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Original article

Tumor necrosis factor-α as a biomarker of infection in total knee arthroplasty

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ABSTRACT. ***Background:*** **Thanks to defense mechanisms, organisms have had to adapt themselves to an adverse natural setting that causes acute and chronic stress. This adaptive response that tries to protect the cells against lethal insults uses its own defense systems.** ***Material and methods:*** **Prospective, observational, descriptive pilot study with analytic components to determine the baseline preoperative TNF levels of 35 patients undergoing total knee arthroplasty due to gonarthrosis. Ten patients with a diagnosis of infected total knee arthroplasty were also included. In order to find differences and possible associations, the Mann-Whitney U test or the Fisher test was used to compare the variables between the non-infected group of patients and the group with the infection complication.** ***Results:*** **We found a statistically significant difference; higher levels of fibrinogen, erythrocyte sedimentation rate, C-reactive protein, TNF-α and temperature were found in the infected patients; temperature was not clinically relevant.** ***Conclusions:*** **In the absence of a diagnostic specificity, the combined determinations of acute phase reactants may be useful to detect the presence and intensity of the inflammatory and infectious processes.**

RESUMEN. ***Antecedentes:*** **Los organismos han tenido que adaptarse gracias al desarrollo de mecanismos de defensa a un entorno natural adverso causante de estrés agudo y crónico. Esta respuesta adaptativa que intenta proteger a las células contra agresiones letales, asocia la formación de sus sistemas de defensa.** ***Material y métodos:*** **Estudio piloto prospectivo observacional descriptivo con componentes analíticos donde se determinan los niveles basales preoperatorios en 35 pacientes sometidos a una artroplastía total de rodilla por presentar gonartrosis. A la vez se estudian 10 pacientes diagnosticados con artroplastía total de rodilla infectadas. Para encontrar diferencias y buscar posibles asociaciones se usó el test U de Mann-Whitney o el test de Fisher para comparar las variables entre el grupo de pacientes no infectados y el grupo con complicación de infección.** ***Resultados:*** **Encontramos una diferencia estadísticamente significativa, mostrando niveles superiores en los pacientes infectados, en el fibrinógeno, velocidad de sedimentación, proteína C reactiva, FNT α y temperatura, no teniendo utilidad clínica esta última.** ***Conclusiones:*** **En ausencia de una especificidad diagnóstica las determinaciones combinadas de los reactantes**

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Palabras clave: factor de necrosis tumoral alfa, biomarcadores farmacológicos, infección, artroplastía, rodilla.

Introduction

The diagnosis of prosthetic infection is a challenge in the daily practice of all orthopedic surgeons, as none of the usual diagnostic tests have a 100% sensitivity and specificity.[1] The combination of several of them within a diagnostic algorithm has improved their yield, but not so much as to eradicate the diagnostic errors that cause great socioeconomic and health costs, especially in false negative cases.

Tumor necrosis factor-α (TNF-α) may be an effective biologic marker of infection in total knee arthroplasties.

The objectives of this paper are:

1. To examine the biology of TNF-α in patients about to undergo total knee replacement surgery.
2. To study the biology of TNF-α in patients with a diagnosis of late chronic complications of an infected knee arthroplasty as a possible diagnostic biomarker of infection.

Material and methods

Observational, prospective, descriptive pilot study with analytical components.

A total of 35 consecutive patients were enrolled starting in March 2003; all of them met the following inclusion criteria:

- Male or female patients, over age 65, with a diagnosis of primary gonarthrosis (Kellgren and Lawrence grades III-IV)[2] about to undergo scheduled surgery for the placement of a total knee prosthesis at *Gregorio Marañón* University General Hospital.
- Patients who gave their written informed consent to participate in the study.
- Concurrently, and starting in the same date, another 10 patients were enrolled, all of whom had undergone total knee replacement and had signs of infection (based on the predictive biomarkers used clinically and the anatomic and microbiologic study of the specimens obtained during the intervention), and met the above mentioned criteria (with the exception of the first one).

Since this is a pilot study, the sample size was not predetermined.

The demographic and clinical variables were obtained by means of patient anamnesis.

A 10 ml blood sample was drawn from each patient; 5 ml were processed in the central lab at *Gregorio Marañón* University General Hospital to quantify C-reactive protein (CRP), fibrinogen, erythrocyte sedimentation rate (ESR), and the leukocyte formula and count. After 15 minutes of rest and coagulation, the remaining 5 ml were spinned for 10 minutes at 2500 rpm to obtain the serum, which was separated in four Eppendorf tubes previously identified. The samples were kept at -40°C until their processing. The tumor necrosis factor-α was analyzed in each one of them.

Tumor necrosis factor measurement method: Commercial ELISA technique (Bender MedSystems BMS223INST®), according to the manufacturer's instructions. The TNF-α concentration was obtained by means of interpolation over a linear regression pattern curve and is expressed in pg/ml. TNF-α is not detected in the serum of healthy subjects.

All the information was collected in a record card designed specifically for the study and was entered in a data base for its later analysis.

Descriptive statistical tests of the measured variables were performed using frequency measures (absolute and percentages) for those measured categorically, and centralization and scatter measures (mean, typical deviation, median and interquartile range) for the quantitative measures.

To compare and look for possible associations, the appropriate statistical tests were performed, whether parametric or non-parametric, using the Mann-Whitney U test (in quantitative variables) or Fisher's test (in categorical variables) to compare the variables between the infected and non-infected primary knee arthroplasties.

The significance was $p < 0.05$. The SPSS 15 statistical software for Windows was used.

This work was carried out in collaboration with the cellular biology lab at the Experimental Medicine and Surgery Unit, *Gregorio Marañón* University General Hospital.

This work was conducted following the good clinical practice guidelines and fully accepting the ethical standards in force (Declaration of Helsinki, 2000 Edinburgh revision).

This protocol was reviewed, approved and sponsored by the Research Committee and the Clinical Research Ethics Committee at *Gregorio Marañón* University General Hospital.

All patients were asked to give their written informed consent by means of a form in order to participate in this project. They were informed about the objective of the

study, the procedures, the potential risks and benefits, the assurance that their participation would be on a voluntary basis, the protection of confidentiality in agreement with the current legislation, and they had the opportunity to ask questions about the study.

Results

Of the 36 patients who underwent primary knee arthroplasty and were selected initially, we could only get complete information for all the variables in 20 of them, due to withdrawal from the study (2 patients), sample alteration during collection or processing (4 patients) or budget limitations (10 patients). The total number of patients with infected total knee arthroplasties was 10.

Following the protocol for taking intraoperative samples for pathologic anatomy and microbiology, after a 2-week period without antibiotics that could mask the results, the prosthetic infection of the patients included in the study was confirmed. *Table 1* shows the different causative agents; infection due to a single microorganism occurred in 4 cases. Seven patients had a culture that was positive for the same microorganism, with the same proportion in the articular fluid and the biopsy culture; in 3 cases it was positive only in the biopsy culture.

Tables 2, 3, 4 and 5 show the results of the demographic characteristics, the associated medical history and the treatment received prior to the intervention in the group of patients in whom a primary total knee prosthesis was implanted (n = 20) and in patients with an infected knee prosthesis (n = 10), as well as their statistical significance.

Table 6 shows the data on the acute phase reactants for both groups, as well as blood pressure and temperature. Fibrinogen, ESR, CRP, TNF-α, and temperature were statistically significantly higher in the group of patients with an infected knee prosthesis.

As *Table 7* and *Chart 1* show, the values for tumor necrosis factor-α are higher for the group of infected arthroplasties.

Discussion

The activation of endothelial cells, the interaction between activated platelets and leukocytes and leukocyte activation are key factors of hemostasis and the tissue inflammatory response to the insults resulting from surgical interventions and accidental trauma. This inflammatory-hemostatic process is usually a localized response at the injury site. Cytokines are released to increase this local response to provide a regulated systemic response. At times this response occurs without control and the systemic inflammatory response syndrome occurs.[3,4]

The activation of coagulation has been positively correlated with the extension of the surgical insult, considering the duration of the intervention and the blood loss, as well as with the increase in platelet activation.

A trauma or a serious burn and major surgery induce a variable of immune response suppression thus increasing the susceptibility to infection. The immunosuppression that occurs after surgery seems to be the result of two mechanisms that include a systemic, excessive and indiscriminate inflammatory response and a failure of the cell-mediated

Table 1. Microorganisms grown in the 10 infected patients.

	GII-1	GII-2	GII-3	GII-4	GII-5	GII-6	GII-7	GII-8	GII-9	GII-10
Staphylococcus aureus		Yes		Yes	Yes	Yes			Yes	
Staphylococcus epidermidis	Yes		Yes					Yes		
Eubacterium limos				Yes						
Enterobacter faecalis				Yes						Yes
Propionibacterium acnes					Yes					
Aspergillus terreus						Yes				
Streptococcus pyogenes A										Yes
Bacillus sp										Yes
Enterobacter cloacae							Yes			

Table 2. Comparison of the demographics of both groups.

Variables	Patients with primary total knee prosthesis N = 20	Patients with infected total knee prosthesis N = 10	Statistical significance (p)
Female sex	75% (15)	80% (8)	1 (*)
Age in years (mean ± SD)	74 ± 5.1	71 ± 4.4	0.482 (**)
[median (IQR)]	[74 (2.5)]	[70 (5.3)]	

Fisher's test (*) Mann-Whitney U test (**)
SD = Standard Deviation

immunity. Therefore, although part of the immune system mounts an overwhelming response to the insult, the rest of it is paralyzed.

A unanimously accepted marker of inflammatory activity is C-reactive protein.[5] It has been found to be elevated in major trauma, infection and uncomplicated scheduled surgeries, with a maximum peak between 24 and 48 hours. The literature shows an increase in CRP in burned patients up to day 6 of their course. Levels remained significantly elevated for as long as three weeks in cases in which more than

Table 3. Comparison of the medical history of both groups. It is important to highlight the higher percentage of non insulin-dependent diabetes mellitus among the patients with an infected knee prosthesis compared to non-infected ones; the prevalence of HT is strikingly higher among non-infected patients.

Variables	Patients with primary total knee prosthesis N = 20	Patients with infected total knee prosthesis N = 10	Statistical significance (p)*
Arthrosis (location outside the knees)	25% (5)	0% (0)	0.140
Ulcus	5.9% (2)	0% (0)	1
Hiatal hernia	5% (1)	0% (0)	1
Allergies	15% (3)	40% (4)	0.181
Previous surgical interventions	80% (16)	100% (10)	0.272
IDDM	10% (2)	10% (1)	1
NIDDM	15% (3)	30% (3)	0.372
HT	75% (15)	50% (5)	0.231
Coronary disease	15% (3)	0% (0)	0.532
Depression	10% (2)	20% (2)	0.584
Rheumatoid arthritis	8.8% (3)	0% (0)	1

*Fisher's test IDDM: insulin-dependent diabetes mellitus NIDDM: non insulin-dependent diabetes mellitus HT: hypertension

Table 4. Comparison between the drug treatments of both groups. We did not find a statistically significant difference in the treatments that the patients in both groups were receiving.

Variables	Patients with primary total knee prosthesis N = 20	Patients with infected total knee prosthesis N = 10	Statistical significance (p)*
Oral anticoagulant	5% (1)	0% (0)	1
Anti-platelet aggregation agents	15% (3)	0% (0)	0.532
Omeprazole	20% (4)	10% (1)	0.640
Analgesics without an antiinflammatory effect	70% (14)	80% (8)	0.682
NSAIDs	25% (5)	30% (3)	1
Opioid patches	10% (2)	0% (0)	0.540

*Fisher's test

Table 5. Comparison of the surgical details in both groups. A trend towards the use of non-posterior stabilized implants is seen in both groups and the infectious complication factor is not considered as an added anesthetic risk when assessing the patients during the presurgical visit conducted by the anesthesiology service. Unlike the non-complicated arthroplasty group, in the infected group two patients required the removal of the fixation material of the failed tibial osteotomies during the stage at which the prosthesis was placed, which led to an increased operative time and a greater insult, which theoretically favors the development of infectious complications.

Variables	Patients with primary total knee prosthesis N = 20	Patients with infected total knee prosthesis N = 10	Statistical significance (p)*
Group A+	52.6% (10)	14.3% (1)	0.178
Group A-	5.3% (1)	0% (0)	1
Group B+	15.8% (3)	14.3% (1)	1
Group O+	26.3% (5)	71.4% (5)	0.069
ASA I	20% (4)	40% (4)	0.384
ASA II	60% (12)	50% (5)	0.705
ASA III	20% (4)	10% (1)	0.640
Insall Burstein II®	30% (6)	20% (2)	0.682
Profix®	70% (14)	80% (8)	0.682
EMO staple	0% (0)	20% (2)	0.103

*Fisher's test

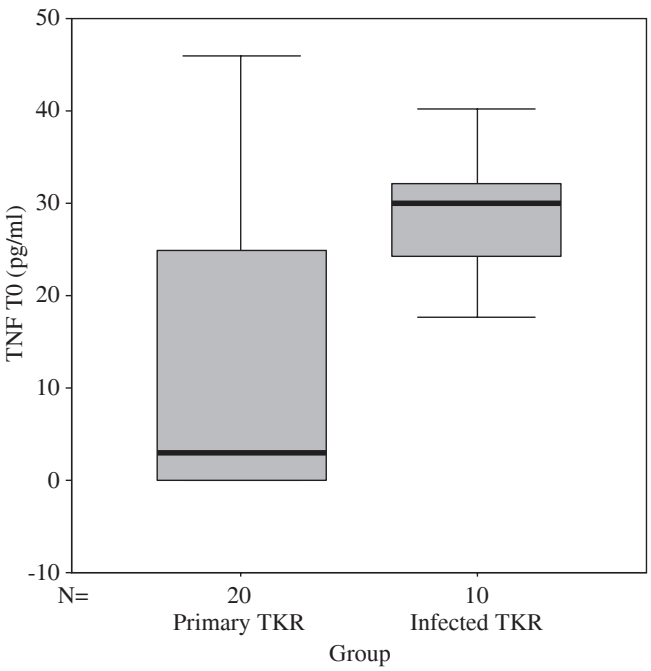
Table 6. Comparison with the measurement of the variables of both groups.

Variables	Patients with primary total knee prosthesis N = 20 (mean ± SD) [median (IQR)]	Patients with infected total knee prosthesis N = 10 (mean ± SD) [median (IQR)]	Statistical significance (p)*
WBC / (mm³)	6,855 ± 2,295 [7,200 (4,050)]	8,400 ± 3,562 [7,200 (3,225)]	0.373
Fibrinogen mg/dl	370.1 ± 100.3 [380.5 (97.8)]	552 ± 172 [522 (211)]	0.001
ESR mm/h	22.3 ± 16.1 [19.5 (17.8)]	54 ± 41.5 [37.5 (76)]	0.039
CRP mg/dl	0.4 ± 0.4 [0.4 (0.5)]	3.8 ± 5.2 [1.7 (5.7)]	0.035
Temperature °C	36.2 ± 0.3 [36.2 (0.5)]	36.7 ± 0.5 [36.8 (0.7)]	0.008
SBP mmHg	144 ± 20 [140 (29.3)]	150 ± 20 [150 (35)]	0.588
DBP mmHg	78 ± 9.9 [80 (10)]	79 ± 11 [80 (20)]	0.650
TNF-α pg/ml	12.16 ± 15.18 [3.06 (25.50)]	27.32 ± 11.39 [29.95 (11.20)]	0.008

*Mann-Whitney U test

Table 7. Distribution of the TNF-α values in both groups.

TNF-α pg/ml	Primary TKR T0	Infected TKR
Mean	12.16	27.32
Median	3.06	29.95
Variance	230.29	129.71
SD	15.18	11.39
Minimum	0.00	0.90
Maximum	45.90	40.20
IQR	25.50	11.20



Mann-Whitney U test. p = 0.008

Chart 1. Comparison of the baseline level of TNF-α in both groups.

30% of the body surface was affected; in cases in which less than 30% of the body surface was affected, a progressive decrease in its levels was observed.

During the past few years a number of clinical researchers have studied the effects of trauma on the cell mediators, especially on the proinflammatory cytokines such as TNF-α.[6,7] Thus an important increase in the synthesis and secretion of all of these inflammatory mediators is seen in trauma. The levels of TNF-α and interleukin-6 (IL6) after elective surgery have been studied and although few changes have been found in TNF-α, an elevation in acute phase protein CRP has been observed following the highest IL6 peak. This correlation has been confirmed in previous studies.[8-10]

Therefore, TNF-α contributes to the acute phase hypermetabolic response that occurs following trauma. Some researchers have attributed an antiinflammatory response to IL6 given that it blunts TNF-α and reduces IL1 activity.[11]

Cancer, ischemia-reperfusion, febrile processes and infection induce an increase in the gene expression and synthesis of heat shock proteins (HSPs). These proteins are also involved in the protective mechanisms against the oxidative stress caused by various causes such as ultraviolet radiation and proinflammatory cytokines including TNF-α, IL1 and IL6.[12-14]

The inflammation mediators, mainly those derived from arachidonic acid, reactive oxygen species and cytokines, are released by the injured tissue. Cytokines may alter the expression of the HSP genes, either directly or indirectly, through their pyrogenic activity. This refers particularly to the following cytokines: IL1 and IL2, interferons, and TNF-α.[15] Numerous relations have been observed between cytokines and stress proteins.[16] The significance of the role

of stress proteins on the development of the immune response is evident.

The stress response inhibits the expression of the inducible form of nitric oxide synthase and the increase in the serum levels of TNF-α. This leads to conclude that the heat shock response may modulate the proinflammatory response and thus protect the individual. It has been shown that the decrease in serum TNF-α is not the result of a decreased synthesis of the latter, but rather of the inhibition of its release by the macrophages due to a probable direct binding of HSP70i (heat shock protein) to TNF-α.[17] Therefore a mechanism through which HSP70i may act as a protective protein in cases of sepsis is the inhibition of the proinflammatory response thus permitting the maintenance of the hemodynamic balance. Other HSP70i-mediated protective mechanisms have been postulated whose overexpression would blunt the apoptosis of the cells submitted to the action of deleterious agents.[18]

The following covariables may modify the results of our project:

1. The overall prevalence of diabetes mellitus is around 5%,[1] but in our population it is approximately 25%. However, the infection rate has not been found to be higher in the people affected by this disease. We need to increase the sample size to study this issue.
2. Some authors have proposed that an ASA III patient is correlated with a 9 fold higher risk of having an infectious complication during primary knee replacement.[1] This selection would partially justify the high rate of infection at the hospital where this study was carried out.
   The risk factors that more firmly have been related with infection in these patients include a prior surgery, another focus of infection at the time of surgery and the presence of immunosuppression. Although the infection rate reported is 2 fold higher in males than in females, in our sample 80% of the infections occurred in females.
3. Nonsteroidal antiinflammatory agents (NSAIDs) are drugs with analgesic, antipyretic and antiinflammatory properties.[19] Most of the effects of these drugs are mediated by the inhibition of the enzyme prostaglandin synthase and by the alteration of the eicosanoid synthesis. The cytokines released by macrophages are the first mediators of the acute inflammatory response and are considered as collaborators of the progression of inflammatory conditions; their levels are elevated in the synovial fluid of patients with rheumatoid arthritis. NSAIDs are capable of activating the HSF (heat shock factors) transcription factors from a latent cytoplasmic form to a nuclear form that can bind to DNA.[19] This way they produce a change in the gene expression of cells which would lead to the suppression of the genes involved in macrophage activation and an increase in the stress protein genes. Upon the activation of HSF1 by the NSAIDs, the former downregulates the IL1β gene promoters thus modulating the febrile response and the acute inflammatory phase.[15] Through a similar mechanism it decreases the synthesis of TNF-α and the adhesion molecules involved in macrophage activation.[5]
4. The most frequently isolated pathogenic bacteria in periprosthetic profound infections are gram positives. The germs most frequently involved in these infections are *Staphylococcus aureus* and *Staphylococcus epidermidis*. This partially coincides with our study because we also found other less aggressive microorganisms involved, so the semiology is less full-blown than in the early acute infections and thus the diagnosis could be delayed. Pain is one of the clinical symptoms that most frequently occurred in our study; we must remember that it is one of the most characteristic markers of prosthetic infection.
   Wilson has reported that the *Staphylococcus aureus* gram positive cocci cause 63% of infections, similar to what Schoifet found in his sample, where they accounted for 58% of infections.[20] Gram negative rods are associated with injuries characterized by long-term draining. Their prevalence goes up in cases of prolonged draining, but *Staphylococci* are still the most frequent organisms. They usually are secondary contaminants and occur as polymicrobial infections. In acute blood-borne infections *Staphylococcus aureus* is the most frequent organism.[21] In 3 out of 4 cases of early acute infections the involved germ is gram positive. The late chronic infection results from an early onset infection that has not been diagnosed or treated before one month. It has a poor response to isolated antibiotic therapy, so a prosthetic exchange is required. It is usually mistaken for an inflammatory or mechanical problem because the bacterial inoculum is small and patchy, and it is associated with coagulase-negative *Staphylococcus*. *Staphylococcus epidermidis* is highly capable of attaching itself to polyethylene. There is a close relation between bacterial antibiotic resistance and the capacity of bacteria to produce adhesion and colonization of the implant surface.

Patients with chronic active fistulas develop mixed infections in which several organisms are isolated. The wrong and continuous administration of antibiotics promotes the appearance of resistant bacteria.

Although it is known that some cytokines like TNF-α are early inflammatory mediators, there are many questions that arise about their role, secretion dynamics and cells responsible for their release during surgical interventions and in the infectious complications of arthroplasty. Mancilla et al. related the IL1, IL6 and TNF-α levels with the prediction of the clinical course of neonates with severe sepsis and found a poor prognosis for those with elevated levels of these cytokines.[11] They also proved that the determination of CRP and TNF-α during the first few hours of life is highly specific but little sensitive in bacterial infections.

TNF-α is found as a reservoir in the surface of the cells that produce it, especially in macrophages and CD4+ T-lymphocytes. It is mobilized in various situations like the contact with bacterial lipopolysaccharides or surgical insults.[22]

Acute phase reactants offer a moderate diagnostic efficacy in case of suspicion of an infectious complication, so

they should support the diagnosis that is based on clinical criteria. There is definitely no single conclusive test to make the diagnosis of prosthetic infection; the presence of several positive tests is what leads to the diagnostic suspicion of infection. These and other tests will continue to be a complement during the follow-up of patients, which is necessarily based on a correct anamnesis and a detailed physical exam. Thanks to the presence of chemiluminescent enzyme immunoassays that are relatively simple to use, it has been possible to eliminate one of the major inconveniences for the determination of these parameters in the clinical practice, due to the complexity of their determination.

Despite the fact that during the conception and development of this study we tried to avoid mistakes, especially concerning the method, every research involves unforeseen or new orientations once one delves deeply into the topic and moves ahead in this exciting undertaking. The following limitations of our study are listed below:

1. One of the main inconveniences is the low prevalence of infection in primary total knee arthroplasties. During the period in which the cases were collected the observed prevalence was 3%, similar to the one reported in the literature by most centers. As a result of this the number of patients included in the research was less than what we wanted. It is true that the studies on prosthetic infections do not usually have large sample sizes and, in the case of certain variables, there is no prior research in the field of orthopedic surgery.
2. Due to the age of the sample population selected, it is reasonable to find concomitant diseases and use of medications, all of which may act as covariables that may affect the final outcome. Eliminating such covariables may prevent this from happening, but then it would be even more difficult to enroll subjects with an infectious complication that, fortunately for society, occurs at a low prevalence. It is likely that the extension of the study by increasing the sample size may provide a greater statistical power to these preliminary results.

There is still much to learn about the biology of acute phase reactants. Delving deeply into the understanding of their regulatory mechanisms may help us use them for the future diagnosis and treatment of various conditions.

References

1. Insall JN: Infection of total knee arthroplasty: *Instr Course Lect* 1986; 35: 319-24.
2. Lawrence JS, Bremner JM, Bier F: Osteoarthrosis. Prevalence in the population and relationship between symptoms and X-ray changes. *Ann Rheum Dis* 1966; 25: 1-24.
3. Cordero I: Respuesta metabólica al estrés anestésico quirúrgico. *Act Med* 2002; 10: 1-6.
4. Voet D, Voet J G: El sistema endocrino. Comunicaciones bioquímicas: Hormonas y neurotransmisores. En: Wiley J e hijos eds: Bioquímica. Barcelona: Omega. 1992: 1234-47.
5. Hata H, Sakaguchi N, Yoshitomi H, Iwakura Y, Sekikawa K et al: Distinct contribution of IL-6, TNF-α, IL-1, and IL-10 to T cell-mediated spontaneous autoimmune arthritis in mice. *J Clin Invest* 2004; 114: 582-8.
6. Benson M, Wennergren G, Fransson M, Cardell L: Altered levels of the soluble IL-1, IL-4 and TNF receptors, as well as the IL-1 receptor antagonist in the intermittent allergic rhinitis. *Int Arch Allergy Imm* 2004; 134: 227-32.
7. Franz A, Bauer K, Schalk A, Garland S et al: Measurement of interleukin 8 in combination with C-reactive protein reduced unnecessary antibiotic therapy in newborn infants: a multicenter, randomized, controlled trial. *Pediatric*s 2004; 114: 1-8.
8. Fransen EJ, Maessen JG, Elenbaas TW, Van Aarnhem EE, Van Dieijen-Visser MP: Enhanced preoperative C-reactive protein plasma levels as a risk factor for postoperative infections after cardiac surgery. *Ann Thorac Surg* 1999; 67: 134-8.
9. Giannoudis PV, Smith MR, Evans RT, Bellamy MC, Guillou PJ: Serum CPR and IL – 6 after trauma. Not predictive of septic complications in 31 patients. *Acta Orthop Scand* 1998; 69: 184-8.
10. Van Dissel J, Van Langevelde P, Westendorp R, Kwappenbe K: Anti-inflammatory cytokine profile and mortality in febrile patients. *Lancet* 1998; 351: 950-3.
11. Mancilla-Ramírez J, Arredondo-García J, Vannier E, Dinarello ChA: Interleucina-1, Interleucina-6 y factor de necrosis tumoral en sepsis neonatal. *Perinatol Reprod Hum* 1996; 10: 230-7.
12. Freitas I, Fernández-Somoza M, Essenfed-Sekler E, Cardier JE: Serum levels of the apoptosis associated molecules, tumor necrosis factor-alpha, tumor necrosis factor type-I receptor and Fas/FasL, in sepsis. *Chest* 2004; 125: 2238-46.
13. Jacob U, Muse W, Eser M, Bardwell JCA: Chaperone activity with a redox switch. *Cell* 1999; 96: 341-52.
14. Wagstaff MJ, Collaco-Moraes Y, Smith J, de Belleroches JS, Coffin RS, Latchman DS: Protection of neuronal cells from apoptosis by Hsp27 delivered with a herpes simplex virus-based vector. *J Biol Chem* 1999; 274: 5061-9.
15. Goldring M, Fukuo K, Kirkhead J, Dudek E, Sandell L: Transcriptional suppression by IL-I and interferon-gamma of type II collagen gene expression in human chondrocytes. *J Cell Biochem* 1994; 54: 85-99.
16. Pockley AG: Heat shock proteins as regulators of the immune response. *The Lance*t 2003; 362: 469-76.
17. Freedman RB, Hirst TR, Tuit MF: Protein disulphide isomerase: building bridges in protein folding. *Trends Biochem Sci* 1994; 19: 331-6.
18. Buzzard KA, Giaccia AJ, Killender M, Anderson RI: Heat shock protein 72 modulates pathways of stress-induced apoptosis. *J Biol Chem* 1998; 273: 17147-53.
19. Housby JN, Cahill CM, Chu B, Bickford K: Non-steroidal anti-inflammatory drugs inhibit the expression of cytokines and induce HSP70 in human monocytes. *Cytokine* 1999; 11: 347-58.
20. Wilson MG, Kelley K, Thornhill TS: Infection as a complication of total knee-replacement arthroplasty: Risk factors and treatment in sixty-seven cases. *J Bone Joint Surg Am* 1990; 72: 878-83.
21. Ayers DC, Dennos DA, Johanson NA, Pellegrini VD: Common complications of total knee arthroplasty. *J Bone Joint Surg* 1997; 79-A: 278-311.
22. Ikebe T, Hirata M, Koga T: Effects of human recombinant tumor necrosis factor-α and interleukin I on the synthesis of glycosaminoglycan and DNA in cultured rat costal chondrocytes. *J Immunol* 1988; 140: 904-11.