Antifibrinolytics attenuate inflammatory gene expression after cardiac surgery

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Objectives: Anti-inflammatory effects of tranexamic acid and aprotinin, used to abate perioperative blood loss, are reported and might be of substantial clinical relevance. The study of messenger ribonucleic acid synthesis provides a valuable asset in evaluating the inflammatory pathways involved.

Methods: Whole-blood messenger ribonucleic acid expression of 114 inflammatory genes was compared pre- and postoperatively in 35 patients randomized to receive either placebo, tranexamic acid, or aprotinin. These results were further confirmed by reverse transcription–polymerase chain reaction.

Results: Of the 23 genes exhibiting independently altered postoperative gene expression levels, 8 were restricted to the aprotinin group only (growth differentiation factor 3, interleukin 19, interleukin 1 family member 7, transforming growth factor α, tumor necrosis factor superfamily 10, tumor necrosis factor superfamily 12, tumor necrosis factor superfamily 13B, vascular endothelial growth factor α), whereas both aprotinin and tranexamic acid altered gene expression of 3 genes as compared with placebo (FMS-related tyrosine kinase 3 ligand, growth differentiation factor 5, interferon-α8). In general, less upregulation of pro-inflammatory, and more upregulation of anti-inflammatory, genes was observed for patients treated with antifibrinolytics. Gene expression affected by aprotinin coded mostly for proteins that function through serine proteases.

Conclusions: This study demonstrates that the use of tranexamic acid and aprotinin results in altered inflammatory pathways on the genomic expression level. We further demonstrate that the use of aprotinin leads to significant attenuation of the immune response, with several inhibitory effects restricted to the use of aprotinin only. The results aid in a better understanding of the targets of these drugs, and add to the discussion on which antifibrinolytic can best be used in the cardiac surgical patient. (J Thorac Cardiovasc Surg 2013;145:1611-6)

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The pharmacologic agents aprotinin and tranexamic acid have proved to reduce substantially perioperative bleeding and transfusion requirements in cardiac surgery.[1] Aprotinin not only abates excess fibrinolysis, but also inhibits serine proteases involved in triggering and signaling pathways.[2] Together with attenuation of chemotactic mediators involved in the coagulation cascade,[3] this explains some of the reported anti-inflammatory effects of aprotinin.[4-6] The routine use of aprotinin was questioned after results from several observational studies indicated an increased risk of serious adverse events.[7,8] When aprotinin-treated, high-risk cardiac surgery patients showed a 50% increase in 30-day mortality compared with placebo or other antifibrinolytics,[9] aprotinin quickly lost its dominant market position in favor of tranexamic acid. This antifibrinolytic is reported to be equally effective in reducing blood loss,[10] and is thought to have similar anti-inflammatory properties as well.[11] Although the impact of aprotinin and tranexamic acid on the coagulation system have been studied extensively, their effects on inflammation remain unclear. A better understanding of the signaling and regulatory pathways involved helps us to determine which positive and negative side effects are associated with their use. This is even more relevant now that the European Medicine Agency has decided that, because the results of the BART study on which this suspension was based were deemed unreliable, the current suspension of the marketing of aprotinin should be lifted.[9,12] We studied pre- and postoperative messenger ribonucleic acid (mRNA) expression levels of

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Abbreviations and Acronyms

CPB = cardiopulmonary bypass
DNA = deoxyribonucleic acid
GDF = growth differentiation factor
GE = gene expression
IFN = interferon
IL = interleukin
MOF = multiorgan failure
mRNA = messenger ribonucleic acid
PCR = polymerase chain reaction
RNA = ribonucleic acid
RT-PCR = reverse transcription–polymerase chain reaction
TGF = transforming growth factor
TNF = tumor necrosis factor
TNFsf = tumor necrosis factor superfamily

114 inflammatory genes in 35 patients randomized to receive either placebo, tranexamic acid, or aprotinin. Our primary end point was a direct comparison of aprotinin and tranexamic acid on genomic expression of genes involved in the inflammatory response, with effects on relevant clinical outcomes serving as secondary outcomes.

METHODS

Patient Enrollment

We performed a single-institution case–control study in which pre- and postoperative whole-blood samples were collected from 199 patients who participated in a double-blind, randomized, placebo-controlled trial evaluating the effectiveness of tranexamic acid and aprotinin between June 2004 and October 2006.[10] Patients were scheduled for primary coronary bypass grafting, valvular surgery, or a combination of the 2 using cardiopulmonary bypass (CPB). Exclusion criteria encompassed active inflammatory disease and preoperative use of corticosteroids. A total of 35 patients (12 placebo, 12 tranexamic acid, 11 aprotinin), matched for age, gender, CPB time, the number of red cell blood products transfused, intraoperative use of steroids, and the incidence of postoperative multiorgan failure (MOF) were analyzed for prospective analysis of differences in pre- and postoperative inflammatory gene expression (GE) profiles. This case–control substudy was approved by our internal review board, and informed consent was obtained from each participant.

Interventions

A conventional operative approach was used in all patients, including midline sternotomy and systemic heparinization. Patients received anesthesia according to a ''fast track'' protocol in which total intravenous anesthesia was used through target-controlled infusion. At the time of the study, 2 of 6 cardiothoracic anesthesiologists employed at our hospital administered corticosteroids routinely to their patients, either 8 mg dexamethasone or 80 mg hydrocortisone. Because anesthesiologists were assigned randomly to the operations, from a patient's perspective, the administration of steroids was random. Either pulsatile or nonpulsatile blood flow was established with a centrifugal pump and hollow-fiber membrane oxygenator primed with crystalloid solution. A tip-to-tip phosphorylcholine coating of all tubing was used. Patients were randomized to receive intraoperatively either high-dose aprotinin (2×10^6 kallikrein inhibiting units aprotinin loading dose, 2×10^6 kallikrein inhibiting units added to the CPB priming solution, and a continuous infusion of 5×10^5 kallikrein inhibiting units/hour during CPB),[13] full-dose tranexamic acid (1 g loading dose, 500 mg added to the CPB system prime and a continuous infusion of 400 mg/hour during CPB),[14] or placebo (0.9% saline solution according to an identical regime). Blood temperature was kept between 35°C and 37°C, and myocardial protection was achieved by intermittent antegrade warm blood cardioplegia according to the Calafiore protocol. Before heparinization and after reversal with protamine, blood from the operative field was retrieved in a cell saver device, but was only washed and returned to the patient if blood loss exceeded 500 mL. Postoperative mediastinal chest tube blood loss was not reinfused.

Blood Sampling

Whole blood samples were withdrawn by vein puncture the day before surgery and 24 hours after the start of CPB. All samples were stored immediately at 4°C and mRNA was stabilized within 24 hours using RNALater (Applied Biosystems/Ambion, Austin, Tex). Ribonucleic acid extraction was performed with the RiboPure-blood kit (Applied Biosystems/Ambion) per the manufacturer's recommendation, including the DNase digestion step to remove residual deoxyribonucleic acid (DNA). Ribonucleic acid quantity and quality were determined using spectrophotometry, for which a 260/280 ratio >1.8 was deemed indicative of ribonucleic acid (RNA) of sufficient quality. In addition, we verified RNA integrity for a random selection of 5 samples with the BioAnalyzer (Agilent Technologies, Santa Clara, Calif).

Arrays

Inflammatory GE analysis was performed with a commercially available oligo GE array (OHS-021; SA Biosciences, Frederick, Md) containing 128 hybridization spots; 114 spots for cytokines and 14 spots for housekeeping genes, positive controls, and negative controls. Amplification and labeling of RNA was performed according to the TrueAMP 2.0 kit protocol (SA Biosciences). Complementary RNA was hybridized for 18 hours on the array. Chemiluminescent signal detection took place using a Chem-iDoc XRS system (Bio-Rad Laboratories, Hercules, Calif), and quantification of spot intensities was conducted using the GEArray Analysis Suite (SA Biosciences). Spot intensities were corrected for the background signal by subtracting from each individual spot intensity the average value of all spot intensities per array. Gene expression values reported are fold changes (ΔGE), where values <1.0 represent a downregulation and values >1.0 represent upregulation of the gene.

Reverse Transcription–Polymerase Chain Reaction (RT-PCR)

Based on between-treatment group differences in ΔGE, 8 genes were selected for confirmation with 2-step reverse transcriptase real-time polymerase chain reaction (PCR). Assays were developed in our laboratory (Table E1). Complementary DNA was constructed using RNA applied to the arrays. Reverse transcription was performed with the SuperScript II reverse transcriptase, using random primers (Invitrogen, Carlsbad, Calif). The 7500 Fast RT-PCR (Applied Biosystems, Foster City, Calif) was used on standard modus and standard program with the GE master mix (Applied Biosystems). Each sample was run 4 times. In each run, a reference sample, consisting of pooled complementary DNA of 6 volunteer blood donors, was taken along in 4 fold. All RT-PCR output threshold cycle values were corrected for this reference sample in the same run.

Clinical Evaluation and Measurements

Preoperative baseline characteristics such as age, weight, gender, logistic EuroSCORE, use of medication, and relevant comorbidity were recorded. The preoperative laboratory investigation included a full coagulation and white blood cell profile. Intraoperatively and 24 hours postoperatively, the type of surgery, operation time, CPB time, crossclamp time,

blood product use, and maximum white blood cell count were recorded. To evaluate the clinical impact of a changed postoperative inflammatory profile, the incidence of the systemic inflammatory response syndrome and MOF was evaluated on a daily basis during the complete period of recovery in the intensive care unit, using the systemic inflammatory response syndrome criteria proposed by the American College of Chest Physicians[15] and the MOF criteria by Knaus and colleauges.[16] All intra- and postoperative measurements were done by caretakers blinded to treatment group assignment.

Statistical Analysis

Clinical data were expressed as median ± interquartile range. Demographic and perioperative clinical characteristics were compared among the 3 treatment groups using 1-way analysis of variance for continuous variables, and the χ^2 test for categoric variables. To test for differences between pre- and postoperative GE values, Wilcoxon signed rank tests were performed. Mann-Whitney *U* tests were performed for between-group comparisons. In addition, for the RT-PCR data, Mann-Whitney *U* tests were performed to test the difference in RQr among the treatment groups. *P* values were calculated using 2-sided tests and were considered significant when <.05. The Benjamini-Hochberg correction was used to correct for the problem of multiple testing during the multiple comparison gene analysis. Statistical analysis was performed using PASW Statistics software, version 17.0.0 (SPSS, Inc, Chicago, Ill).

RESULTS

Patients

Patient demographics are described in Table 1. Preoperative white blood cell count, platelet count, and coagulation profiles were within the normal range for all patients (data not shown). Patients were matched with respect to age, gender, CPB time, the number of blood products transfused, intraoperative use of steroids, and the incidence of postoperative MOF. No substantial differences in terms of the type of surgery performed, the operation time, the time on CPB, and the myocardial arrest time were detected. In the postoperative patient variables, an independent lower maximum leukocyte count was seen in patients treated with either tranexamic acid ($P = .040$) or aprotinin ($P = .018$), with no important difference between them. No ischemic stroke or seizures were seen.

Arrays

Quantity and quality of RNA was good for all samples (data not shown). After hybridization and subtraction of the background signal, 39 of 114 genes on the array differed with respect to pre- and postoperative GE across 1 of the 3 treatment groups, with 23 genes showing significant changes between pre- and postoperative GE levels in at least 1 of the groups. Eleven of the 23 genes showed important between-group differences when compared with placebo: 8 genes showed differences in the aprotinin group only (growth differentiation factor [GDF] 3, interleukin [IL]-19, IL-1 family member 7, vascular endothelial growth factor α downregulated, transforming growth factor [TGF]-α, tumor necrosis factor superfamily [TNFsf] 10, TNFsf12, TNFsf13 upregulated), whereas 3 genes (FMS-related tyrosine kinase 3 ligand downregulated, GDF-5, and interferon [IFN]-α8 upregulated) showed differences in both aprotinin- and tranexamic acid-treated patients. Two genes (platelet-derived growth factor α and TNFsf13 upregulated) showed a trend ($P < .10$) toward independence when aprotinin-treated patients were compared with placebo (data are described in Table E2).

Gene Selection for RT-PCR Confirmation

Based on functional relevance and the amount of up- or downregulation (Table E2), 6 genes were selected for confirmation with RT-PCR: FLT3-ligand, GDF-5, IFN-α8, TGF-α1, TNFsf10, and TNFsf13. We further selected 4 additional genes: CD40 ligand, TNF, IL-8, and TNFsf13b. CD40 ligand was chosen for its implication in platelet activation and reports on diminished platelet activation in aprotinin-treated patients.[17] Interleukin 8 had our special interest because array data indicated postoperative downregulation in all patient groups, whereas increased plasma levels have been related to patient outcome after cardiac surgery.[18,19] Tumor necrosis factor and TNFsf13B were further chosen for their close functional connection to TNFsf13.

Confirmation RT-PCR

Reverse transcription-PCR assays were developed successfully for 8 selected genes. Unfortunately, GDF-5 and IFN-α8 GE could not be confirmed with RT-PCR because of technical difficulties during assay development. Results of the RT-PCR confirmation are presented in Table 2. Reverse transcription-PCR results were remarkably similar to GEArray results, the only difference being more pronounced upregulation in RT-PCR-confirmed upregulated genes. No independent ΔGE for IL-8, TNFsf10, and TNFsf13B could be found for patients treated with aprotinin. Also, TNF GE did not change substantially in tranexamic acid-treated patients. When we tested for between-group differences (placebo vs tranexamic acid or aprotinin), TGF upregulation was less for patients treated with aprotinin (ΔGE 1.99 vs 5.37, respectively; $P = .019$). The already noted unchanged GE of TNFsf10 in aprotinin-treated patients was confirmed and proved relevant when compared with placebo (median ΔGE, 0.99 vs 1.98; $P = .042$). For TNFsf13, a trend toward independently lower upregulation in GE was seen in patients treated with aprotinin (ΔGE, 1.68 vs 2.47; $P = .074$). None of the genes tested on RT-PCR showed important differences between patients treated with tranexamic acid and placebo.

Differences in Array GE Between Patients Developing MOF Versus Non-MOF Patients

We tested further for differences in GEArray patterns of patients developing MOF (n = 16) compared with patients not developing MOF (n = 19). Seventeen of the 39 genes detectable on the array showed no substantial up- or downregulation after surgery, leaving 22 genes for further

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TABLE 1. Demographic and clinical data of 35 patients

Characteristic	Placebo (n = 12)	Tranexamic acid (n = 12)	Aprotinin (n = 11)	*P* value
Age, years	61.4 (53.3-73.4)	65.3 (54.7-75.3)	68.6 (61.0-73.6)	.819
Gender, male/female, n	11/1	11/1	8/3	.331
EuroSCORE, %	2.5 (1.0-3.3)	2.6 (1.6-5.4)	3.3 (1.5-4.5)	
Left ventricular ejection fraction, n				.585
Moderate	5	5	3	
Poor	1	0	0	
Diabetes mellitus, n				.621
Type 1	0	1	0	
Type 2	1	1	2	
Preoperative medication, n				
Acetylsalicylic acid	7	6	5	.820
Calcium antagonists	2	2	0	.355
Statins	6	8	7	.676
Operation				
Type of surgery, n				
CABG only	5	5	3	.716
Valve only	5	6	7	.570
CABG and valve	2	1	1	.777
Duration of myocardial arrest, minutes	121 (68-169)	99 (91-138)	100 (70-172)	.840
Duration of CPB, minutes	167 (123-222)	139 (114-205)	152 (119-206)	.712
Corticosteroids, n	3	2	3	.813
Operation and 24 h postoperative				
PRBC, units/patients transfused	21/8	8/6	7/5	.586
FFP, units/patients transfused	13/5	5/2	8/4	.460
Platelets, units/patients transfused	3/3	3/2	2/2	.904
Highest leukocyte count, g/dL	16.1 (12.8-19.6)	12.5 (11.0-13.1)	11.1 (10.3-13.1)	.027
Total ICU stay				
Use of CVVH	1	2	0	.344
Use of IABP	0	2	0	.104
Myocardial infarction	0	2	0	.104
Incidence of SIRS, n	12	12	11	.388
Incidence of MOF, n	6	5	5	.574
Mortality, n	1	0	0	.373

Data are reported as median (interquartile range). *CABG*, Coronary artery bypass grafting; *CPB*, cardiopulmonary bypass; *PRBC*, packed red blood cell; *FFP*, fresh frozen plasma; *ICU*, intensive care unit; *CVVH*, continuous veno-venous hemofiltration; *IABP*, intra-aortal balloon pump; *SIRS*, systemic inflammatory response syndrome; *MOF*, multi-organ failure; *EuroSCORE,* European System for Cardiac Operative Risk Evaluation.

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analysis. Gene expression IL-12a, IL-17b, inhibin α, and inhibin-β subunit A proved importantly altered after surgery, an effect not seen in the analysis of antifibrinolytic mediated GE. In contrast to non-MOF patients, MOF patients showed unchanged GE inhibin α, inhibin-β subunit A, and platelet-derived growth factor β 24 hours after surgery, whereas IL-12a, IL-17b, and TNFsf10 GE was changed. However, direct comparisons between MOF and non-MOF patients yielded no independent differences (data are described in Table E3).

The Effect of Perioperative Corticosteroids on Array GE

Last, we tested for the effect of intraoperative administration of corticosteroids on inflammatory GE. A total of 8 patients (23%) received steroids intraoperatively, equally distributed across the 3 treatment groups ($P = .813$). Of the 39 detectable genes, 20 genes had a substantially altered GE in either corticosteroid- or noncorticosteroid-treated patients (data are described in Table E5). Again, overall differences in GE were less (ΔGE range, 0.46-1.73) than the differences observed in patients treated with either antifibrinolytic; only lymphotoxin-β and TNFsf13 expression differed, with stronger lymphotoxin-β downregulation in the corticosteroid group (ΔGE, 0.66 vs 0.85; $P = .003$), and less TNFsf13 upregulation (1.18 vs 1.27, $P = .023$).

DISCUSSION

The effects of aprotinin and tranexamic acid on surgical bleeding have been studied extensively, with reports indicating aprotinin to be most effective,[20] whereas others conclude there is hardly any difference between them.[21] More important is the issue of whether the use of antifibrinolytics is clinically advantageous. The contrary may be true; several studies reported higher incidences of complications after the use of aprotinin[7-9] and tranexamic acid.[22]

TABLE 2. ⊿GE RT-PCR

Gene	Group	n	⊿GE, median (IQR)	*P* value pre- vs post	*P* value vs placebo
CD40lg	PL	12	0.39 (0.26-0.51)	.002	
	TX	12	0.39 (0.15-0.62)	.008	
	AP	11	0.24 (0.11-0.37)	.006	
FLT3lg	PL	12	0.26 (0.15-0.37)	.002	
	TX	12	0.34 (0.13-0.54)	.003	.133
	AP	11	0.34 (0.20-0.47)	.003	
IL-8	PL	12	0.28 (−0.02-0.58)	.019	
	TX	12	0.31 (−0.42-0.66)	.012	
	AP	11	0.72 (0.14-1.30)	.182	
TGF-α1	PL	12	5.37 (3.09-7.66)	.002	
	TX	12	4.05 (1.33-6.78)	.002	
	AP	11	1.99 (0.66-3.33)	.008	.019
TNF	PL	12	0.71 (0.51-0.92)	.005	
	TX	12	0.78 (0.36-1.19)		
	AP	11	0.62 (0.49-0.75)	.010	
TNFsf10	PL	12	1.98 (1.16-2.80)	.012	
	TX	12	2.10 (1.35-2.85)	.008	
	AP	11	1.00 (0.38-1.61)		.042
TNFsf13	PL	12	2.47 (1.13-3.81)	.003	
	TX	12	1.99 (1.06-2.92)	.002	
	AP	11	1.68 (1.01-2.35)	.075	.074
TNFsf13B	PL	12	4.98 (2.51-7.44)	.004	
	TX	12	5.20 (0.62-9.79)	.002	
	AP	11	3.65 (1.02-6.28)	.004	

The differences in ⊿GE after surgery between placebo and either tranexamic acid or aprotinin were tested with the Mann-Whitney *U* test. Only *P* values <.20 are reported here. *GE*, Gene expression; *RT-PCR*, reverse transcription–polymerase chain reaction; *IQR*, interquartile range; *CD40lg*, CD40 ligand; *FLT3lg,* FMS-like tyrosine kinase 3 ligand; *PL*, placebo; *TX*, tranexamic acid; *AP*, aprotinin; *IL*, interleukin; *TGF*, transforming growth factor; *TNF*, tumor necrosis factor; *TNFsf*, TNF super family member.

Anti-inflammatory properties of both aprotinin and tranexamic acid have been observed in studies evaluating plasma cytokine concentrations.[23-26] Seen as messengers orchestrating the immune response, cytokines play a key role in the inflammatory response after cardiac surgery. Here we used the approach to evaluate the immune modulating properties of antifibrinolytics by studying protein synthesis at its earliest level—namely, that of transcription of the gene. This study compared the effects of tranexamic acid and aprotinin on the genomic expression level in cardiac surgery. We used an inflammatory pathway RNA gene expression array to identify unique profiles related to the use of antifibrinolytics. The results of RT-PCR confirmed the array technique, indicating that the GEArray is a suitable tool for screening for genes of interest.

We found that both antifibrinolytics alter inflammatory GE; less formation of macrophage colonies is reflected by a diminished upregulation of colony stimulating factor. Tranexamic acid and aprotinin induce a deviation toward a stronger humoral immune response through independently lower expression of IFN-α8, further aided by less downregulation of FMS-related tyrosine kinase 3 ligand. Common antifibrinolytic effects were also seen; aprotinin and tranexamic acid induce less upregulation of GDF-5, involved in both plasminogen activity and migration of endothelial cells. The independently lowered TGF-α1 expression is possibly a result of lower thrombin concentrations, and it reaches independence in the aprotinin group only, perhaps through aprotinin's additional inhibition of chemotrypsin. Last, we noticed upregulation of vascular endothelial growth factor α involved in synthesis of antiplasminogen activator, thus reducing fibrinolysis and clot preservation. However, most effects on GE profiles were seen in aprotinin-treated patients only with upregulation of IL-19, a cytokine with properties similar to anti-inflammatory IL-10, and IL-1 family member 7, thereby diminishing induction of proinflammatory IFN-γ. Only aprotinin-treated patients lack upregulation of a whole range of TNFsf subclass proteins involved in apoptosis induction, B-cell proliferation, and proinflammatory cellular signaling pathways. Last, aprotinin-treated patients exhibit lower upregulation of platelet-derived growth factor α, reflecting less platelet activation, thrombin generation, and chemotaxis of monocytes and neutrophils.

Altogether, the use of tranexamic acid and aprotinin led to less upregulation of proinflammatory genes and more upregulation of anti-inflammatory genes. Considering the GE impacted exclusively in the aprotinin group, we found these to include genes coding for proteins that function through serine proteases and genes with important anti-inflammatory functioning.

Neither this study, nor the original randomized, controlled trial from which this patient group was derived, was powered to detect independent between-group differences in clinical outcomes. However, analysis of GE array profiles revealed differences between patients who developed MOF and patients who did not. Gene expression altered exclusively in MOF patients includes downregulation of IL-12α, a cytokine that augments the cytolytic activity of natural killer cells, and IL-17β, depressing the release of TNF-α and IL-1β (Table E3). We further noticed downregulation of inhibin-β A, released concurrently with TNF-α early in sepsis, prior to IL-6.[27] Although these differences were independent when compared with baseline values, no independent differences between MOF and non-MOF patients were seen.

Our results provide insight into the cellular mechanisms through which tranexamic acid and aprotinin exert their immune modulating effects. We identified several genetic pathways expressed differentially in patients receiving either tranexamic acid or aprotinin, with statistical independence when tested against placebo. Furthermore, several GE profiles were unique for the use of aprotinin only, with most effects induced by aprotinin's serine protease inhibition. Our study demonstrates these associations despite the small sample size, reflecting the strength of the relationship between antifibrinolytic status and particular pathway regulation.

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However, our study has limitations, too. Although patient groups were well matched according to use of corticosteroids, type of surgery, operation duration, and other relevant patient characteristics, this study lacked true randomization to the intraoperative use of corticosteroids. An additional analysis of the effects of corticosteroids on GE, however, showed only limited effects. Second, our small sample size makes it difficult to determine whether the attenuation of the inflammatory gene expression by antifibrinolytics results in clinical better recovery. Third, one can argue whether transfusions are responsible for the favorable inflammatory response seen in antifibrinolytic-treated patients. An additional analysis concentrating on GE profiles between transfused and nontransfused patient indicated small nonindependent differences (Table E4). Last, the relatively long time frame during which we studied alterations in GE might be responsible for the relatively limited number of genes with altered postoperative GE, because many plasma proteins can be measured only shortly after cardiac surgery. The fact that GE is affected 24 hours after randomization to placebo, tranexamic acid, or aprotinin, however, indicates the powerful and long-lasting effects of the use of antifibrinolytics in cardiac surgery.

CONCLUSIONS

This study demonstrates that the use of tranexamic acid and aprotinin alters inflammatory GE profiles. We further demonstrate that the use of aprotinin leads to independent attenuation of the immune response, with several inhibitory effects restricted to the use of aprotinin only, and not observed in tranexamic acid-treated patients. It is unclear from these results whether this modulated immune response plays a causal role in presumed beneficial or detrimental clinical effects after surgery. However, better understanding of the targets of these drugs enable possible adjustment of pharmacologic properties, allowing for more precise alteration of hemostasis after cardiac surgery. Our results can add further to the discussion on which antifibrinolytic, if any, can best be used in the cardiac surgical patient.

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References

1. Henry DA, Moxey AJ, Carless PA, O'Connell D, McClelland KM, Henderson KM, et al. Anti-fibrinolytic use for minimising perioperative allogeneic blood transfusion. *Cochrane Database Syst Rev*. 1999;4:CD001886.
2. McEvoy MD, Reeves ST, Reves JG, Spinale FG. Aprotinin in cardiac surgery: a review of conventional and novel mechanisms of action. *Anesth Analg*. 2007;105:949-62.
3. Levi M, Van Der Poll T. Inflammation and coagulation. *Crit Care Med*. 2010;38: S26-34.
4. Day JR, Taylor KM, Lidington EA, Mason JC, Haskard DO, Randi AM, et al. Aprotinin inhibits proinflammatory activation of endothelial cells by thrombin through the protease-activated receptor 1. *J Thorac Cardiovasc Surg*. 2006; 131:21-7.
5. Hsia TY, McQuinn TC, Mukherjee R, Deardorff RL, Squires JE, Stroud RE, et al. Effects of aprotinin or tranexamic acid on proteolytic/cytokine profiles in infants after cardiac surgery. *Ann Thorac Surg*. 2010;89:1843-52.
6. Graham EM, Atz AM, Gillis J, Desantis SM, Haney AL, Deardorff RL, et al. Differential effects of aprotinin and tranexamic acid on outcomes and cytokine profiles in neonates undergoing cardiac surgery. *J Thorac Cardiovasc Surg*. 2012;143:1069-76.
7. Karkouti K, Beattie WS, Dattilo KM, McCluskey SA, Ghannam M, Hamdy A, et al. A propensity score case–control comparison of aprotinin and tranexamic acid in high-transfusion-risk cardiac surgery. *Transfusion*. 2006;46:327-38.
8. Mangano DT, Miao Y, Vuylsteke A, Tudor IC, Juneja R, Filipescu D, et al. Mortality associated with aprotinin during 5 years following coronary artery bypass graft surgery. *JAMA*. 2007;297:471-9.
9. Fergusson DA, Hebert PC, Mazer CD, Fremes S, MacAdams C, Murkin JM, et al. A comparison of aprotinin and lysine analogues in high-risk cardiac surgery. *N Engl J Med*. 2008;358:2319-31.
10. Later AF, Maas JJ, Engbers FH, Versteegh MI, Bruggemans EF, Dion RA, et al. Tranexamic acid and aprotinin in low- and intermediate-risk cardiac surgery: a non-sponsored, double-blind, randomised, placebo-controlled trial. *Eur J Cardiothorac Surg*. 2009;36:322-9.
11. Robertshaw HJ. An anti-inflammatory role for tranexamic acid in cardiac surgery? *Crit Care*. 2008;12:105.
12. DeAnda A Jr, Spiess BD. Aprotinin revisited. *J Thorac Cardiovasc Surg*. 2012; 144:998-1002.
13. Royston D, Bidstrup BP, Taylor KM, Sapsford RN. Effect of aprotinin on need for blood transfusion after repeat open-heart surgery. *Lancet*. 1987;2:1289-91.
14. Dowd NP, Karski JM, Cheng DC, Carroll JA, Lin Y, James RL, et al. Pharmacokinetics of tranexamic acid during cardiopulmonary bypass. *Anesthesiology*. 2002;97:390-9.
15. American College of Chest Physicians/Society of Critical Care Medicine Conference. definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Chest*. 1992;101:1644-55.
16. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. Prognosis in acute organ-system failure. *Ann Surg*. 1985;202:685-93.
17. Mengistu AM, Rohm KD, Boldt J, Mayer J, Suttner SW, Piper SN. The influence of aprotinin and tranexamic acid on platelet function and postoperative blood loss in cardiac surgery. *Anesth Analg*. 2008;107:391-7.
18. Calafiore AM, Teodori G, Di GG, Bosco G, Mezzetti A, Lapenna D, et al. Intermittent antegrade cardioplegia: warm blood vs cold crystalloid: a clinical study. *J Cardiovasc Surg (Torino)*. 1994;35:179-84.
19. Sablotzki A, Friedrich I, Muhling J, Dehne MG, Spillner J, Silber RE, et al. The systemic inflammatory response syndrome following cardiac surgery: different expression of proinflammatory cytokines and procalcitonin in patients with and without multiorgan dysfunctions. *Perfusion*. 2002;17:103-9.
20. Porte RJ, Leebeek FW. Pharmacological strategies to decrease transfusion requirements in patients undergoing surgery. *Drugs*. 2002;62:2193-211.
21. Casati V, Guzzon D, Oppizzi M, Bellotti F, Franco A, Gerli C, et al. Tranexamic acid compared with high-dose aprotinin in primary elective heart operations: effects on perioperative bleeding and allogeneic transfusions. *J Thorac Cardiovasc Surg*. 2000;120:520-7.
22. Murkin JM, Falter F, Granton J, Young B, Burt C, Chu M. High-dose tranexamic acid is associated with nonischemic clinical seizures in cardiac surgical patients. *Anesth Analg*. 2010;110:350-3.
23. Pruefer D, Makowski J, Dahm M, Guth S, Oelert H, Darius H, et al. Aprotinin inhibits leukocyte–endothelial cell interactions after hemorrhage and reperfusion. *Ann Thorac Surg*. 2003;75:210-5.
24. Greilich PE, Brouse CF, Whitten CW, Chi L, Dimaio JM, Jessen ME. Antifibrinolytic therapy during cardiopulmonary bypass reduces proinflammatory cytokine levels: a randomized, double-blind, placebo-controlled study of epsilon-aminocaproic acid and aprotinin. *J Thorac Cardiovasc Surg*. 2003;126: 1498-503.
25. Tassani P, Augustin N, Barankay A, Braun SL, Zaccaria F, Richter JA. High-dose aprotinin modulates the balance between proinflammatory and anti-inflammatory responses during coronary artery bypass graft surgery. *J Cardiothorac Vasc Anesth*. 2000;14:682-6.
26. Jimenez JJ, Iribarren JL, Lorente L, Rodriguez JM, Hernandez D, Nassar I, et al. Tranexamic acid attenuates inflammatory response in cardiopulmonary bypass surgery through blockade of fibrinolysis: a case control study followed by a randomized double-blind controlled trial. *Crit Care*. 2007;11:R117.
27. Jones KL, de Kretser DM, Patella S, Phillips DJ. Activin A and follistatin in systemic inflammation. *Mol Cell Endocrinol*. 2004;225:119-25.

APPENDIX E1.

The primer search was conducted with Primer express software (version 2.0; Applied Biosystems, Inc, Foster City, Calif). To confirm the uniqueness of the primer sequences for the gene of interest, a nucleotide blast was performed at http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi?PAGE=Nucleotides&PROGRAM=blastn&MEGABLAST=on&BLAST_PROGRAMS=megaBlast&PAGE_TYPE=BlastSearch&SHOW_DEFAULTS=on.

Genes and sequences are depicted in Table E1. All probes were labeled with fluorescein amidite dye and had nonfluorescent minor groove binder quenchers.

TABLE E1. Sequences of primers and probes for the reverse transcription–polymerase chain reaction

Selected genes	REFSEQ ID	Forward primer	Reverse primer	Probe
CD40lg	NM_000074	CCA GGT GCT TCG GTG TTT GT	CCA GTG CCA TGG CTC ACT T	AAT GTG ACT GAT CCA AGC
FLT3lg	NM_001459	TGG AGC GGC TCA AGA CTG T	TTC ACG CGC TCC AGC AA	TGG GTC CAA GAT GC
IL-8	NM_000584	CAC CGG AAG GAA CCA TCT CA	AGA GCC ACG GCC AGC TT	TGT GTG TAA ACA TGA CTT C
TGF-α	NM_003236	TCC CTT GGG CCA GAT ATG TG	TCC GTT GAT TGG TCT CTA AGC A	TTG AGG CTT GAC TGT AGC AT
TNF	NM_000594	CTT TGG GAT CAT TGC CCT GT	GGA GGC GTT TGG GAA GGT T	AGG AGG ACG AAC ATC C
TNFsf10	NM_003810	GCT CTG GGC CGC AAA AT	AGG AAT GAA TGC CCA CTC CTT	ACT CCT GGG AAT CAT
TNFsf13	NM_003808	GCA GGA ACA GAG GCG TCT TC	TGG GAA TGA AAA GGG AAA AGT G	TTT GGC TCC CCG TTC C
TNFsf13B	NM_006573	GGC CCC AAC CTT CAA AGT TC	GCG TGA CTG CTC CCT TTC TG	AGT AGT GAT ATG GAT GAC TCC

CD40lg, CD40 ligand; *FLT3lg*, FMS-like tyrosine kinase 3 ligand; *IL-8*, interleukin 8; *TGF-α*, transforming growth factor alpha; *TNF*, tumor necrosis factor; *TNFsf*, TNF super family member; *REFSEQ ID,* NCBI reference sequence ID.

TABLE E2. Fold changes in cytokine gene expression on the array for patients receiving placebo (PL), tranexamic acid (TX), and aprotinin (AP)

Gene	Medication group	n	RQ, median (IQR)	*P* value, pre vs post	*P* value vs group 1
CSF1	PL	12	0.89 (0.85-1.01)	.005	
	TX	12	0.96 (0.86-1.07)	NS	NS
	AP	11	1.06 (0.79-1.21)	NS	NS
CD40lg	PL	12	0.68 (0.58-0.78)	.004	
	TX	12	0.77 (0.58-0.90)	.002	NS
	AP	11	0.71 (0.57-0.84)	.004	NS
FLT3lg	PL	12	0.52 (0.43-0.59)	.002	
	TX	12	0.72 (0.51-0.91)	.003	.033
	AP	11	0.69 (0.56-0.76)	.021	.027
GDF3	PL	12	0.93 (0.83-1.24)	NS	
	TX	12	1.02 (0.79-1.19)	NS	NS
	AP	11	1.24 (1.05-1.41)	.033	.049
GDF5	PL	12	2.23 (1.64-3.44)	.002	
	TX	12	1.38 (1.07-1.76)	.005	.013
	AP	11	1.11 (1.06-1.48)	.003	.002
IFN-α8	PL	12	1.32 (1.13-1.40)	.002	
	TX	12	1.08 (1.02-1.19)	.010	.008
	AP	11	0.98 (0.94-1.07)	NS	.001
TXLN-α	PL	12	0.96 (0.82-1.08)	NS	
	TX	12	1.01 (0.86-1.10)	NS	NS
	AP	11	0.88 (0.76-0.97)	.033	NS
IL-19	PL	12	0.95 (0.86-1.19)	NS	
	TX	12	1.07 (0.92-1.21)	NS	NS
	AP	11	1.19 (1.01-1.44)	.026	.049
IL-1β	PL	12	1.10 (0.95-1.36)	.041	
	TX	12	1.24 (1.01-1.35)	.006	NS
	AP	11	1.33 (0.90-1.53)	.026	NS
IL-1F7	PL	12	0.97 (0.90-1.10)	NS	
	TX	12	1.05 (0.93-1.16)	NS	NS
	AP	11	1.14 (1.02-1.26)	.033	.027
IL-8	PL	12	0.50 (1.80-0.91)	.034	
	TX	12	0.43 (0.23-0.61)	.015	NS
	AP	11	0.58 (0.38-0.69)	.041	NS
LT-β	PL	12	0.86 (0.63-1.00)	.012	
	TX	12	0.79 (0.59-1.17)	.028	NS
	AP	11	0.83 (0.64-1.15)	.041	NS
PDGF α	PL	12	1.23 (1.13-1.33)	.004	
	TX	12	1.12 (1.06-1.24)	.019	NS
	AP	11	1.10 (1.07-1.27)	.013	NS
TGF-α	PL	12	1.60 (1.51-1.85)	.003	
	TX	12	1.43 (1.17-1.60)	.002	NS
	AP	11	1.16 (0.98-1.39)	.016	.014
TNF	PL	12	0.87 (0.76-0.98)	.015	
	TX	12	0.92 (0.83-1.11)	NS	NS
	AP	11	0.75 (0.67-0.94)	.016	NS
TNFsf10	PL	12	1.40 (1.07-1.73)	.041	
	TX	12	1.36 (1.18-1.61)	.010	NS
	AP	11	0.93 (0.68-1.34)	NS	.036
TNFsf12	PL	12	1.21 (0.99-1.47)	.028	
	TX	12	1.04 (0.85-1.14)	NS	NS
	AP	11	0.92 (0.77-1.03)	NS	.003
TNFsf13	PL	12	1.21 (1.08-1.35)	.006	
	TX	12	1.18 (1.06-1.26)	.015	NS
	AP	11	1.08 (0.93-1.21)	NS	NS
TNFsf13b	PL	12	1.87 (1.24-4.00)	.002	
	TX	12	1.72 (1.49-2.27)	.002	NS
	AP	11	1.24 (0.74-1.67)	NS	.027
TNFsf14	PL	12	1.27 (1.12-1.45)	.005	
	TX	12	1.34 (1.04-1.52)	.023	NS
	AP	11	1.07 (1.05-1.27)	.021	NS
TNFsf9	PL	12	0.71 (0.41-1.08)	.034	
	TX	12	0.68 (0.57-0.95)	.023	NS
	AP	11	0.75 (0.65-0.98)	.016	NS
VEGF α	PL	12	0.93 (0.90-0.98)	.019	
	TX	12	1.02 (0.90-1.08)	NS	NS
	AP	11	1.03 (0.93-1.08)	NS	.036
VEGF β	PL	12	0.87 (0.72-0.93)	.034	
	TX	12	0.84 (0.72-1.07)	.023	NS
	AP	11	0.70 (0.67-0.88)	.006	NS

Gene expression (GE) values on the array. Pre- and postoperative values are expressed as fold changes compared with the average expression on each array. The difference in GE values are fold changes after surgery compared with before surgery. *CSF1*, Colony stimulating factor-1; *RQ*, relative quantitation; *IQR*, interquartile range; *CSF*, cerebrospinal fluid; *PL*, placebo; *TX*, tranexamic acid; *AP*, aprotinin; *NS*, not significant; *CD40lg*, CD40 ligand; *FLT3lg*, FMS-like tyrosine kinase 3 ligand; *GDF*, growth differentiation factor; *IFN*, interferon; *TXLN*, taxilin; *IL*, interleukin; *LT*, lymphotoxin; *PDGF*, platelet-derived growth factor; *TGF*, transforming growth factor; *TNF*, tumor necrosis factor; *TNFsf*, TNF super family member; *VEGF*, vascular endothelial growth factor.

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TABLE E3. Fold changes in cytokine gene expression on the array for patients developing and not developing MOF

Gene	MOF	n	RQ, median (IQR)	*P* value, pre vs post	Between-group *P* value
FLT3lg	No	19	0.67 (0.51-0.84)	.001	
	Yes	16	0.55 (0.49-0.69)	.000	NS
GDF5	No	19	1.38 (0.11-1.85)	.000	NS
	Yes	16	1.56 (1.11-3.44)	.001	
IFN-α8	No	19	1.07 (1.00-1.15)	.003	NS
	Yes	16	1.13 (1.07-1.36)	.005	
IL-12α	No	19	1.00 (0.94-1.15)	NS	NS
	Yes	16	0.94 (0.91-1.02)	.023	
TXLN-α	No	19	0.59 (0.80-1.04)	.018	NS
	Yes	16	0.99 (0.88-1.08)	NS	
IL-17β	No	19	0.95 (0.86-1.23)	NS	NS
	Yes	16	0.92 (0.76-1.02)	.049	
IL-1β	No	19	1.17 (0.98-1.35)	.003	NS
	Yes	16	1.21 (0.94-1.43)	.011	
IL-8	No	19	0.50 (0.24-0.64)	.004	NS
	Yes	16	0.48 (0.23-0.67)	.011	
INH-α	No	19	1.08 (1.01-1.16)	.013	NS
	Yes	16	1.06 (0.97-1.17)	NS	
INH-βA	No	19	1.04 (0.99-1.16)	.040	NS
	Yes	16	0.95 (1.12-1.07)	NS	
LT-β	No	19	0.83 (0.74-1.15)	.027	NS
	Yes	16	0.77 (0.57-0.95)	.001	
PDGF-α	No	19	1.22 (1.09-1.30)	.001	NS
	Yes	16	1.13 (1.07-1.24)	.002	
PDGF-β	No	19	1.10 (0.99-1.30)	.013	NS
	Yes	16	1.04 (0.84-1.21)	NS	
TGF-α	No	19	1.30 (1.11-1.57)	.001	NS
	Yes	16	1.49 (1.88-1.73)	.001	
TNF	No	19	0.90 (0.80-1.00)	.016	NS
	Yes	16	0.82 (0.70-1.02)	.010	
TNFsf10	No	19	1.30 (0.92-1.46)	NS	NS
	Yes	16	1.34 (1.06-1.69)	.034	
TNFsf13	No	19	1.18 (1.00-1.23)	.008	NS
	Yes	16	1.14 (1.07-1.26)	.001	
TNFsf13B	No	19	1.49 (1.07-1.76)	.001	NS
	Yes	16	1.81 (1.29-3.10)	.002	NS
TNFsf14	No	19	1.24 (1.04-1.53)	.005	
	Yes	16	1.17 (1.06-1.41)	.001	NS
CD40lg	No	19	0.69 (0.55-0.80)	.000	
	Yes	16	0.73 (0.61-0.92)	.001	NS
TNFsf9	Yes	19	0.74 (0.55-1.11)	.011	
	No	16	0.65 (0.47-0.86)	.000	NS
VEGF β	Yes	19	0.83 (0.70-0.91)	.005	
	No	16	0.81 (0.69-1.05)	.023	NS

Gene expression (GE) values on the array. Pre- and postoperative values are expressed as fold changes compared with the average expression on each array. The difference in GE values are fold changes after surgery compared with before surgery. *MOF*, Multiorgan failure; *RQ*, relative quantitation; *IQR*, interquartile range; *FLT3LG*, FMS-like tyrosine kinase 3 ligand; *NS*, not significant; *GDF*, growth differentiation factor; *IFN*, interferon; *IL*, interleukin; *TXLN*, taxilin; *INH*, inhibin; *LT*, lymphotoxin; *PDGF*, platelet-derived growth factor; *TGF*, transforming growth factor; *TNF*, tumor necrosis factor; *TNFsf*, TNF super family member; *CD40lg*, CD40 ligand; *VEGF*, vascular endothelial growth factor.

TABLE E4. Fold changes in cytokine gene expression on the array for patients transfused and not transfused with either packed red blood cells, fresh frozen plasma, or thrombocytes

Gene	Transfused	n	RQ, median (IQR)	*P* value, pre vs post	Between-group *P* value
FLT3lg	No	14	0.73 (0.54-0.88)	.001	
	Yes	21	0.56 (0.48-0.70)	.000	NS
GDF3	No	14	1.03 (0.90-1.22)	.030	
	Yes	21	1.03 (0.79-1.33)	NS	NS
GDF5	No	14	1.48 (1.21-1.92)	.001	
	Yes	21	1.38 (1.08-2.36)	.000	NS
IFN-α8	No	14	1.08 (1.03-1.25)	.016	
	Yes	21	1.13 (0.98-1.32)	.001	NS
IL-19	No	14	1.07 (0.96-1.20)	.022	
	Yes	21	1.05 (0.84-1.25)	NS	NS
IL-1	No	14	1.25 (1.02-1.35)	.030	
	Yes	21	1.20 (0.96-1.43)	.002	NS
IL-1F7	No	14	1.04 (0.93-1.14)	.006	
	Yes	21	1.08 (0.93-1.18)	NS	NS
IL-8	No	14	0.52 (0.27-0.63)	.002	
	Yes	21	0.42 (0.21-0.85)	.013	NS
INH-α	No	14	1.04 (0.99-1.14)	.008	
	Yes	21	1.08 (0.98-1.16)	NS	NS
LT-β	No	14	0.88 (0.55-1.18)	.026	
	Yes	21	0.83 (0.65-0.95)	.002	NS
PDGF α	No	14	1.21 (1.03-1.25)	.004	
	Yes	21	1.22 (0.10-1.32)	.001	NS
PDGF β	No	14	0.99 (1.57-1.26)	.019	
	Yes	21	1.07 (0.88-1.20)	NS	NS
TGF-α	No	14	1.43 (1.19-1.65)	.005	
	Yes	21	1.41 (1.09-1.76)	.000	NS
TNF	No	14	0.88 (0.79-1.11)	NS	
	Yes	21	0.85 (0.72-0.99)	.003	NS
TNFsf10	No	14	1.32 (1.03-1.43)	NS	
	Yes	21	1.33 (0.82-1.75)	.005	NS
TNFsf13	No	14	1.14 (1.05-1.28)	.009	
	Yes	21	1.17 (1.03-1.26)	.001	NS
TNFsf13b	No	14	1.69 (1.47-2.00)	.004	
	Yes	21	1.50 (1.07-2.76)	.000	NS
TNFsf14	No	14	1.23 (1.05-1.62)	.019	
	Yes	21	1.11 (1.04-1.44)	.000	NS
CD40lg	No	14	0.79 (0.67-0.86)	.001	
	Yes	21	0.66 (0.55-0.77)	.000	NS
CD70	No	14	1.05 (0.97-1.11)	.035	
	Yes	21	1.03 (0.91-1.13)	NS	NS
TNFsf9	No	14	0.74 (0.58-1.02)	.008	
	Yes	21	0.70 (0.46-0.96)	.009	NS
VEGF β	No	14	0.84 (0.70-1.04)	.001	
	Yes	21	0.75 (0.69-0.89)	.007	NS

Gene expression (GE) values on the array. Pre- and postoperative values are expressed as fold changes compared with the average expression on each array. The difference in GE values are fold changes after surgery compared with before surgery. *RQ*, Relative quantitation; *IQR*, interquartile range; *FL3lg*, FMS-like tyrosine kinase 3 ligand; *NS*, not significant; *GDF*, growth differentiation factor; *IFN*, interferon; *IL*, interleukin; *INH*, inhibin; *LT*, lymphotoxin; *PDGF*, platelet-derived growth factor; *TGF*, transforming growth factor; *TNF*, tumor necrosis factor; *TNFsf*, TNF super family member; *CD40lg*, CD40 ligand; *VEGF*, vascular endothelial growth factor.

TABLE E5. Fold changes in cytokine gene expression on the array for patients that received corticosteroids intraoperatively and those who did not

Gene	Steroids	n	RQ, median (IQR)	*P* value, pre vs post	Between-group *P* value
CSF3	No	27	0.93 (0.87-0.99)	.037	
	Yes	8	1.03 (1.01-1.12)	NS	NS
FLT3lg	No	27	0.63 (0.51-0.83)	<.001	
	Yes	8	0.58 (0.36-0.69)	.012	NS
GDF5	No	27	1.48 (1.11-2.30)	<.001	
	Yes	8	1.35 (1.06-1.76)	.012	NS
IFN-α8	No	27	1.13 (1.03-1.33)	.001	
	Yes	8	1.05 (0.98-1.27)	.012	NS
TXLN-α	No	27	0.95 (0.80-1.08)	.022	
	Yes	8	0.91 (0.75-1.03)	NS	NS
IL-1β	No	27	1.28 (1.03-1.36)	<.003	
	Yes	8	1.04 (0.94-1.48)	NS	NS
IL-8	No	27	0.50 (0.24-0.64)	<.001	
	Yes	8	0.46 (0.22-1.84)	NS	NS
INH-α	No	27	1.05 (0.99-1.16)	NS	
	Yes	8	1.12 (1.04-1.23)	.036	NS
INH-β	No	27	1.01 (0.97-1.04)	NS	
	Yes	8	1.06 (0.96-1.16)	.017	NS
LT-β	No	27	0.85 (0.71-1.15)	.003	
	Yes	8	0.69 (0.54-0.82)	.012	.003
PDGF-α	No	27	1.13 (1.07-1.30)	<.001	
	Yes	8	1.20 (1.11-1.24)	.012	NS
TGF-α	No	27	1.42 (1.13-1.64)	<.001	
	Yes	8	1.40 (1.08-1.71)	.017	NS
TNF	No	27	0.91 (0.76-1.00)	.004	
	Yes	8	0.79 (0.72-0.85)	NS	NS
TNFsf10	No	27	1.35 (1.00-1.62)	.016	
	Yes	8	1.04 (0.74-1.32)	NS	NS
TNFsf13	No	27	1.18 (1.02-1.26)	.001	
	Yes	8	1.27 (1.06-1.30)	.025	.023
TNFsf13B	No	27	1.73 (1.37-2.70)	<.001	
	Yes	8	1.26 (0.75-1.50)	NS	NS
TNFsf14	No	27	1.24 (1.05-1.50)	<.001	
	Yes	8	1.12 (0.94-1.30)	NS	NS
CD40lg	No	27	0.94 (0.57-0.84)	<.001	
	Yes	8	0.64 (0.57-0.74)	.017	NS
TNFsf9	No	27	0.70 (0.53-0.98)	.001	
	Yes	8	0.81 (0.65-0.81)	.036	NS
VEGF-β	No	27	0.85 (0.68-1.00)	.001	
	Yes	8	0.71 (0.70-0.82)	.017	NS

RQ, Relative quantitation; *IQR*, interquartile range; *CSF*, cerebrospinal fluid; *NS*, not significant; *FL3lg*, FMS-like tyrosine kinase 3 ligand; *GDF*, growth differentiation factor; *IFN*, interferon; *TXLN*, taxillin; *IL*, interleukin; *INH*, inhibin; *LT*, lymphotoxin; *PDGF*, platelet-derived growth factor; *TGF*, transforming growth factor; *TNF*, tumor necrosis factor; *TNFsf*, TNF super family member; *CD40lg*, CD40 ligand; *VEGF*, vascular endothelial growth factor α.

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