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TNF-α gene promoter polymorphisms and risk of venous thromboembolism in gastrointestinal cancer patients undergoing chemotherapy

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Background: TNF-α has been proposed as a predictive factor for venous thromboembolism (VTE). Genetic polymorphisms could regulate TNF-α production. However, the relationship between *TNFA* gene variants and VTE is not clarified. This study aims to investigate the predictive role of five different *TNFA* gene promoter SNPs, or their haplotype combination(s), for a first VTE episode in gastrointestinal cancer out-patients treated with chemotherapy.

Patients and methods: Serum TNF-α levels and *TNFA* -863C/A, -857C/T, -376G/A, -308G/A and -238G/A gene promoter polymorphisms were retrospectively evaluated in 314 subjects, including 157 controls and 157 Caucasian patients with histologically diagnosed GI cancers beginning chemotherapy delivery (5-fluorouracil either as monotherapy or in combination with platinum compounds or irinotecan).

Results: Haplotype analysis showed that a five-loci haplotype (CTGGG haplotype) has higher frequency in GI cancer patients who developed VTE ($n = 15$) during chemotherapy [odds ratio = 2.7, 95% confidence interval (CI) 1.04–7.11, $P = 0.04$]. GI patients who remained VTE-free did not differ in CTGGG haplotype frequency from controls. No association was observed between serum TNF-α levels and *TNFA* haplotype, but both were independent predictors of VTE. Approximately 20% of GI cancer patients carrying the CTGGG haplotype developed VTE compared with 4% of the remaining 101 patients (hazard ratio = 5.6, 95% CI 1.8–17.6, $P = 0.003$).

Conclusion: These results suggest that *TNFA* might represent a candidate gene contributing to VTE pathogenesis in GI cancer patients and suggest that VTE risk during chemotherapy might be genetically identified. Validation studies are needed for translation into clinical practice.

Key words: gastrointestinal cancer, venous thromboembolism, chemotherapy, tumour necrosis factor-α, single nucleotide polymorphisms

introduction

Recent epidemiological and clinical data support the concept of an intensive cross-talk between the inflammatory network and procoagulant state of patients with cancer [1]. Among the pro-inflammatory gene products involved in such interactions, tumour necrosis factor-α (TNF-α) represents a key player [2, 3]. Earlier studies showed that TNF-α infusion was rapidly followed by thrombin generation [4, 5], eventually associated to autoptic findings of deep vein thrombosis (DVT) [5].

Subsequent studies confirmed an association between venous thromboembolism (VTE) and markers of inflammation [6], TNF-α possibly representing a risk determinant for VTE, as individuals with detectable levels had a 2-fold increased risk in the Leiden Thrombophilia Study [7]. Recently, we demonstrated that elevated TNF-α levels increase VTE risk through impairment of the anticoagulant protein C system in metastatic colorectal cancer (mCRC) treated with chemotherapy [8].

TNF-α production is regulated at many levels and can be controlled by genetic polymorphisms, especially in the promoter region [9]. In particular, *TNFA* -308G/A [10] and -857C/T [11, 12] single nucleotide polymorphisms (SNPs) were associated with increased cytokine production. Interestingly, both SNPs would represent a risk factor for CRC [13] or gastric cancer [14, 15]. Nonetheless, to the best of our knowledge, only rare studies focused on the association between *TNFA* SNPs and

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clinical outcome of gastrointestinal (GI) cancer patients [15], and one study only investigated the possible association of *TNFA* -308G/A with VTE, rejecting the hypothesis of their relationship [16]. Based on the above reported observations, we decided to resume the original theory that *TNFA* SNPs might be involved in VTE risk and retrospectively evaluated whether five different *TNFA* gene promoter SNPs, or their haplotype combination(s), could be predictive of a first VTE in GI cancer out-patients in whom chemotherapy might act as thrombotic trigger.

patients and methods

patients and sample collection

A cohort of 157 Caucasian patients with histologically diagnosed GI cancer, treated at the Medical Oncology Unit of the 'Tor Vergata' Clinical Center, was enrolled. Patients were required to be at the start of a first chemotherapy regimen. Patient characteristics are summarized in Table 1. Exclusion criteria were ECOG-PS>2, therapeutic doses of any heparin before enrolment or treatment with anticoagulant drugs, age <18 years or unwillingness to provide informed consent. No patient received prophylactic treatment with any anticoagulant drug after chemotherapy start. All patients were seen regularly at their scheduled chemotherapy visits or at the occurrence of clinically suspected VTE. DVT was confirmed by venography or colour-coded duplex sonography (in proximal DVT only). Pulmonary embolism (PE) was diagnosed by spiral computed tomography.

One hundred and fifty-seven sex-matched unrelated controls (68 ± 16 years) from the same geographical area of the patients were recruited from healthy individuals enrolled in the Interinstitutional Multidisciplinary Biobank (BioBIM) of the IRCCS San Raffaele, Rome, Italy.

Written informed consent was obtained from each participating subject, and the study was carried out under the appropriate institutional ethics approvals and in accordance with the principles embodied in the Declaration of Helsinki.

Table 1. Patient characteristics

Age (years), mean ± SD (range)	62 ± 10 (30–83)
Gender	
Male	95 (61%)
Female	62 (39%)
Venous thromboembolism	
Pulmonary embolism	4 (3%)
Deep venous thrombosis	11 (7%)
Cumulative frequency	15 (10%)
Site of primary	
Colorectal	132 (84%)
Gastric	25 (16%)
Class of risk[a]	
Low	113 (72%)
Intermediate	36 (23%)
High	8 (5%)
Stage of disease	
Primary	61 (39%)
Metastatic	96 (61%)
Performance status (ECOG)	
0	137 (87%)
1	19 (12%)
2	1 (1%)
Body mass index (BMI), mean ± SD (range)	25.7 ± 4.2 (18.0–39.7)
Chemotherapy	
Neo-adjuvant	3 (2%)
Adjuvant	55 (35%)
First-line	99 (63%)
Chemotherapy regimen	
Irinotecan/fluorouracil based	70 (45%)
Oxaliplatin/fluorouracil based	58 (37%)
Fluorouracil monotherapy	16 (10%)
Cisplatin/fluorouracil based	13 (8%)
Drugs	
Erythropoietin stimulating agents	2 (1%)
Bevacizumab	59 (38%)
Prophylactic myeloid growth factors	2 (1%)
Corticosteroids	23 (15%)
Length of follow-up (months), median (IQR)	12 (7–21)
Bilirubin (mg/dl), median (IQR)	0.50 (0.40–0.72)
Creatinine (mg/dl), median (IQR)	0.80 (0.70–0.99)

[a]According to Khorana et al. [19].

sample collection and TNF-α determination

Blood samples were obtained by all subjects at time of enrolment, processed, aliquoted and stored at −80°C in the facilities of the BioBIM. Storage conditions were carefully maintained and aliquots were limited to one freeze-thaw cycle to ensure proper quality.

TNF-α levels were measured by enzyme-immunoassay (R&D Systems, MN) according to the manufacturers' instructions. All samples were assayed in duplicate and those showing values above the standard curve were re-tested with appropriate dilutions. All measurements were ascertained while blinded to the patient outcome.

molecular analyses *TNFA* gene promoter polymorphisms

DNA was isolated from EDTA anticoagulated whole blood using MagNA Pure LC instrument (Roche Diagnostics) and the MagNA Pure LC total DNA isolation kit I (Roche Diagnostics) according to the manufacturer's instructions.

TNFA (OMIM # 191160) -863C/A (rs 1800630) and -857C/T (rs 1799724) SNPs were determined by a 132-bp PCR amplification spanning from nucleotide (nt)-1117 to nt-985; -376G/A (rs1800750), -308G/A (rs1800629) and -238G/A (rs361525) gene promoter polymorphisms were determined by a 380-bp PCR amplification spanning from nt-667 to nt-287 based on the first translating nt of *TNFA* Ensembl sequence (Ensembl Transcript ID ENST00000229681).

Standard PCR was carried out using the primers: F5′-CCTCTGGGG AGATGTGACCA-3′ and R5′-AGGTCCTGGAGGCTCTTTCAC-3′ (for -863C/A and -857C/T) and F5′-AGGACTCAACACAGCTTTTCCC-3′ and R5′-TCTGGAGGAAGCGGTAGTGG-3′ (for 376G/A, -308G/A and -238G/A), in a GeneAmp PCR System 9700 (Applied Biosystems, CA) using HotStarTaq Master Mix (HotStarTaq Master Mix Kit, QIAGEN, Inc., CA) and an annealing temperature of 60°C for both reactions. Direct sequencing analyses were carried out to exclude pre-analytical and analytical errors on both strands using Big Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems), run on an ABI 3130 Genetic Analyzer (Applied Biosystems), and repeated on PCR products obtained from new nucleic acid extractions.

statistical analysis

Data are presented as percentages, mean ± SD, or median and interquartile ranges (IQR). Allelic frequencies were estimated by gene counting and genotypes were scored. Frequencies of each *TNFA* SNPs genotype were compared with those expected for a population in Hardy–Weinberg

equilibrium. The significance of the differences of observed alleles and genotypes between groups were tested using a free web-based application for association studies in multiple inheritance models (http://bioinfo.iconcologia.net/snpstats/start.htm)[17]. Haplotype frequencies and associations were calculated with Haploview (version 4.1).

Differences between percentages were assessed by χ^2 test. Student's unpaired *t*-test and ANOVA test were used for normally distributed variables. Appropriate non-parametric tests (Mann–Whitney *U*-test and Kruskal–Wallis test) were employed for all the other variables. Survival curves were calculated by the Kaplan–Meier and log-rank methods. Cox-proportional hazards analysis was used to evaluate the association between clinical variables and VTE-free survival. Time to event (TTE) was calculated from the date of enrolment until the event date or study end, with the event being any DVT or PE.

A total of 157 patients entered the study on the hypothesis that this number will detect a difference with a probability of 98%, at a two-sided 5% significance level, if the true hazard ratio (HR) is 2. This was based on the assumption of an accrual period of 2 years, an elapsed time between cycles within 30 days and a median TTE of 2.5 months. Only two-tailed probabilities were used for testing statistical significance. Only *P*-values lower than 0.05 were regarded as statistically significant. Calculations were made using a computer software package (Statistica 8.0, StatSoft, Inc., OK) or free web-based applications (http://statpages.org/).

results

The promoter region of the *TNFA* gene was screened for -863C/A, -857C/T, -376G/A, -308G/A and -238G/A SNPs using PCR amplification and direct sequencing analysis in 314 subjects, including 157 GI cancer patients and 157 unrelated controls. Genotypes and corresponding allele frequencies did not differ between patient and controls (supplementary Table S1, available at *Annals of Oncology* online). Both groups were in Hardy–Weinberg equilibrium.

All patients were followed-up for a median period of 12 months, or until time of event. Data were collected by two independent oncologists. No patient underwent surgery during follow-up, nor was admitted to clinic for an acute medical illness requiring thromboprophylaxis. VTE occurred in 10% (4 PE and 11 DVT) of GI cancer patients (median TTE: 3 months), in agreement with previous reports [8, 18]. In particular, seven (three non-fatal sub-segmental PE and 4 DVT) patients were incidentally diagnosed with asymptomatic VTE at time of CT scan for restaging. The remaining eight patients had symptomatic VTE. No patient had an asymptomatic VTE on outset.

SNPs analysis of observed allele and genotype frequencies showed a significant association in a co-dominant inheritance model of the *TNFA* -857 T allele [odds ratio (OR) = 6.5; $P = 0.003$] in GI cancer patients who developed VTE during chemotherapy compared with those who did not (supplementary Table S2, available at *Annals of Oncology* online). Furthermore, haplotype frequency estimation in the overall population of 314 subjects showed the presence of a five-loci (-863C/-857 T/-376G/-308G/-238G) haplotype (CTGGG haplotype), which frequency was significantly higher in GI cancer patients who developed VTE compared with those who did not (OR = 2.7, $P = 0.04$) (Table 2). GI patients who remained VTE free during chemotherapy did not differ in CTGGG haplotype frequency from controls (Table 2). The prevalence of the other observed haplotypes was comparable between cases and controls. Linkage disequilibrium (LD) analysis, defined by the D′ coefficient, revealed no LD between all five *TNFA* promoter SNPs.

Pre-chemotherapy serum TNF-α levels were higher in GI cancer patients compared with controls [5.0 (2.1–12.6) versus 1.1 (0.1–7.0) pg/ml, $P < 0.0001$], as previously shown [8], and in metastatic compared with non-metastatic GI cancer ($P = 0.046$). Pre-chemotherapy serum TNF-α levels were higher in GI cancer patients who developed VTE during chemotherapy compared with those who did not [14.8 (3.4–52.5) versus 4.8 (1.9–10.1) pg/ml, $P = 0.017$], as previously reported [8]. Although, no association could be observed between serum TNF-α levels and *TNFA* genotypes or haplotypes (data not shown) both were independent predictors of VTE at multivariate analysis (regression coefficient for CTGGG haplotype = 0.754, $P < 0.0001$; regression coefficient for serum TNF-α levels = 0.337, $P < 0.05$).

Of interest, 11 (20%) of 56 GI cancer patients carrying CTGGG haplotype developed VTE compared with 4 (4%) of the remaining 101 patients ($P = 0.003$). Univariate Cox-proportional hazards survival analysis showed that the presence of the CTGGG haplotype was able to significantly predict VTE with a HR of 5.6 [95% confidence interval (CI) 1.8–17.6; $P = 0.003$]. These values were substantially unmodified after adjustment for age, sex, site of primary, metastasis, ECOG-PS, concurrent treatment with bevacizumab and class of risk

Table 2. TNF-α gene promoter haplotype analysis and their corresponding frequencies in gastrointestinal cancer patients ($n = 157$) and control subjects ($n = 157$)

Haplotypes					Frequency					
						Cancer patients				
-863C/A	-857C/T	-376G/A	-308G/A	-238G/A	Control subjects	All patients	Without VTE	With VTE	OR (C.I.)	*P**
C	C	G	G	G	0.53	0.52	0.52	0.47	1.00	NA
C	T	G	G	G	0.18	0.19	0.19	0.37	2.72 (1.04–7.11)	0.04
A	C	G	G	G	0.13	0.14	0.14	0.10	0.75	0.67
C	C	G	A	G	0.11	0.06	0.06	NA	0.00	1.00
C	C	G	G	A	0.01	0.05	0.05	0.03	0.71	0.74
C	C	A	G	A	0.02	0.03	0.03	0.03	1.81	0.61

*P-value for haplotype association with venous thromboembolism (VTE).

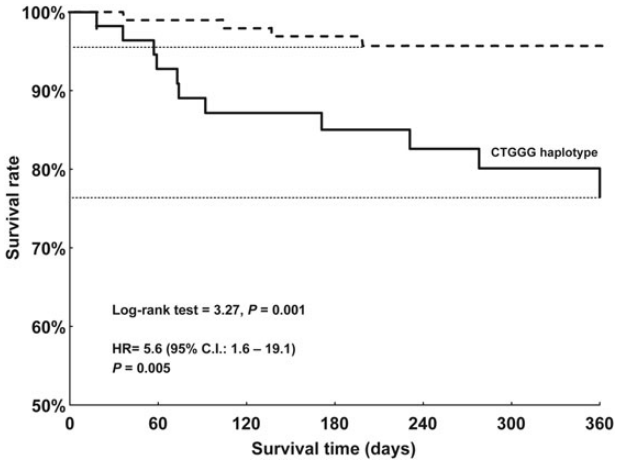

Figure 1. Kaplan–Meier analysis of VTE-free survival time in gastrointestinal cancer patients undergoing chemotherapy and categorized on the presence (solid line) or absence (dotted line) of the CTGGG haplotype.

according to Khorana [19] (HR 5.6; 95% CI 1.6–19.1; $P = 0.005$) (supplementary Table S3, available at *Annals of Oncology* online). Kaplan–Meier curves for patients stratified on the basis of the presence of the CTGGG haplotype showed that patients who carried the haplotype had a worst 1-year VTE-free survival (79%) compared with patients who did not carry CTGGG haplotype (96%, Log-rank test = 3.43, $P = 0.001$)(Figure 1).

discussion

These results demonstrate, for the first time to our knowledge, that *TNFA* gene promoter SNPs may predispose GI cancer patients to develop a first VTE episode during chemotherapy. In particular, the *TNFA* -857 SNP was associated to VTE in a co-dominant inheritance model, with the T allele predisposing to VTE. Moreover, the presence of a five-loci CTGGG haplotype was associated to VTE both in haplotype association studies and in Cox proportional hazards analysis. Our results are in agreement with a former study demonstrating a lack of association between the *TNFA* -308G/A SNP and VTE [16]. Conversely, the findings here reported extend these previous observations suggesting that *TNFA* sequence variants different from -308 G/A SNP, either alone or as a specific haplotype combination, might be involved in VTE onset, at least under particular triggers, such as chemotherapy.

Chemotherapy is associated with a 2- to 6-fold increased VTE risk, especially during the first 3 months [8, 18], as the result of haemostatic activation, possibly related to an increased inflammatory status [8]. Indeed, experimental and clinical evidences support the capability of anti-cancer drugs to induce an adaptive stress response in the neoplastic environment [20]. TNF-α is a product of the acute phase response, and its production is regulated at many levels under stress conditions and is under control of genetic polymorphisms [9]. In particular, *TNFA* -308A allele [10], -857 T allele and -863A allele [11, 12] have been associated with increased cytokine production. Thus, it was tempting to hypothesize that variations in TNF-α production (as a functional consequence of *TNFA* gene promoter polymorphisms) could be responsible for VTE onset under triggering conditions, i.e. chemotherapy. Accordingly, this study was designed as a case–control study in which the contribution of *TNFA* SNPs to the development of VTE was investigated in association to serum TNF-α levels. However, the increased frequency of the *TNFA* -857 T allele or the CTGGG haplotype in GI cancer patients who developed VTE did not associate with increased serum TNF-α levels. These results are in agreement with recent findings that could not confirm any functional relevance of any single *TNFA* SNPs, or the ancestral haplotype of the *TNFA* gene promoter -238G/-308G/-857 T, on transcriptional as well as post-translational levels, at least in healthy individuals [21]. Moreover, a meta-analysis conducted by the same authors confirmed the lack of association between TNF-α production and the *TNFA* SNPs analysed [21].

Thus, it is logical to conclude that the increased VTE frequency found in carriers of the CTGGG haplotype may be due to mechanisms different from increased TNF-α production. Indeed, the *TNFA* SNPs could affect the production of other cytokines, as the gene lies with other inflammatory genes within the major histocompatibility complex, a genetic region characterised by strong linkage disequilibrium. Another explanation might come from the fact that TNF-α was measured before chemotherapy start and, due to the retrospective nature of the study, no serial measurements during treatment were carried out. Thus, we may not exclude the possibility that chemotherapy might have acted as a trigger for sustained production of TNF-α only in carriers of the *TNFA* -857 T allele or CTGGG haplotype during early cycles of chemotherapy, a timing that is consistent with data demonstrating an increase of TNF-α after the first two cycles of platinum-based chemotherapy [22].

There are of course other limitations to acknowledge. First of all, the study was a retrospective analysis. However, all eligible consecutive patients within the designated timeframe were included, and all measurements were carried out while blinded to the patient outcome. Moreover, recruitment was carried out in a single Institution, which might have posed further limitation as the primary and most obvious shortcoming of single-centre studies is their potentially limited external validity. A final issue is represented by the relatively small sample size, ultimately leading to a small number of patients who actually developed VTE and large confidence intervals in the statistically significant findings. This might have affected the 'true' incidence of VTE or site-related rates, which, however, was not the purpose of our study.

In conclusion, the results here reported suggest that *TNFA* might represent a candidate gene contributing to VTE pathogenesis in GI cancer patients. To the best of our knowledge, this is the first evidence of an association between *TNFA* gene promoter SNPs and VTE. At present, we may hypothesize that chemotherapy elicits an adaptive response (with disruption of vascular homeostasis and/or cytokine up-regulation) mostly in individuals who carry the *TNFA* -857 T allele, or the CTGGG haplotype. Specifically designed studies are required to validate this hypothesis. Nonetheless, our present study suggests an involvement of *TNFA* SNPs in the

pathophysiology of VTE and indicates that GI cancer patients at increased VTE risk might be genetically identified and offered enhanced prophylaxis. Further multicentre prospective and validation cohort studies involving larger numbers of patients are needed to fully establish not only the predictive value of *TNFA* SNPs analysis during chemotherapy, but also the economic impact of its routine use to identify at-risk cancer patients, which may benefit from thromboprophylaxis.

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disclosure

The authors have declared no conflicts of interest.

references

1. ten Cate H, Falanga A. Overview of the postulated mechanisms linking cancer and thrombosis. Pathophysiol Haemost Thromb 2008; 36: 122–130.
2. Ku DH, Arkel YS, Paidas MP et al Circulating levels of inflammatory cytokines (IL-1 beta and TNF-alpha), resistance to activated protein C, thrombin and fibrin generation in uncomplicated pregnancies. Thromb Haemost 2003; 90: 1074–1079.
3. Guadagni F, Ferroni P, Palmirotta R et al TNF/VEGF cross-talk in chronic inflammation-related cancer initiation and progression: an early target in anticancer therapeutic strategy. In Vivo 2007; 21: 147–161.
4. Bauer KA, ten Cate H, Barzegar S et al Tumor necrosis factor infusions have a procoagulant effect on the hemostatic mechanism of humans. Blood 1989; 74: 165–172.
5. Wakefield TW, Greenfield LJ, Rolfe MW et al Inflammatory and procoagulant mediator interactions in an experimental baboon model of venous thrombosis. Thromb Haemost 1993; 69: 164–172.
6. Fox EA, Kahn SR. The relationship between inflammation and venous thrombosis. A systematic review of clinical studies. Thromb Haemost 2005; 94: 362–365.
7. Reitsma PH, Rosendaal FR. Activation of innate immunity in patients with venous thrombosis: the Leiden Thrombophilia Study. J Thromb Haemost 2004; 2: 619–622.
8. Ferroni P, Riondino S, Portarena I et al Association between increased tumor necrosis factor-alpha levels and acquired activated protein C resistance in patients with metastatic colorectal cancer. Int J Colorectal Dis 2012; 27: 1561–1567.
9. Wilson AG, Di Giovine FS, Blankemore M et al Single base polymorphism in the tumor necrosis factor alpha gene detected by NcoI restriction for the PCR product. Hum Mol Genet 1992; 1: 353.
10. Wilson AG, Symons JA, McDowell TL et al Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc Natl Acad Sci USA 1997; 94: 3195–3199.
11. Higuchi T, Seki N, Kamizono S et al Polymorphism of the 50-flanking region of the human tumor necrosis factor (TNF) -alpha gene in Japanese. Tissue Antigens 1998; 51: 605–612.
12. Soga Y, Nishimura F, Ohyama H et al Tumor necrosis factor-alpha gene (TNFalpha) -1031/-863–857 single-nucleotide polymorphisms (SNPs) are associated with severe adult periodontitis in Japanese. J Clin Periodontol 2003; 30: 524–531.
13. Fan W, Maoqing W, Wangyang C et al Relationship between the polymorphism of tumor necrosis factor-α-308 G>A and susceptibility to inflammatory bowel diseases and colorectal cancer: a meta-analysis. Eur J Hum Genet 2011; 19: 432–437.
14. Gorouhi F, Islami F, Bahrami H et al Tumour-necrosis factor-A polymorphisms and gastric cancer risk: a meta-analysis. Br J Cancer 2008; 98: 1443–1451.
15. Tahara T, Shibata T, Nakamura M et al Effect of IL-1β and TNF-α polymorphisms on the prognosis and survival of gastric cancer patients. Clin Exp Med 2011; 11: 211–217.
16. Brown K, Luddington R, Baglin T. A common polymorphism in the tumour necrosis factor-alpha gene associated with high TNF levels is not a risk factor for venous thromboembolism. Br J Haematol 1998; 101: 480–482.
17. Sole` X, Guino` E, Valls J et al SNPStats: a web tool for the analysis of association studies. Bioinformatics 2006; 22: 1928–1929.
18. Di Nisio M, Ferrante N, De Tursi M et al Incidental venous thromboembolism in ambulatory cancer patients receiving chemotherapy. Thromb Haemost 2010; 104: 1049–1054.
19. Khorana AA, Kuderer NM, Culakova E et al Development and validation of a predictive model for chemotherapy-associated thrombosis. Blood 2008; 111: 4902–4907.
20. Tiligada E. Chemotherapy: induction of stress responses. Endocr Relat Cancer 2006; 13: S115–S124.
21. Mekinian A, Tamouza R, Pavy S et al Functional study of TNF-α promoter polymorphisms: literature review and meta-analysis. Eur Cytokine Netw 2011; 22: 88–102.
22. Derin D, Soydinç HO, Guney N et al Serum levels of apoptosis biomarkers, survivin and TNF-alpha in nonsmall cell lung cancer. Lung Cancer 2008; 59: 240–245.