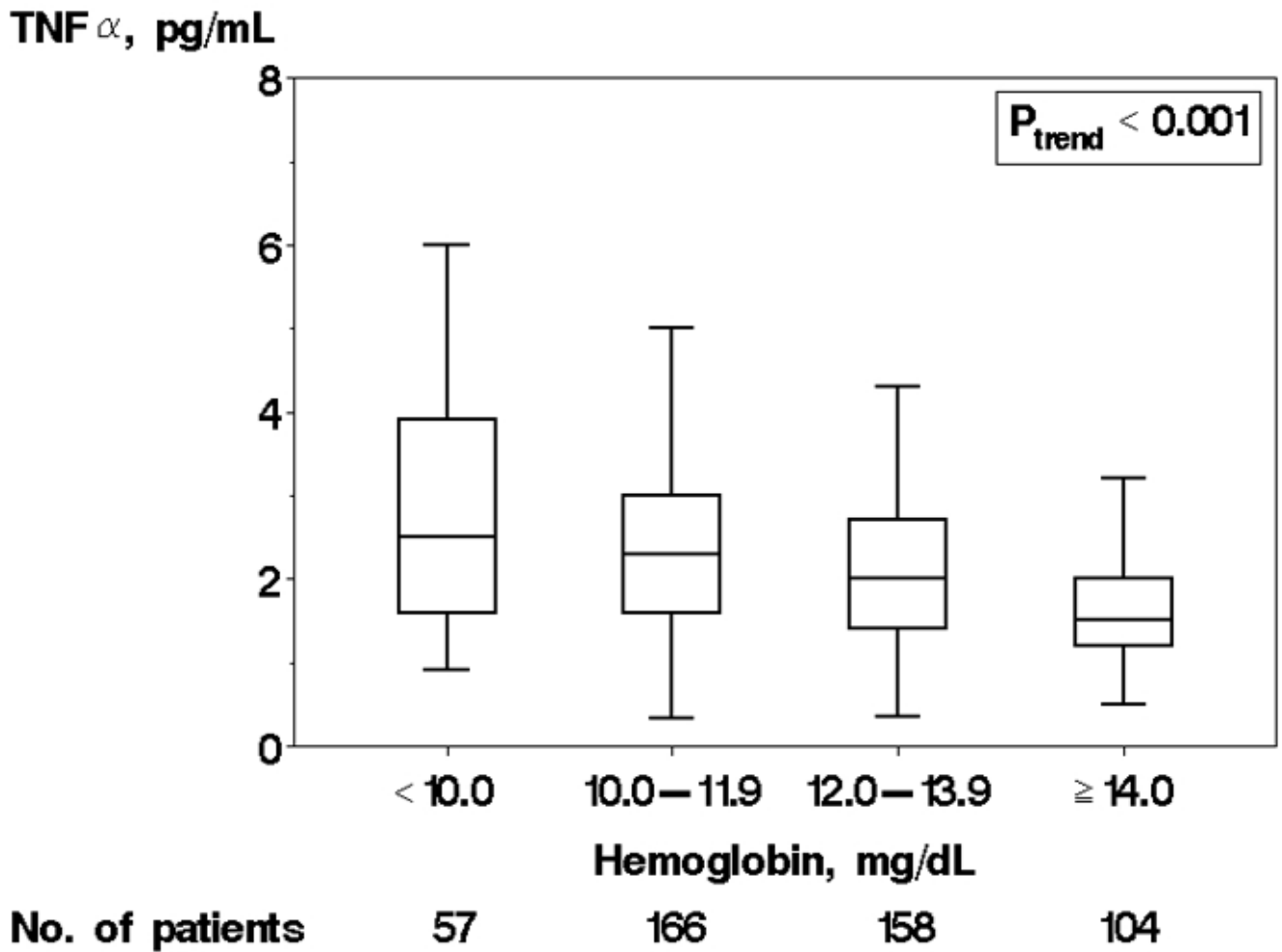
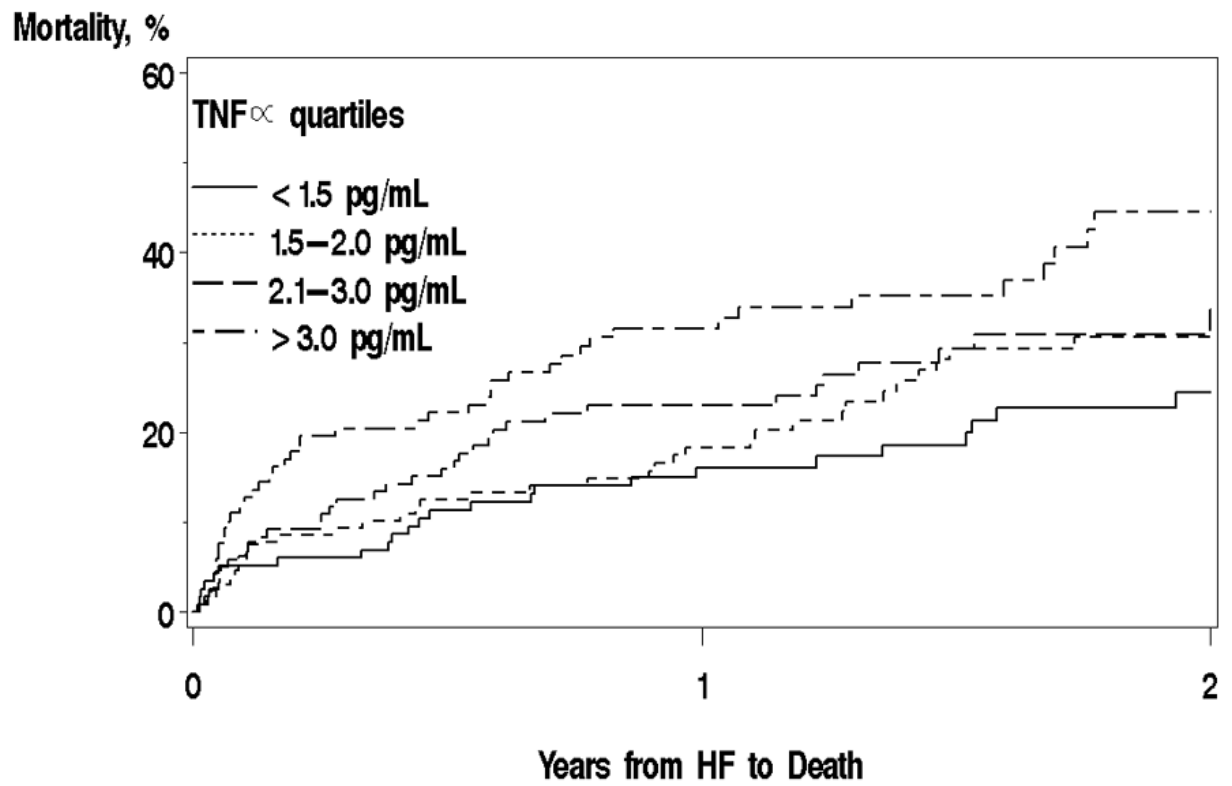


Figure 1.

Tumor Necrosis Factor α (TNF α) levels by Creatinine Clearance and Hemoglobin. Caption: TNF α levels by creatinine clearance (A) and hemoglobin (B) are shown. Each box displays the median, 25th percentile, and 75th percentile values; horizontal bars represent 1.5 times the interquartile range.

No.= number



Number at risk			
< 1.5 pg/mL	118	79	42
1.5–2.0 pg/mL	130	89	43
2.1–3.0 pg/mL	120	76	25
> 3.0 pg/mL	118	61	25

Figure 2.
Kaplan-Meier Mortality Curves by TNF α Quartile.
Caption: Mortality during follow-up was analyzed by TNF α quartile.
HF= heart failure

Table 1

Baseline Patient Characteristics

Characteristics	Overall (n=486)	Quartile 1 TNF α <1.5 pg/mL (n=118)	Quartile 2 1.5 TNF α <2.1 pg/mL (n=130)	Quartile 3 2.1 TNF α <3.1 pg/mL (n=120)	Quartile 4 TNF α \geq 3.1 pg/ mL (n=118)	P _{trend}
Age (yrs), mean (SD)	76.7 (13.0)	74.4 (12.5)	77.0 (14.0)	78.2 (11.8)	77.1 (13.2)	0.09
Male	236 (48.6)	52 (44.1)	65 (50.0)	64 (53.3)	55 (46.6)	0.60
EF	49.2 (16.4)	47.9 (17.4)	47.7 (16.4)	50.3 (16.2)	51.1 (15.6)	0.07
EF \geq 50%	266 (54.8)	61 (51.7)	66 (50.8)	69 (58.0)	70 (59.3)	0.14
Risk Factors						
Hypertension	391 (80.4)	86 (72.9)	109 (83.8)	98 (81.7)	98 (83.1)	0.09
Current smoker	40 (8.2)	20 (17.1)	5 (3.8)	10 (8.3)	5 (4.2)	0.003
Hyperlipidemia	326 (67.1)	78 (66.1)	89 (68.5)	79 (65.8)	80 (67.8)	0.91
Diabetes	148 (30.5)	30 (25.4)	42 (32.3)	42 (35.0)	34 (28.8)	0.50
BMI (kg/m ²), mean (SD)	29.2 (7.9)	29.5 (8.3)	29.3 (7.9)	28.8 (7.7)	29.2 (7.8)	0.64
Comorbidities						
Prior MI	118 (24.8)	26 (22.0)	36 (28.3)	23 (19.3)	33 (29.7)	0.46
Prior CAD	261 (53.7)	58 (49.2)	66 (50.8)	66 (55.0)	71 (60.2)	0.07
COPD	140 (28.8)	42 (35.6)	29 (22.3)	39 (32.5)	30 (25.4)	0.30
Anemia	258 (53.2)	42 (35.6)	63 (48.8)	74 (61.7)	79 (66.9)	<0.001
Severe renal disease	81 (17.2)	4 (3.5)	16 (12.6)	25 (21.6)	36 (31.3)	<0.001
Creatinine clearance (ml/min), mean (SD)	58.8 (33.9)	70.2 (33.2)	61.0 (33.4)	56.2 (35.0)	47.7 (30.6)	<0.001
C- Reactive Protein (mg/dL)	4.34 (6.61)	2.41 (4.39)	3.64 (5.48)	4.39 (6.17)	7.01 (8.91)	<0.001
Atrial fibrillation/Flutter	155 (31.9)	44 (37.3)	43 (33.1)	37 (30.8)	31 (26.3)	0.07
NYHA class 3 or 4	355 (73.0)	89 (75.4)	101 (77.7)	81 (67.5)	84 (71.2)	0.20
Charlson Index \geq 3	316 (66.5)	64 (54.2)	83 (65.4)	87 (73.1)	82 (73.9)	0.001
Moderate or severe diastolic dysfunction	369 (75.9)	84 (71.2)	98 (75.4)	95 (79.2)	92 (78.0)	0.17

* All values are reported as n(%) unless otherwise noted

BMI = body mass index, CAD= coronary artery disease, COPD= chronic obstructive pulmonary disease, EF= ejection fraction, MI= myocardial infarction, NYHA= New York Heart Association

Table 2
Hazard Ratios (95% CI) for Death Associated with TNF α Quartile

	Quartile 1 TNF α <1.5 pg/mL	Quartile 2 1.5 TNF α <2.1 pg/mL	Quartile 3 2.1 TNF α <3.1 pg/mL	Quartile 4 TNF α 3.1 pg/mL	P value (trend)
Unadjusted	1 (reference)	1.34 (0.82, 2.19)	1.47 (0.89, 2.44)	2.10 (1.30, 3.38) [†]	0.002
Model 1	1	1.24 (0.76, 2.04)	1.37 (0.83, 2.28)	1.90 (1.18, 3.08) [†]	0.007
Model 2	1	1.46 (0.87, 2.43)	1.57 (0.92, 2.64)	2.37 (1.43, 3.93) [†]	<0.001
Model 3	1	1.27 (0.75, 2.14)	1.26 (0.73, 2.16)	1.88 (1.09, 3.25) [*]	0.028

Model 1 adjusted for age, sex, and EF

Model 2 adjusted for above plus hypertension, hyperlipidemia, hyperlipidemia, smoking status, body mass index, diabetes mellitus, coronary artery disease, chronic obstructive pulmonary disease, New York Heart Association functional class, atrial fibrillation/flutter

Model 3 adjusted for above plus creatinine clearance, anemia

* p<0.05;

[†] p<0.01

Table 3

Area under receiver operating characteristic curves predicting 6-month mortality

	Area under the curve Overall (n=486)		P value
	Model without TNF α	Model with TNF α	
Unadjusted	0.500	0.600 (0.534,0.666)	<0.01
Model 1 [*]	0.671 (0.610,0.732)	0.700 (0.642,0.758)	0.035
Model 2 [†]	0.687 (0.627,0.747)	0.723 (0.667,0.779)	0.030
Model 3 [‡]	0.742 (0.689, 0.795)	0.777 (0.728,0.826)	0.018

* Model 1- Age, sex

† Model 2- Above + risk factors (hypertension, hyperlipidemia, diabetes mellitus, tobacco use, prior coronary artery disease)

‡ Model 3- Above + New York Heart Association functional class, ejection fraction