Tumor Necrosis Factor Alpha Influences the Inflammatory Response After Coronary Surgery

Mohamad N. Bittar, MD, MRCS, John A. Carey, FRCS, James B. Barnard, BS, MRCS, Vera Pravica, MD, Abdul K. Deiraniya, FRCS, FETCS, Nizar Yonan, MD, FRCS, and Ian V. Hutchinson, PhD

Department of Cardiothoracic Surgery, South Manchester University Hospitals NHS Trust, Wythenshawe Hospital, and School of Biological Sciences, University of Manchester, Manchester, United Kingdom

Background. **A systemic inflammatory response is not uncommonly observed after coronary revascularization. Tumor necrosis factor alpha is one of a number of modulators of this response. A functional polymorphism within the TNFα gene at position G-308A has been associated with increased TNFα levels. The relationship between predicted TNFα genotype and circulating TNFα levels in patients undergoing coronary revascularization surgery has yet to be defined. We examined the relationship between TNFα G-308A polymorphism, TNFα postoperative levels, and clinical outcome after coronary revascularization surgery.**

Methods. **We obtained DNA from 96 consecutive patients who underwent elective coronary revascularization. Patients were genotyped for TNFα G-308A polymorphism using sequence specific primer–polymerase chain reaction (SSP-PCR). Tumor necrosis factor alpha levels were measured on serum samples taken 3 hours postoperatively using enzyme-linked immunosorbent assay (ELISA).**

Results. **The prevalence of AA, AG, and GG TNFα-308 genotype was 12%, 40%, and 48%, respectively. Patients homozygous for A had higher circulating levels of TNFα ($p = 0.009$). Higher levels of TNFα were significantly associated with prolonged intensive care unit stay ($p = 0.008$), increase usage of an inotropic agent ($p = 0.024$), increased mortality risk ($p = 0.018$), and diabetes ($p = 0.019$). These remained statistically significant after risk stratification.**

Conclusions. **Patients of the AA-308 TNFα genotype showed significantly higher TNFα plasma levels. Higher plasma levels of TNFα were associated with less favorable outcome after coronary revascularization surgery. It may prove useful to utilize TNFα serum levels as a marker for identifying high-risk patients in the future.**

(Ann Thorac Surg 2006;81:132–8)

Coronary revascularization is associated with a systemic inflammatory response syndrome (SIRS) that is known to affect the outcome after cardiac surgery. In its mildest form, it has little clinical impact. However, in the most severe form, it is potentially fatal [1]. Many factors could trigger this response including surgical trauma, contact of blood with foreign surfaces, lung injury caused by the cardiopulmonary bypass machine, and reperfusion injury [2–4]. Inflammatory cells produce cytokines that mediate various stages of inflammation and are capable of stimulating many cells, including smooth muscle cells, fibroblasts, and endothelial cells [5]. Many researchers focused on the role of cytokines, particularly interleukin (IL)-6, IL-8, IL-10, and tumor necrosis factor alpha (TNFα), in the SIRS caused by coronary surgery. A polymorphism within the TNFα gene at position -308 is associated with increased TNFα levels and high mortality rate in severe trauma and sepsis cases [6].

We have investigated whether the magnitude of TNFα release in response to coronary revascularization is related to the presence of a certain allele in the functional polymorphism at position -308. Furthermore, we investigated whether it is the G-308A polymorphism or the TNFα postoperative levels that is related to the clinical outcome.

Material and Methods

Study Patients

A prospective study of 96 patients undergoing first-time elective coronary revascularization was conducted at Wythenshawe Hospital (Manchester, United Kingdom). Patients with unstable angina, recent myocardial infarction (within 30 days), preexisting autoimmune diseases, or renal failure were excluded from the study. Patients on immunosuppressive therapy or anti-inflammatory agents were also excluded from the study. Antiplatelet therapy was routinely stopped 7 days before surgery. Patients gave informed consent to the collection and storage of blood, isolation of DNA, and determination of cytokine gene polymorphism. Ethical approval was obtained from the South Manchester Medical Research Ethics Committee.

Surgical Procedure

All operations were performed by consultant grade surgeons through midline sternotomy approach. Cardiopul-

Accepted for publication July 13, 2005.

Address correspondence to Dr Bittar, Department of Cardiac Surgery, Wythenshawe Hospital, Southmoor Rd, Manchester M239LT, United Kingdom; e-mail: mbittar@doctors.org.uk.

Published by Elsevier Inc

0003-4975/06/$32.00
doi:10.1016/j.athoracsur.2005.07.037

monary bypass was instituted in 82 patients by cannulation of the right atrium and ascending aorta. Myocardial protection was provided by intermittent antegrade with or without retrograde blood cardioplegia. Aprotinin was not used. In 14 patients, the coronary revascularization was performed without the use of CPB with the aid of intracoronary shunt (Intravascular Arteriotomy Cannula; Medtronic, Minneapolis, MN) and stabilizer (Medtronic Octopus 2 Tissue Stabilization System). Perioperative anticoagulation with heparin was reversed after CPB with the use of protamine sulphate.

Sample Collection

Venous blood samples were collected preoperatively in the outpatient clinic and 3 hours after surgery in the intensive care unit in ethylenediamine-tetra acid (EDTA) Vacutainer tubes (Becton Dickinson, Plymouth, UK). Samples were centrifuged within 30 minutes of collection at 3,000 rpm for 10 minutes. Plasma and blood cells were separated and stored separately at – 80°C.

Genotyping

EXTRACTION OF DNA. The DNA was obtained from EDTA anticoagulated blood using the double lysis method. Briefly, 4 mL collected blood was centrifuged; blood cells were transferred to 13 mL polypropylene tubes containing 9 mL Lysis buffer 1 (155 mM ammonium chloride, 10 mM potassium hydrogen carbonate, 1 mM EDTA) and mixed for 15 minutes. Samples were centrifuged and supernatants removed. The cell pellets were then resuspended and lysed again. A nuclear membrane lysis step was performed using 25 mM EDTA and 2% SDS in a 3 mL volume and 1 mL 10M ammonium acetate. Samples were centrifuged and supernatant added to propan-2-ol to wash DNA. This was finally centrifuged and resuspended in 350 μl double-distilled water.

SEQUENCE-SPECIFIC PRIMER–POLYMERASE REACTION. The polymerase chain reaction is a method of gene amplification. Polymerase chain reaction amplifies the chosen DNA in the test tube. Two short single-stranded DNA fragments, or primers, delineate the segment to be amplified. The primers initiate the amplification, which proceeds in successive copying cycles, each of which double the number of DNA segments in the reaction. A cycle begins when heat melts the double-stranded DNA template into single strands. The primers hybridize to their complementary sequences on the separated strands of the template. This process can be repeated until it incorporates all the primers into double-stranded DNA. It is possible to make millions of copies of a DNA segment in a matter of hours with PCR.

The DNA was amplified using specific oligonucleotide primers based on the published sequence (GenBank accession number AF 005485, Genosys, Suffolk, UK). The primer sequences used for TNFα genotyping are shown in Table 1.

Each PCR reaction mixture comprised 1.25 U Thermoprime Plus DNA polymerase, 75 mM Tris-HCl, 20 mM (NH4) $2SO_4$, 2.0 mM $MgCl_2$, 0.01% Tween 20, and 0.2 mM each for dATP, dCTP, dGTP, and Dttp (all ABgene, Epsom, United Kingdom). Precipitant and red dye were also present for electrophoresis. This reaction mixture was added to human growth hormone primers (control) in a ratio of 10 μl:1 μl. Ten microliters of this was then mixed with 1.5 μl DNA in a thin-walled PCR tube; 5 μl of this mastermix was added to 5 μl of specific G (5′-ATAGGTTTTGAGGGGCATGG-3′)or A (5′-ATAGGTTTTGAGGGGCATGA-3′) primers in separate tubes. Cycling conditions included an initial denaturation of 95°C for 1 minute followed by 95°C for 15 seconds, 65°C for 50 seconds, and 72°C for 40 seconds (10 cycles), followed by 95°C for 50 seconds, 59°C for 50 seconds, and 72°C for 50 seconds (20 cycles). A final holding temperature at 4°C was performed. The PCR products were electrophoresed on a 2% agarose gel (Life Technologies) and visualized using ethidium bromide (Life Technologies) under ultraviolet illumination [7, 8].

Table 1. Primer Sequences Used for the TNF Sequence-Specific Primer Genotyping Method

Primer Position	Primer Sequence	Tm
TNF-308 A	5′-ATAGGTTTTGAGGGGCATGA-3′	60°C
TNF-308 G	5′-ATAGGTTTTGAGGGGCATGG-3′	
Generic antisense	5′-TCTCGGTTTCTTCTCCATCG-3′	
HGH (sense)	′5-GCCTTCCCAACCATTCCCTTA-3′	60°C
HGH (antisense)	′5-TCAGGATTTCTGTTGTGTTTC-3′	

HGH = human growth hormone; Tm = melting temperature; TNF = tumornecrosis factor.

Enzyme-Linked Immunosorbent Assay

Serum levels of TNFα were measured using a solid phase sandwich enzyme-linked immunosorbent assay (ELISA, IDS Ltd, Tyne and Wear, UK). A monoclonal TNFα antibody was coated onto the wells of a 96-well plate. Standards of known TNFα concentration were then added along with control samples and patient serum. After washing, a biotinylated polyclonal TNFα antibody was added and incubated. After washing, streptavidin-coupled peroxidase was added. After incubation, a wash step was performed to remove a chromogenic peroxidase. Finally, tetramethylbenzidine (TMB) substrate was added that changed color directly in proportion to the amount of TNFα present. The color (absorbance) was then measured using a Dynex Technologies MRX plate reader (DYNEX Technologies Ltd, Worthing, West Sussex, UK) with a primary wavelength of 450 nm and a reference wavelength of 620 nm.

Statistical Analysis

The χ^2 test was used to analyze relationship between categorical data. Nonparametric Mann-Whitney *U* test and Kruskal-Wallis test, as appropriate, were used to compare TNFα levels and different outcomes between subgroups. Nonparametric testing was chosen because (apart from age, which was analyzed using one-way analysis of variance) the data did not follow a normal

Table 2. Patients' Preoperative Characteristics

	With CPB	Without CPB	*p* Value
Age, years	62 ± 9.5	62 ± 12.5	0.8
Male sex	67 (82%)	10 (72%)	0.4
Previous MI	38 (46%)	6 (43%)	1.0
Hypertension	40 (49%)	6 (43%)	0.7
Type II diabetes mellitus	18 (22%)	5 (35%)	0.3
COPD	10 (12%)	2 (14%)	0.6
Previous stroke	4 (5%)	1 (7%)	0.5
Previous PTCA	12 (15%)	5 (35%)	0.1
Smoking history			0.3
Former smoker	60 (73%)	12 (86%)	
Current smoker	11 (13.5%)	1 (7%)	
Never smoked	11 (13.5%)	1 (7%)	

Age data presented as mean ± SD; other data presented as numbers and proportions.

COPD = chronic obstructive pulmonary disease; CPB = cardiopulmonary bypass; MI = myocardial infarction; PTCA = percutaneous transluminal coronary angioplasty.

distribution. Association between two continuous variables were determined using Spearman rank correlation. Tumor necrosis factor alpha levels are given as median and range values. A χ^2 test was used to compare the observed numbers of each genotype with those expected for a population to establish if they were in the Hardy-Weinberg equilibrium. Regression analyses were used to adjust for confounding factors. All statistical analysis was performed with the SPSS computer package (SPSS, Chicago, Illinois). All tests were two sided. Significance was established at a value of p less than 0.05.

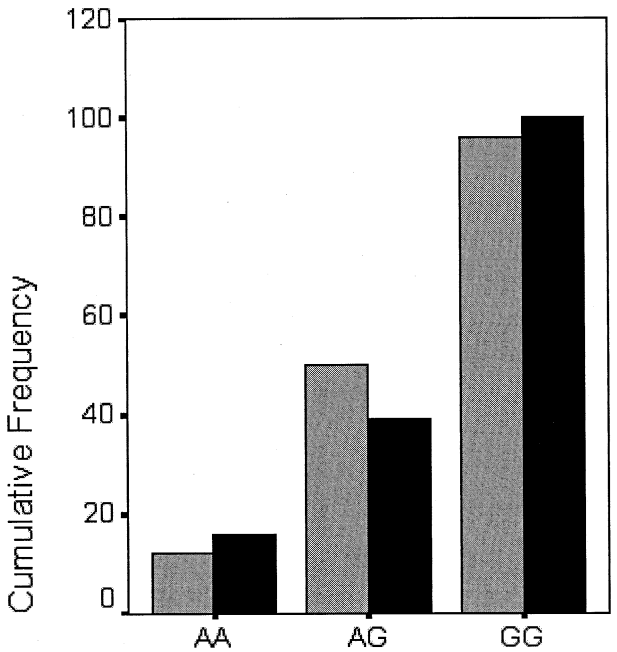

Fig 1. Tumor necrosis factor alpha genotypes prevalence. (Black bars = normal population; gray bars = study patients.)

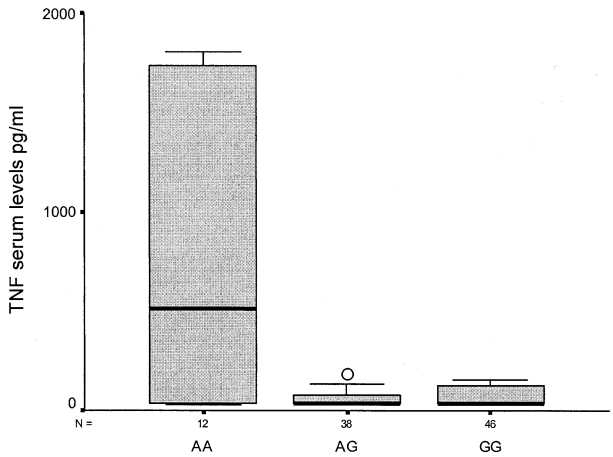

Fig 2. Tumor necrosis factor alpha (TNF) genotypes ($p = 0.009$) and postoperative serum levels.

Results

Patients undergoing elective coronary revascularization were recruited. Patients' baseline characteristics are shown in Table 2. All planned procedures were completed, and patients left the operating theater in sinus rhythm. The median number of grafts per patient was 3 (range, 1 to 5), the mean aortic cross clamp time was 52 ± 31 minutes, and CPB time was 80 ± 50 minutes. There were two in-hospital deaths.

The TNFα serum levels were not detected preoperatively, whereas all patients had detectable levels of TNFα 3 hours postoperatively. The median TNFα serum level was 37 pg/mL (range, 25 to 3,512 pg/mL). Tumor necrosis factor alpha levels were comparable between patients operated on with or without the use of CPB: TNFα levels in CPB patients were 37 pg/mL (range, 25 to 3,512 pg/mL) versus 48 pg/mL (range, 33 to 4,985 pg/mL) in the non-CPB patients ($p = 0.45$).

The prevalence of the AA, AG, and GG genotypes in the study population were 12%, 40%, and 48%, respec-

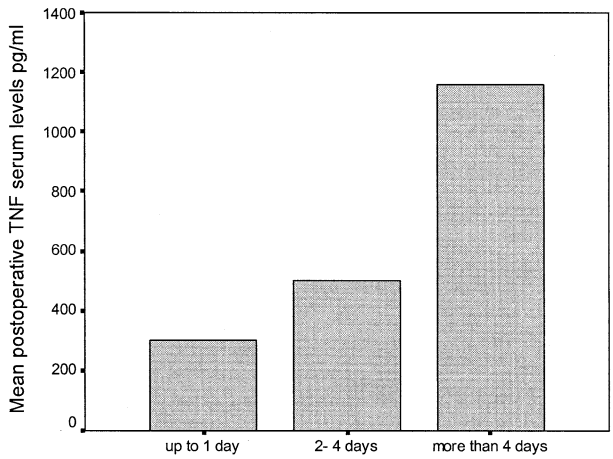

Fig 3. Tumor necrosis factor alpha (TNF) postoperative serum levels and intensive care unit stay ($p = 0.008$).

Table 3. Preoperative Characteristics: Demographic Data by Genotype Groups[a]

	AA (n = 12)	AG (n = 38)	GG (n = 46)	*p* Value
Age, years	62 ± 11	62 ± 10	62 ± 9	0.9
Male sex	10 (83%)	31 (81%)	36 (78%)	0.8
Previous MI	6 (50%)	20 (52%)	18 (40%)	0.4
Hypertension	7 (68%)	17 (44%)	22 (47%)	0.7
Diabetes mellitus	4 (33%)	7 (18%)	12 (26%)	0.5
COPD	1 (8%)	5 (13%)	6 (13%)	0.8
Previous stroke	0	3 (8%)	2 (4%)	0.5

[a] Age data are presented as mean ± SD; other data are numbers and proportions.

COPD = chronic obstructive pulmonary disease; MI = myocardial infarction.

tively, which was not different from normal population as predicted by Hardy-Weinberg equilibrium (Fig 1). Patients with AA genotypes had higher circulating levels of TNFα postoperatively compared with AG and GG genotype, which reached high statistical significance TNFα levels: AA = 511 pg/mL (range, 30 to 4,985), AG = 38 pg/mL (range, 25 to 3,309), and GG = 37 pg/mL (range, 25 to 3,512; $p = 0.009$). These results remained statistically significant after risk adjustment for age, sex, blood loss, ventilation time, duration of CPB, and aortic cross-clamp time (Fig 2, Tables 3 and 4).

Higher levels of TNFα were associated with prolonged intensive care unit stay (Kruskal-Wallis test, $p = 0.008$), increased usage of inotropes (Mann-Whitney test, $p = 0.024$), and higher incidence of death (Mann-Whitnet test, $p = 0.018$), Diabetic patients had significantly higher levels of TNFα compared with nondiabetic patients ($p = 0.019$). These results remained statistically significant after risk adjustment to age, sex, aortic cross-clamp time, and duration of CPB using multiple linear regression analyses (Figs 3 through 6).

Comment

Inflammation is involved in all stages of atherosclerotic development. It is a cause and a consequence of ischemic heart disease [9–11]. Tumor necrosis factor alpha is a proinflammatory mediator in the pathogenesis of the SIRS [12]. It has been previously demonstrated that TNFα plays a major role in SIRS secondary to infection, burns, trauma, hemorrhagic shock, and pancreatitis [5]. Tumor necrosis factor alpha influences the outcome of other inflammatory processes, including allograft rejection, ischemia-reperfusion injury, delayed-type hypersensitivity, and granuloma development. Excessive production of TNFα may lead to organ dysfunction and death [13]. Tumor necrosis factor alpha acts by binding to specific receptors on cell surfaces, blocking these receptors provide a protective mechanism. Anti-TNFα therapy has become a well-recognized treatment modality in several inflammatory conditions such as Crohn's disease and rheumatoid arthritis [14, 15].

In patients undergoing coronary revascularization surgery, different stimuli such as general anesthesia, surgical wounds, heparin administration, CPB, and protamine administration are thought to play a role in the genesis of this response [16]. Previous reports confirmed the increase of TNFα levels in response to cardiac surgery at different time points reaching a peak at 3 hours after surgery and degraded rapidly with a short half-life [3, 4, 17].

This study has focused on the aspects of the relationship between TNFα genotype, TNFα postoperative levels, and postoperative outcome. We hypothesized that polymorphisms in the TNFα gene -308 may account for the variation observed in TNFα plasma concentrations after cardiac revascularization. Our results showed a highly significant positive association between TNFα levels and AA genotype. The distribution of TNFα G-308A genotypes in this cohort were close to the normal distribution predicted by the Hardy-Weinberg equilibrium. Although we found a significant association between the AA genotype and the postoperative TNF levels, we were unable to demonstrate a significant association between TNFα genotype and clinical outcome. This could be related to the small number of patients with the AA genotype in the study group.

We examined the relationship between TNFα postoperative levels and clinical outcome, and we found that

Table 4. Postoperative Outcome: Demographic Data by Genotype Groups[a]

	AA (n = 12)	*AG (n = 38)*	*GG (n = 46)*	*p* Value
Blood loss, mL	630 (230–2,020)	560 (120–1,370)	610 (200–3,500)	0.2
Ventilation time, hours	11 (4–86)	11 (4–20)	9 (0–15)	0.2
Intensive care unit stay, hours	24 (24–48)	24 (24–96)	24 (24–408)	0.4
Hospital stay, days	7 (2–10)	7 (4–16)	6 (5–17)	0.1
Inotropes usage, number	6 (50%)	25 (65%)	13 (34%)	0.1

[a] Data are presented as the median (range) where appropriate.

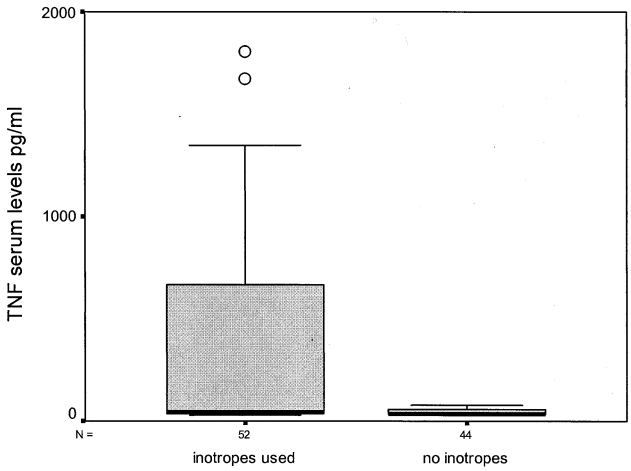

Fig 4. Tumor necrosis factor alpha (TNF) postoperative serum levels and the usage of inotropic support (p = 0.024).

patients who required inotropic support and stayed longer in the intensive care unit had significantly raised serum levels of TNFα. These findings are consistent with previously published data showing raised TNFα levels were associated with an increased incidence of sepsis and prolonged intensive care stay in trauma patients [18].

The TNFα G-308A genotype's frequency was comparable in the type 2 diabetic and nondiabetic patients. However, we found significantly higher levels of TNFα in diabetic patients postoperatively regardless of their genotype. This finding could be related to the impaired endothelial function, which is observed in the diabetic patient and its association with increased inflammatory reaction and subsequently augmented TNFα levels. These findings are supported by a recent study in diabetic patients with atherosclerotic disease [19]. Whether there is a genetic predisposition for diabetes is the subject of an ongoing debate. Previous reports showed that the AA genotype is a predictor of conversion from impaired glucose tolerance to type 2 diabetes and that

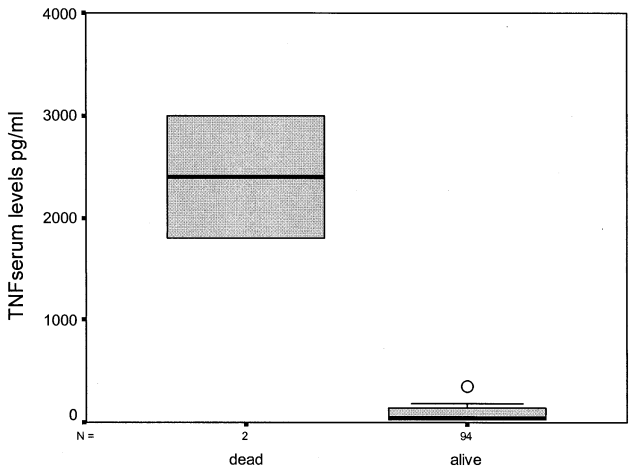

Fig 5. Tumor necrosis factor alpha (TNF) postoperative serum levels and mortality in the study group population (p = 0.018).

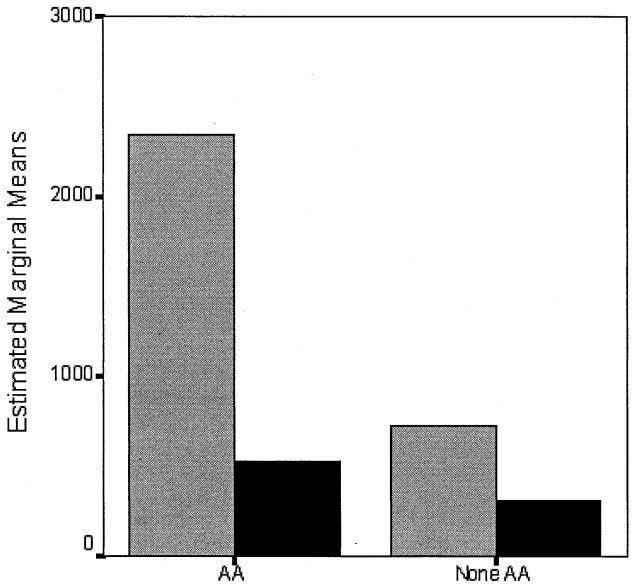

Fig 6. Tumor necrosis factor alpha (TNF) postoperative serum levels and diabetes mellitus (TNFα genotype, p = 0.019). (Black bars = nondiabetic patients; gray bars = diabetic patients.)

TNFα is a key player in the development of insulin resistance [20, 21]. There were two in-hospital deaths. Both patients had significantly raised TNFα serum levels. This finding is consistent with a previously published report [22].

The TNFα response is just a part of the spectrum of inflammatory markers observed after revascularization. Interleukin-6 and IL-10 were studied in this setting and their postoperative levels were determinants of clinical outcome [23]. As the IL-6 response is also genetically determined, genetic profiles may allow us to target specific therapies in the future [24, 25].

Although the inclusion of off-pump coronary artery bypass graft surgery patients introduced heterogeneity to the study, statistical analysis revealed the two groups were comparable in their preoperative parameters and risk factors. Therefore, it was appropriate to include them in the study.

In summary, TNFα G-308A polymorphism determines postoperative TNFα serum levels. Increased TNFα levels are associated with less favorable outcome after coronary revascularization surgery, and, therefore, TNFα genotyping and level monitoring may be useful for identifying patients with an increased risk of developing organ dysfunction and death after cardiac surgery.

References

1. Rothenburger M, Soeparwata R, Deng MC, et al. Prediction of clinical outcome after cardiac surgery: the role of cytokines, endotoxin, and anti-endotoxin core antibodies. Shock 2001;16(Suppl 1):44–50.
2. Butler J, Baigrie RJ, Parker D, et al. Systemic inflammatory responses to cardiopulmonary bypass: a pilot study of the effects of pentoxifylline. Respir Med 1993;87:285–8.

3. Misoph M, Babin-Ebell J. Interindividual variations in cytokine levels following cardiopulmonary bypass. Heart Vessels 1997;12:119–27.
4. Wei M, Kuukasjarvi P, Laurikka J, et al. Cytokine responses in patients undergoing coronary artery bypass surgery after ischemic preconditioning. Scand Cardiovasc J 2001;35:142–6.
5. Pannen BH, Robotham JL. The acute-phase response. New Horiz 1995;3:183–97.
6. Tomasdottir H, Hjartarson H, Ricksten A, Wasslavik C, Bengtsson A, Ricksten SE. Tumor necrosis factor gene polymorphism is associated with enhanced systemic inflammatory response and increased cardiopulmonary morbidity after cardiac surgery. Anesth Analg 2003;97:944–9 [and Table of Contents].
7. Aldener-Cannava A, Olerup O. HLA-DOB1 "low-resolution" typing by PCR amplification with sequence-specific primers (PCR-SSP). Eur J Immunogenet 1994;21:447–55.
8. Perrey C, Turner SJ, Pravica V, Howell WM, Hutchinson IV. ARMS-PCR methodologies to determine IL-10, TNF-alpha, TNF-beta and TGF-beta 1 gene polymorphisms. Transpl Immunol 1999;7:127–8.
9. Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. Circulation 2000;101:1767–72.
10. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 2000;342:836–43.
11. Jenny NS, Tracy RP, Ogg MS, et al. In the elderly, interleukin-6 plasma levels and the -174G>C polymorphism are associated with the development of cardiovascular disease. Arterioscler Thromb Vasc Biol 2002;22:2066–71.
12. Cain BS, Meldrum DR, Dinarello CA, Meng X, Banerjee A, Harken AH. Adenosine reduces cardiac TNF-alpha production and human myocardial injury following ischemia-reperfusion. J Surg Res 1998;76:117–23.
13. Strieter RM, Kunkel SL, Bone RC. Role of tumor necrosis factor-alpha in disease states and inflammation. Crit Care Med 1993;21:S447–63.
14. Casellas i Jorda F. TNF-alpha inhibitors in inflammatory bowel disease [translation]. Med Clin (Barc) 2004;123:627–34.
15. Ohshima S, Mima T, Sasai M, et al. Tumour necrosis factor alpha (TNF-alpha) interferes with Fas-mediated apoptotic cell death on rheumatoid arthritis (RA) synovial cells: a possible mechanism of rheumatoid synovial hyperplasia and a clinical benefit of anti-TNF-alpha therapy for RA. Cytokine 2000;12:281–8.
16. Liebold A, Keyl C, Birnbaum DE. The heart produces but the lungs consume proinflammatory cytokines following cardiopulmonary bypass. Eur J Cardiothorac Surg 1999;15:340–5.
17. Tonnesen E, Christensen VB, Toft P. The role of cytokines in cardiac surgery. Int J Cardiol 1996;53(Suppl):1–10.
18. Majetschak M, Flohe S, Obertacke U, et al. Relation of a TNF gene polymorphism to severe sepsis in trauma patients. Ann Surg 1999;230:207–14.
19. Antoniades C, Tousoulis D, Tountas C, et al. Vascular endothelium and inflammatory process, in patients with combined type 2 diabetes mellitus and coronary atherosclerosis: the effects of vitamin C. Diabet Med 2004;21:552–8.
20. Kubaszek A, Pihlajamaki J, Komarovski V, et al. Promoter polymorphisms of the TNF-alpha (G-308A) and IL-6 (C-174G) genes predict the conversion from impaired glucose tolerance to type 2 diabetes: the Finnish Diabetes Prevention Study. Diabetes 2003;52:1872–6.
21. Fernandez-Real JM, Lainez B, Vendrell J, et al. Shedding of TNF-alpha receptors, blood pressure, and insulin sensitivity in type 2 diabetes mellitus. Am J Physiol Endocrinol Metab 2002;282:E952–9.
22. Yu BJ, Li JS, Zhang DL, Tang XM. The associations of the single nucleotide polymorphisms on TNF and CD14 promoters with the mortality of infection, systematic inflammatory response syndromec and sepsis in surgical patients [translation]. Zhonghua Yi Xue Za Zhi 2003;83:2132–6.
23. Gaudino M, Di Castelnuovo A, Zamparelli R, et al. Genetic control of postoperative systemic inflammatory reaction and pulmonary and renal complications after coronary artery surgery. J Thorac Cardiovasc Surg 2003;126:1107–12.
24. Bittar MN, Carey JA, Barnard J, et al. Interleukin-6 G-174C polymorphism influences outcome following coronary revascularisation surgery. Heart Surg Forum 2005;8:E140–5.
25. Gaudino M, Andreotti F, Zamparelli R, et al. The -174G/C interleukin-6 polymorphism influences postoperative interleukin-6 levels and postoperative atrial fibrillation. Is atrial fibrillation an inflammatory complication? Circulation 2003;108(Suppl 1):195–9.

INVITED COMMENTARY

Bittar and colleagues discuss whether the inflammatory response (particularly after coronary bypass operations) can be predicted by genetic phenotyping [1].

The bypass circuit (ie, blood foreign surface interface) causes an intense pro-inflammatory reaction. This response seems to be macrophage and endothelial-cell induced. The effect of tumor necrosis factor α (TNFα) by itself seems to be negative inotropic and pro-inflammatory. Some cytoprotective effects have been proposed as well. TNFα acts pro-inflammatory by self amplification as well as by induction and expression of other pro-inflammatory cytokines. The myocardium constitutively expresses a low level of TNFα. Although macrophages are activated at the blood foreign surface interface, an increase of myocardial TNF after ischemia and reperfusion has also been demonstrated. It is unclear if TNFα gene expression and peptide synthesis occur during global ischemia. Hence, the source of TNFα is multifactorial. It is intriguing to merge molecular genetics with molecular mechanisms of the systemic inflammatory response. However, the systemic inflammatory response involves a multitude of humeral and cellular mechanisms, and it would be an oversimplification to assume that one molecule is responsible for this very complex phenomenon.

Genetic polymorphism is defined as the presence of multiple alleles at a gene locus in appreciable frequencies in a population. The TNF gene is embedded within the class three major histocompatibility complex on the short arm of chromosome 6. Whether TNF gene polymorphism influences TNFα expression in patients undergoing coronary artery bypass grafting with or without cardiopulmonary bypass has been the object of a number of studies. The results have been conflicting.

How can we explain these discrepancies? First when planning or reading one of these studies, the goal at the onset has to be kept in mind. To study gene polymorphism and its possible influence on outcome as a conse-

0003-4975/06/$32.00
doi:10.1016/j.athoracsur.2005.08.021