

Response of Serum Proteome in Patients Undergoing Infrarenal Aortic Aneurysm Repair

Pietro Amedeo Modesti, M.D., Ph.D., Tania Gamberi, Sc.D.,† Cristina Bazzini, M.D.,‡ Marina Borro, Sc.D.,† Salvatore Mario Romano, Ph.D.,§ Giulia Elisa Cambi, Sc.D.,‡ Andrea Corvi, Eng.D.,‖ Walter Dorigo, M.D.,# Laura Paparella, M.D.,†† Carlo Pratesi, M.D.,** Marco Carini, M.D.,§§ GianFranco Gensini, M.D.,* Alessandra Modesti, Ph.D.‡‡*

Background: **Postoperative organ dysfunction in conventional surgery for abdominal aortic aneurysm (AAA) is associated with a complex inflammatory reaction, with activation of coagulation and fibrinolysis. A prospective,observational study was performed to define the complex plasma proteomic changes after AAA repair and to identify factor(s) that may affect myocardial function in uncomplicated procedures.**

Methods: **Ten patients undergoing infrarenal AAA repair were investigated. Eight subjects subjected to major abdominal surgery served as controls. Hemodynamic changes were continuously monitored by using the pressure recording analytical method technique. The time course of plasma proteins was investigated after induction of anesthesia and at different times after surgery (6 h, 12 h, 24 h, 36 h) by using two-dimensional difference gel electrophoresis, matrix-assisted laser desorption/ionization-time of flight mass spectrometry, and Western blot. The effects of plasma on the functional properties of isolated rat ventricular myocytes were also investigated.**

Results: **In AAA patients alone, 18 spots were found to change more than two-fold in expression level, spot identification revealing an increased thrombin generation 6 h after surgery. At the same time cardiac cycle efficiency significantly reduced *versus* baseline (−0.5 ± 0.9 *vs.* 0.18 ± 0.3 in AAA patients, $P < 0.01$; 0.4 ± 0.1 *vs.* 0.2 ± 0.3 in control surgery, not significant; $P < 0.01$ group × time interaction at ANOVA). Plasma obtained 6 h after AAA surgery dose-dependently inhibited contractile function of control rat myocytes (percent shortening fell by 51% with 10% of AAA plasma and was abolished with 20% of AAA plasma, $P < 0.001$ for both). The inhibitory response was abolished by thrombin antagonism.**

Conclusions: **These findings show for the first time the possible role of thrombin generation within the complex activation of inflammatory response in causing hemodynamic instability in the early postoperative period after AAA surgery.**

WITH the population steadily aging, the prevalence of high-risk patients among adults undergoing vascular surgery is increasing each year.[1] The different prospective indices for the prediction of perioperative cardiac risk in noncardiac surgery all include abdominal aortic operation as a major predictor of perioperative mortality and morbidity.[2,3] There is great interest both in understanding the causes and in optimizing perioperative treatment strategies in this clinical setting to minimize cardiovascular risk.

Postoperative organ dysfunction in conventional surgery for aortic aneurysm is associated with a particular biologic response strictly related to an ischemia/reperfusion injury.[4,5] After aortic surgery, a complex inflammatory reaction ensues, with activation of coagulation and fibrinolysis. In particular, the unwanted inflammatory response[6,7] has been reported to be associated with perioperative organ dysfunction,[4,8] whereas the extensive thrombin generation[9–11] is considered to be a major contributor to thrombotic complications occurring in the early postoperative period, including myocardial infarction, lower extremity and intestinal ischemia, stroke, and venous thrombosis.[12] However, the use of protamine to reverse the anticoagulant effects of heparin in patients undergoing vascular surgery has been reported to be associated with increased pulmonary artery pressure and heart rate and systemic vascular resistance and decrease in blood pressure and cardiac output.[13] The impaired myocardial oxygen consumption and function after protamine administration were found to be independent of myocardial ischemia.[14–16] Although protamine may directly affect isolated myocyte contractile function,[16] the hypothesis that thrombin may directly depress cardiac function might be considered. Experiments performed to investigate the effects of thrombin on the functional properties of cardiomyocytes yielded conflicting results, showing either an increase[17,18] or a reduction in myocyte contraction strength.[19,20]

The current study was therefore performed to investigate (1) serial changes in plasma proteome after abdominal aortic aneurysm repair, (2) the effects of plasma obtained from operated patients on rat myocyte contractility, and (3) the factor responsible for cryptic hemodynamic alterations.

Materials and Methods

Subjects Investigated

Two groups of patients scheduled for elective aortic reconstructive surgery for infrarenal abdominal aortic aneurysm repair (AAA, n = 10) or nonlaparoscopic elective abdominal surgery (gastrectomy, 1; intestinal resection, 1; nephrectomy, 6) (AS, n = 8), were investigated (table 1). The preoperative examination included phys-

* Professor of Internal Medicine, ‡ Research Fellow and Resident in Internal Medicine, § Assistant Professor of Medical Technologies, Department of Critical Care Medicine and Don C. Gnocchi Foundation, † Research Fellow, ‡‡ Associate Professor of Biology, Department of Biochemical Sciences, ‖ Professor of Biomechanics, Department of Industrial Mechanics and Technologies, # Assistant Professor of Vascular Surgery, ** Professor of Vascular Surgery, Department of Vascular Surgery, †† Anesthesiologist, §§ Professor of Urology, Department of Urology, University of Florence, Florence, Italy.

Received from Department of Critical Care Medicine, University of Florence, Florence, Italy. Submitted for publication February 2, 2009. Accepted for publication June 2, 2009. Supported in part by grant no. 2003063257-006 from the "Ministero dell' Universita' e della Ricerca Scientifica e Tecnologica," Rome, Italy (to Dr. P. A. Modesti) and by grant no. 239, 2005 from the University of Florence, Florence, Italy (to Dr. P. A. Modesti). Presented in part at the American Heart Association Scientific Session, Chicago, Illinois, November 15, 2006.

Address correspondence to Dr. Modesti: Clinica Medica e Cardiologia, Dept. of Critical Care Medicine; University of Florence, Viale Morgagni 85, 50134 Florence, Italy. pamodesti@unifi.it.

Table 1. Clinical Characteristics and Operative Details of Subjects Investigated

Clinical Characteristics	AAA	AS	*P* Value
Gender, men/women	6/4	6/2	
Age, yrs	68 ± 6	66 ± 7	NS
Weight, kg	72 ± 8	79 ± 11	NS
Height, cm	170 ± 5	172 ± 5	NS
Systolic blood pressure, mmHg	128 ± 9	119 ± 14	NS
Diastolic blood pressure, mmHg	78 ± 10	74 ± 9	NS
Heart rate, beats/min	71 ± 10	75 ± 5	NS
Smokers, n	6	4	
Creatinine, mg/dl	1.03 ± 0.3	0.9 ± 0.1	NS
Creatinine clearance, ml/min	74 ± 14	73 ± 21	NS
Ejection fraction, %	56 ± 6	57 ± 4	NS
Cardiac mass, g/m^2	119 ± 33	115 ± 23	NS
Operative details			
Intraoperative blood losses, ml	480 ± 200	310 ± 160	NS
Fluids administered, ml · kg^{-1}· h^{-1}	11.5 ± 3	10.1 ± 2	NS
Colloids/crystalloid ratio	0.39 ± 0.20	0.29 ± 0.17	NS
Cross-clamping time, min	53 ± 17	—	
Duration of surgery, min	240 ± 60	190 ± 80	NS

Data are presented as mean ± SD.

AAA = abdominal aortic aneurysm repair; AS = abdominal surgery; NS = not significant.

ical examination, standard 12-lead electrocardiography, and dobutamine stress echocardiography. Exclusion criteria were diabetes, evidence of peripheral vascular disease, recent history of myocardial infarction, intestinal chronic inflammatory disease, widespread cancer, left ventricular ejection fraction less than 40%, symptomatic congestive heart failure, significant segmental wall motion abnormalities before dobutamine stress echocardiography, new wall motion abnormalities detected on dobutamine stress echocardiography, and inability to understand informed consent.

Experimental protocols were approved by the institutional review board. All the patients gave their informed written consent to participate in the study.

Experimental Protocol

Infrarenal AAA repair was performed by using standard surgical technique; the details of surgical and anesthetic procedure and of intraoperative monitoring have been previously presented.[5] Aortic clamping lasted 30 to 60 min. AAA patients received 30 IU/kg of heparin intravenously before clamping, and low molecular weight heparin (nadroparin 4,000 IU sc) for 15 days from the first postoperative day. AS patients received low–molecular weight heparin (nadroparin 2,500 IUsc) before surgery and 2,500–4,000 U, depending on weight, from the first postoperative day for 1 week. No subject received protamine. Transthoracic echocardiography was repeated at the end and at 24 and 48 h after the end of surgery. Venous blood samples (3 ml) were collected in K_2EDTA-containing tubes after induction of anesthesia (baseline) and at 6, 12, 24, and 36 h after surgery as previously reported.[5] Blood was centrifuged at 4°C, 1,000*g* for 10 min, and the isolated plasma samples were stored at −80°C until use.

Hemodynamic Monitoring

Hemodynamic changes were continuously monitored by using a system based on online, real-time, digital processing of intraarterial radial pressure waveform analysis. A standard arterial catheter was inserted in the radial artery. Pressure signals were obtained at 1,000 Hz by means of an analogic-digital DAQ Card-700 (National Instruments Corp., Austin, TX) and recorded on a personal computer. The Pressure Recording Analytical Method technique, based on analysis of the peripheral artery waveform morphology, has been described elsewhere.[21,22] Pressure Recording Analytical Method is based on a software (Mostcare; Vytek, Padova, Italy) that analyzes the pressure signal obtained *via* the analogic card by means of a routine that identifies the characteristic points of the pressure wave during each beat (diastolic, systolic, dicrotic, and resonant pressure points during the systolic and end-diastolic phase). The morphologic analysis of the beat allows determination of the stroke volume that, multiplied by cardiac frequency, provides the cardiac output value. During spontaneous circulation, Pressure Recording Analytical Method results are based on the simultaneous evaluation of the pulsatile and continuous components of cardiac output flow, as expressed by the arterial waveform.[21,22]

Proteomic Studies

Two-dimensional Electrophoresis. An aliquot of 6.25 μl of human plasma was mixed with 10 μl of a solution containing Sodium Dodecyl Sulfate (10% w/v) and dithioerythritol (2.3% w/v). The sample was heated to 95°C for 5 min and then diluted to 500 μl with a solution containing urea (8 M), 3-cholamidopropyl dimethylammonium-1-propane sulfonate (4% w/v), Tris (40 mM), dithioerythritol (65 mM), and a trace of bromophenol blue.[23] Two-dimensional electrophoresis was performed by using the Immobiline polyacrylamide system. Briefly, 60 μg of the final diluted plasma sample for the analytical gels and 500 μg for the preparative gels were loaded onto a commercial nonlinear immobilized pH gradient from pH 3 to pH 10 of 18 cm length (Amersham Biosciences, Uppsala, Sweden). Isoelectric focusing was carried out on the Ettan Immobilized pH gradient phor system (Amersham Biosciences). Immobilized pH gradient strips were rehydrated in 350 μl of lysis buffer and 0.2% (v/v) carrier ampholyte for 1 h at 0 V and for 8 h at 30 V at 16°C. The strips were focused according to the following electrical conditions at 16°C: 200 V for 1 h, from 300 to 3,500 V in 30 min, 3,500 V for 3 h, from 3,500 to 8,000 V in 30 min, and 8,000 V until a total of 80,000 V per h was reached. After isoelectric focusing separation, the strips were equilibrated with 10 ml of a solution containing 50 mM Tris-HCl pH 6.8, 6 M urea, 30% (v/v) glycerol, 2% (w/v) Sodium Dodecyl Sulfate, and 2% (w/v) dithioerythritol for 12 min and with 10 ml of a solution containing 50 mM Tris-HCl pH 6.8, 6 M urea, 30%

(v/v) glycerol, 2% (w/v) Sodium Dodecyl Sulfate, 2.5% (w/v) iodoacetamide, and a trace of bromophenol blue for 5 min. Immobilized pH gradient strips were then placed on 12% SDS-PAGE gels (18 cm × 20 cm × 1.5 mm) and overlaid with a solution of 0.5% (w/v) agarose. The second-dimensional SDS-PAGE was carried out at 40 mA constant current at 10°C until the dye front reached the bottom of the gel. Analytical gels were stained with ammoniacal silver nitrate as previously described.[24] The mass spectrometry–preparative gels were stained according to a silver staining protocol compatible with mass spectrometry.[25]

Image Acquisition and Data Analysis. The stained gels were scanned using a 300S laser densitometer (4,000–65,000 pixels, 12 bits/pixel; Molecular Dynamics, Sunnyvale, CA). Image analysis used ImageMaster 2D Platinum 6.0 software (GE Healthcare, Little Chalfont, United Kingdom). Image spots were detected, matched, and then manually edited. Gel images of a plasma sample drawn from the basal time (baseline) were used as reference. Each spot intensity volume was processed by background subtraction and total spot volume normalization. Relative spot volumes (%*V*) (*V* = integration of optical density over the spot area; %*V* = *V* single spot/*V* total spots) were used for quantitative analysis to decrease experimental errors. The % volume of the baseline was taken as 1.00 when the comparison was made between groups with the use of the software.

The threshold for considering a spot "of interest" was set at a twofold change. This quantitative difference was selected by considering the recognized detection error range in the reproducibility of two-dimensional electrophoresis gels. Only the significantly different spots were selected for mass spectrometry analysis.

Protein Identification by Peptide Mass Fingerprinting. The spots of interest were manually excised from gels and destained in 30 mM potassium ferricyanide/100 mM sodium thiosulfate/H_2O solution (1:1:2, by volume). Gel pieces were then washed six times with deionized water, equilibrated with 200 mM ammonium bicarbonate for 20 min, washed with deionized water, dehydrated twice with acetonitrile for 20 min, and in-gel digested with trypsin (Trypsin Gold, mass spectrometry grade; Promega, Milan, Italy) 10 μg/ml at 30°C for 16 h in 50 mM ammonium bicarbonate buffer pH 8.0, according to the manufacturer's protocol. Peptides were extracted from the gel with 50% acetonitrile/5% trifluoroacetic acid (two steps, 20 min at room temperature each), dried, resuspended with 0.1% trifluoroacetic acid in 50% acetonitrile, mixed with matrix (C18 cyano-4-hydroxycinnamic acid), and analyzed by using Voyager-DE0 matrix-assisted laser desorption/ionization-time of flight mass spectrometer (Applied Biosystems, Foster City, CA).§§

§§ Swiss Prot. Available at: http://www.expasy.org./sprot; accessed May 12, 2009.

Western Blot Analysis. Plasma protein samples (40 μg), obtained at different times after AAA and AS from all patients (before and 6 h, 12 h, 24 h, and 36 h after surgery), were separated by 12% SDS-PAGE and transferred onto polyvinylidene fluoride membrane (Millipore, Billerica, MA).Western blot was performed by using a polyclonal antibody against Thrombin (sc-59717; Santa Cruz Biotechnology, Santa Cruz, CA) and peroxidase-conjugated secondary antibody. The reaction was detected by chemioluminescence with an ECL kit (Amersham Bioscience). The amount of each band was quantified with a densitometer software (Quantity One; Bio-Rad, Hercules, CA) and normalized by using the total protein amount detected by Ponceau red solution (Ponceau S solution; Sigma-Aldrich, St. Louis, MO). Data are shown as mean ± SD of each normalized intensity of band obtained from all patients studied at different times after AAA and AS. A two-tailed Student *t* test was performed by using ORIGIN 6.0 software (Microcal, Northampton, MA) to determine whether the relative change was statistically significant.

Rat Myocyte Isolation and Contractile Function Studies

The effects of plasma obtained at different times on myocyte contractile function was investigated by using cardiomyocytes isolated from rat hearts. The investigation complied with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1985). Cardiomyocytes were isolated from adult male Wistar rats weighing 180–230 g by the enzymatic method, as previously reported.[26] Nonmyocytes accounted for less than 2% of the cells.

Cardiomyocyte systolic performance was examined by using an IonOptix MyoCam system (IonOptix, Milton, MA). Briefly, after 2 h of stabilization, isolated myocytes were placed in a Warner chamber mounted on the stage of an inverted microscope (IX-50; Olympus, Center Valley, PA) and superfused (1 ml/min at 36°C) with a buffer containing the following (in mM): 131 NaCl, 4 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 10 HEPES, at pH 7.4. The myocyte being studied was displayed on the computer monitor with an IonOptix MyoCam camera (IonOptix), which rapidly scans the image area every 8.3 ms so that the amplitude and velocity of shortening/relengthening are recorded with a good level of accuracy. Edge-detection software (IonOptix) was used to measure cell length during shortening and relengthening. Parameters computed from the digitized contraction profiles included percent shortening, maximal velocity of shortening, and maximal velocity of relengthening. Cells were field-stimulated with suprathreshold voltage and at a frequency of 0.5 Hz. A minimum of 15 cells from each isolation was studied (at least 60 cells/group).

The functional effects of plasma (5%, 10%, and 20%) obtained in all patients at baseline and at 30 min and 6,

Table 2. Perioperative Hemodynamic Changes in Two Groups of Subjects Investigated

	AS					AAA					ANOVA (Group × Time)
	Bas	6 h	12 h	18 h	24 h	Bas	6 h	12 h	18 h	24 h	
SBP, mmHg	62 ± 9	52 ± 8	49 ± 9	51 ± 6	47 ± 8	57 ± 16	55 ± 17	53 ± 14	44 ± 9.1	48 ± 8.3	NS
DBP, mmHg	126 ± 8	107 ± 16	101 ± 7	106 ± 15	107 ± 12	134 ± 29	113 ± 18	89 ± 16	106 ± 12	109 ± 10	NS
DicBP, mmHg	96 ± 16	68 ± 17	58 ± 11	62 ± 6	57 ± 13	84 ± 18	74 ± 13	66 ± 12	63 ± 11	66 ± 8	NS
HR, bpm	70 ± 8	67 ± 10	69 ± 8	68 ± 6	65 ± 5	65 ± 11	71 ± 8	76 ± 9	70 ± 12	71 ± 8	NS
SVR, dynes s/cm^5	1,201 ± 67	1,004 ± 73	997 ± 213	1,636 ± 347	996 ± 160	1,220 ± 480	1,210 ± 311	1,090 ± 166	1,073 ± 160	1,019 ± 107	NS
CCE, units	0.2 ± 0.3	0.4 ± 0.1	0.3 ± 0.3	–0.1 ± 0.4	0.4 ± 0.2	0.18 ± 0.3	–0.5 ± 0.9*	–0.2 ± 0.5	0.2 ± 0.3	0.3 ± 0.1	0.01
CO, l/min	4.9 ± 1.1	5.3 ± 1.4	4.9 ± 0.8	4.6 ± 0.6	5.0 ± 0.7	5.4 ± 1.3	5.0 ± 1.5	4.7 ± 0.7	4.8 ± 1	5.2 ± 0.8	NS
dP/dt, 10^3 mmHg/s	0.9 ± 0.3	1.1 ± 0.2	0.9 ± 0.2	0.9 ± 0.3	1.0 ± 0.3	1.5 ± 0.5	1.2 ± 0.4	0.9 ± 0.4*	0.9 ± 0.2	1.0 ± 0.2	0.01

Values are presented as mean ± SD.

* $P < 0.01$ *vs*. baseline (Tukey test).

AAA = abdominal aortic aneurism repair (n = 10); AS = abdominal surgery (n = 8); Bas = baseline; CCE = cardiac cycle efficiency; CO = cardiac output; DBP = diastolic blood pressure; DicBP = blood pressure corresponding to dicrotic incisure; dP/dt = changes in arterial pressure *vs*. time; HR = heart rate; NS = not significant; SBP = systolic blood pressure; SVR = systemic vascular resistance.

12, 24, and 36 h after declamping (for AAA surgery) or after the end of surgery (for AS surgery) on myocytes preincubated (5 min) with buffer, heparin (0.5 U/ml), or melagatran (0.5 μmol/l), a direct, competitive, reversible, and noncovalent thrombin inhibitor that does not require endogenous cofactors,[27] were studied.

Statistical Analysis

Continuous variables are presented as means ± SD and compared with Student *t* test. Categorical variables were expressed as counts and percentages, and Fischer exact test was used for comparison. Hemodynamic changes and responses of plasma proteins were assessed with

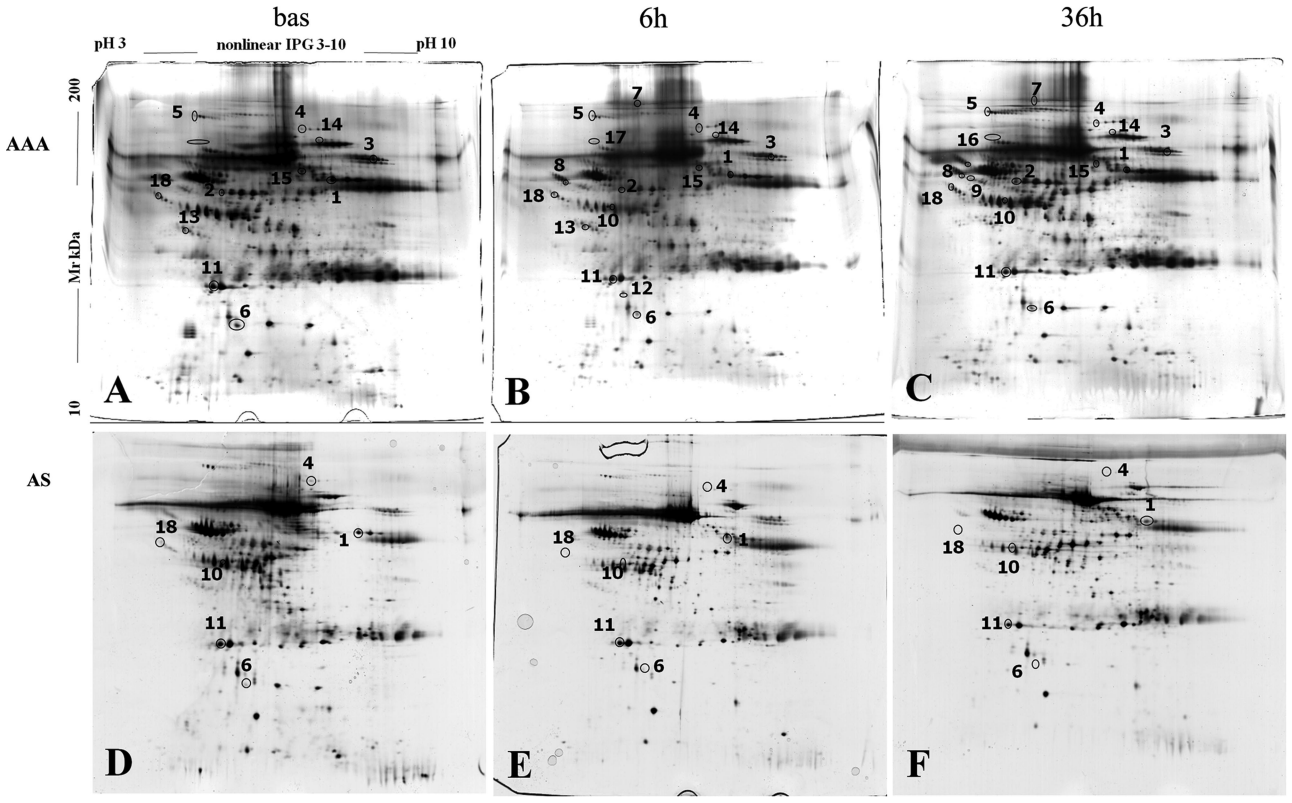

Fig. 1. Representative two-dimensional electrophoresis of plasma obtained before (baseline) and at 6 and 36 h after abdominal aortic aneurysm surgery (AAA) (*panels A, B, C*) or abdominal surgery (AS) (*panels D, E, F*) using a pH range of 3 to 10. Spots marked by their numbers show the location of the proteins that were altered in their expression (table 3). bas. = baseline; Mr = relative mass; nonlinear IPG = nonlinear immobilized pH gradient.

Table 3. Identification of Analyzed Proteins

Spot - Protein Name	AC	Identification method	Theoretical pI/Mr (kDa)	ExperimentalpI/Mr (kDa)	Seq Cov %
1 - Fibrinogen β chain	P02675	PI.map, MS	7.15/51.57	6.47/52.57	22%
2 - Fibrinogen γ chain	P02679	PI.map	5.24/48.48	5.22/47.78	
3 - Fibrinogen α/α–E chain	P02671	PI.map, MS	5.79/91.36	6.82/63.09	18%
4 - Complement factor B	P00751	PI.map	6.66/83.00	6.12/77.55	
5 - Ceruloplasmin	P00450	PI.map	5.41/120.08	5.99/114.98	
6 - Haptoglobin β chain	P00738	PI.map, MS	6.13/43.35	5.10/40.93	29%
7 - α-2-macroglobin	P01023	PI.map	5.95/160.80	5.42/151.42	
8 - α-2-HS-glycoprotein	P02765	PI.map, MS	5.43/39.19	4.48/54.26	26%
9 - α-1 antitrypsin	P01009	PI.map, MS	5.37/44.32	4.92/58.13	28%
10 - Apolipoprotein A-IV	P06727	PI.map, MS	5.18/43.40	5.12/47.03	27%
11 - Apolipoprotein A-I	P02647	PI.map, MS	5.45/28.83	5.12/22.90	24%
12 - Retinol binding protein	P02753	PI.map, MS	5.27/21.07	5.24/20.41	30%
13 - Clusterin	P10909	PI.map	5.89/50.06	5.47/40.48	
14 - Transferrin	P02787	PI.map, MS	6.70/75.18	6.36/71.90	20%
15 - Serum albumin	P02768	PI.map, MS	5.85/67.24	6.12/61.73	20%
16 - α-1-antichymotripsin	P01011	PI.map	5.33/47.35	4.60/56.74	
17 - Thrombin	P00734	PI.map, WB	5.64/70.03	5.00/69.46	
18 - Leucine rich α-2-glycopr.	P02750	PI.map, MS	6.45/37.18	4.78/54.98	29%

ANOVA for repeated measurements (group and time as main effects, and group $\times$ time interaction) followed by *post hoc* analyses (Tukey test) to compare later points to baseline or groups to each other at various time points.

In studies performed with isolated myocytes, responses of different variables in the two groups were assessed with a generalized linear model where controlled sources of variation included in the model were the concentration of plasma added, the time point at which plasma was withdrawn, and group. A further model was fitted allowing interaction terms among time, group, and concentration of plasma added. *Post hoc* analyses were then performed to discriminate the group and times responsible for differences. Analysis was performed on an intention-to-treat basis.

All P values were two-tailed, with statistical significance set at 0.01, and confidence intervals (CI) were calculated at the 99% levels. All statistical analyses were performed with the SPSS 15.0 software (SPSS, Inc., Chicago, IL).

Results

Clinical Outcomes

Patients undergoing infrarenal AAA repair and AS, respectively, were comparable in terms of demographics and preoperative risk factors (table 1). The mean intraoperative blood loss and duration of anesthesia were also comparable in the two groups (table 1). The mean time of muscle ischemia (aortic clamping) in patients undergoing AAA was 53 $\pm$ 17 min (range 30–70 min) with no ischemia–reperfusion in the other group.

No patients had ST-segment changes at electrocardiography at any time during the study or received cardiovascular drugs or other support to cardiac function in the perioperative observation period. No patients died in the operating room, had to return to the operating room within 6 days, or had a postoperative stay less than 48 h. The time course assessment of cardiac systolic function (left ventricular ejection fraction) at transthoracic echocardiography performed before and after surgery did not reveal any differences between groups. Likewise, neither cardiac output nor systolic and diastolic pressure changes differed between groups (ns at ANOVA test for all) (table 2). Conversely, ANOVA test revealed a significantly different curve for changes of both in cardiac cycle efficiency (CCE) and maximal rate of rise in the arterial pulse curve (dP/dt) measured in patients undergoing AAA and AS surgery ($P < 0.01$ for both variables) (table 2). In particular, *post hoc* analyses revealed a significant reduction of CCE and dP/dt in AAA patients at 6 h and 12 h, respectively ($P < 0.01$ *vs.* baseline for both). Conversely, no significant changes were observed in patients undergoing AS surgery.

Table 3. Continued

%V (mean ± SD)					
Baseline	6 h	12 h	24 h	36 h	ANOVA (time) *P* Value
0.31 ± 0.10	0.63 ± 0.27	0.86 ± 0.10*	0.64 ± 0.10	0.26 ± 0.15	0.0001
0.32 ± 0.17	0.65 ± 0.14*	0.61 ± 0.08*	0.30 ± 0.08	0.34 ± 0.07	0.0001
0.12 ± 0.04	0.45 ± 0.09*	0.34 ± 0.07*	0.09 ± 0.04	0.15 ± 0.06	0.0001
0.03 ± 0.02	0.44 ± 0.07*	0.25 ± 0.08*	0.08 ± 0.01	0.08 ± 0.01	0.0001
0.04 ± 0.02	0.05 ± 0.02	0.03 ± 0.01	0.20 ± 0.09*	0.64 ± 0.01*	0.0001
0.33 ± 0.08	0.22 ± 0.08	0.09 ± 0.02*	0.07 ± 0.02*	0.06 ± 0.02*	0.0001
0	0.01 ± 0.001	0.01 ± 0.008	0.70 ± 0.08*	0.70 ± 0.09*	0.0001
0	0.08 ± 0.01*	0.06 ± 0.02*	0.02 ± 0.009	0.02 ± 0.007	0.0001
0	0	0	0.04 ± 0.007	0.13 ± 0.034*	0.0001
0	0.04 ± 0.01*	0.04 ± 0.007*	0.06 ± 0.009*	0.08 ± 0.01*	0.0001
0.03 ± 0.006	0.03 ± 0.005	0.07 ± 0.01	0.24 ± 0.09*	0.6 ± 0.07*	0.0001
0	0.02 ± 0.006*	0.05 ± 0.009*	0.03 ± 0.009*	0	0.0001
0.05 ± 0.01	0.07 ± 0.008*	0.03 ± 0.008*	0*e	0*	0.0001
0.06 ± 0.01	0.06 ± 0.01	0.199 ± 0.04*	0.05 ± 0.007	0.04 ± 0.005	0.0001
0.03 ± 0.008	0.04 ± 0.007	0.06 ± 0.008	0.21 ± 0.07*	0.27 ± 0.09*	0.0001
0	0	0	0.2 ± 0.08*	0.7 ± 0.1*	0.0001
0	0.008 ± 0.01*	0.003 ± 0.005*	0.003 ± 0.01*	0	0.0001
0.02 ± 0.009	0.06 ± 0.01	0.9 ± 0.08	0.47 ± 0.07*	1.5 ± 0.3*	0.000

List of differentially expressed proteins identified in plasma from 10 patients after aortic aneurysm repair: Acute Phase Proteins (spot 1–9), Transport Proteins (10–15), Protease and Protease inhibitors (16–17), Other (18).

* $P < 0.01$ *versus* baseline (Tukey test).

AC = accession number for SwissProt/TrEMBL entry; Experimental pI/Mr = values of isoelectric point and relative mass calculated with Master 2D Platinum 6.0 (GE Healthcare, Little Chalfont, United Kingdom) by using identified proteins with known parameters as references; MS = mass spectrometry; Pl.map = plasma map of SWISS-PROT 2D database; Seq Cov % = percentage of amino acid sequence coverage of matched peptides in the identified proteins; Theoretical pI/Mr = predicted isoelectric point and relative mass according to protein sequence; %V = relative spot volumes of patients undergoing aortic surgery (*V* = integration of optical density over the spot area; %*V* = *V* single spot/*V* total spots); WB = Western blot.

Proteomic Profiling of Plasma Samples in AAA Subjects

More than 900 spots were detected in each gel, ranging from 10 to 200 kDa with a pH between 3 and 10 (fig. 1). When gels corresponding to the basal time were used as reference in bidimensional electrophoresis (2DE) gel analysis, the densities of 100 spots were found to change after AAA surgery. The densities of only 18 of these 100 spots changed constantly in all patients studied. These spots were densitometrically measured and identified by comparison with those in the SWISS-2D database plasma map (fig. 1).

Identification of Differentially Expressed Proteins. Of the 18 differentially expressed proteins, 12 were also identified by either matrix-assisted laser desorption/ionization-time of flight mass spectrometry (n = 11) or Western blot (n = 1) analysis selected on the basis of spot relative abundance, high variation, and resolution on preparative gels. In particular, six protein spots with insufficient quantities were not processed at matrix-assisted laser desorption/ionization analysis. The locations of differentially expressed spots were marked with numbers in representative gels shown in figure 1. All the identified proteins are described, and their relative amounts are reported in table 3.

The volume of protein spot No. 10 significantly increased *versus* baseline at 6 h, 12 h, 24 h, and 36 h ($P <$ 0.0001 for all); protein spots Nos. 12 and 17 were changed *versus* baseline at the same confidence level (99%) after 6 h, 12 h, and 24 h; the volumes of five protein spots (Nos. 2, 3, 4, 8, 13) changed significantly *versus* baseline at 6 and 12 h.

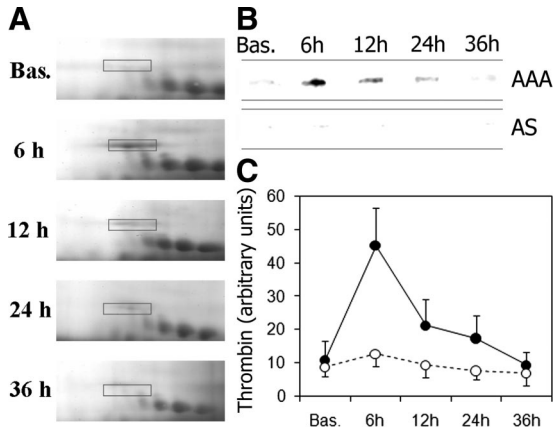

Fig. 2. (*A*) Representative pattern of thrombin in the plasma of a patient operated on for abdominal aortic aneurysm repair before (bas. = baseline) and at 6, 12, 24, and 36 h after surgery. Images were obtained from the two-dimensional electrophoresis gels using a pH range of 3 to 10. (*B*) Representative Western blots for thrombin performed in plasma samples obtained before (bas. = baseline) and 6, 12, 24 and 36 h after abdominal aortic aneurysm surgery (AAA) or abdominal surgery (AS). (*C*) Densitometric assay of Western blots performed in plasma from AAA (*filled circles*) and AS patients (*empty circles*).

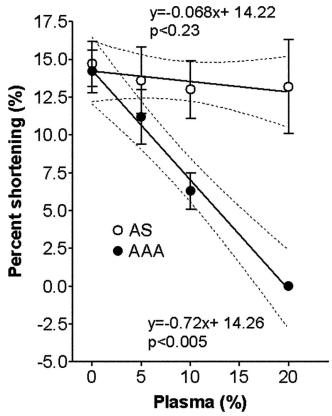

Fig. 3. Concentration-dependent effects on control rat myocyte sarcomere shortening (%) of plasma obtained 6 h after surgery from patients undergoing abdominal aortic aneurysm surgery (AAA, $P < 0.005$) or abdominal surgery (AS, *P* value not significant).

Two protein spots (Nos. 1 and 14) showed a relative volume change in expression at 12 h that was significant at the 99% confidence level *versus* baseline.

A third group of protein spots were significantly changed after 12 h. In particular, protein spot No. 6 reduced significantly at the 99% confidence level at 12 h ($P = 0.008$), 24 h ($P = 0.002$), and 36 h ($P = 0.001$), when compared to baseline; six spots (Nos. 5, 7, 11, 15, 16, 18) changed significantly at 24 and 36 h *versus* baseline ($P < 0.01$), spot No. 9 only at 36 h ($P < 0.0001$).

Comparative analysis revealed that AAA repair induces a severe change in the expression level of a number of plasma proteins. At 6 h after declamping, a contemporary increase in clusterin, complement factor B, and fibrinogen γ and α/α-E chain occurred. The fibrinogen β-chain was significantly increased at 12 h. All isoforms of fibrinogen and complement factor B returned to baseline levels 24 h after surgery (fig. 1, table 3). The expression level of transferrin showed a peak of increase after 12 h. A delayed peak at 24–36 h was observed for ceruloplasmin. Conversely, haptoglobin showed a progressive reduction in plasma levels with minimum values at 36 h.

Six hours after aortic declamping, plasma proteome analysis revealed the presence of thrombin, α-2-HSglycoprotein, and retinol-binding-protein, which were all absent at the basal time and showed peaks at 12 and 24 h. α-1-antitrypsin, α-1-antichymotripsin, and α-2-macroglobulin appeared in 2DE only 24 h after reperfusion. Apolipoprotein A-IV, not detectable at baseline, appeared at 6 h and progressively increased up to 36 h. A similar pattern was shown by the spot of isoform Apolipoprotein A-I, already present at baseline and slowly increasing with a peak at 36 h.

Table 4. Effects of Human Plasma on Rat Cardiomyocyte Function

		+AS Plasma			
Time	Buffer	5%	10%	20%	20% + Heparin
Peak velocity of sarcomere shortening, μ/s					
Baseline	−12.7 ± 0.4	−12.0 ± 0.7	−12.1 ± 0.5	−12.0 ± 0.3	−12.8 ± 2.8
30 min	−12.2 ± 1.8	−12.0 ± 1.8	−11.9 ± 1.6	−11.9 ± 1.5	−11.6 ± 1.2
6 h	−13.0 ± 1.7	−11.4 ± 2.0	−11.1 ± 2.4	−10.7 ± 2.6	−12.4 ± 1.9
12 h	−13.1 ± 1.1	−11.0 ± 0.9	−10.6 ± 0.8	−10.2 ± 1.0	−12.7 ± 2.1
24 h	−12.7 ± 1.6	−12.7 ± 1.5	−12.5 ± 1.2	−12.5 ± 1.3	−11.8 ± 0.1
36 h	−11.9 ± 0.8	−11.7 ± 0.5	−12.0 ± 0.8	−11.8 ± 0.5	−11.3 ± 2.9
Generalized linear model analysis	—	—	—	—	—
Peak velocity of sarcomere relengthening, μ/s					
Baseline	11.5 ± 0.9	11.1 ± 1.2	11.1 ± 1.2	11.5 ± 1.1	11.8 ± 2.0
30 min	11.8 ± 1.0	11.6 ± 1.0	11.6 ± 1.3	11.8 ± 0.9	11.5 ± 0.8
6 h	12.7 ± 1.8	11.4 ± 2.1	10.9 ± 1.7	10.3 ± 2.2	12.3 ± 2.8
12 h	13.0 ± 0.8	11.2 ± 0.9	10.7 ± 1.1	10.2 ± 0.9	12.0 ± 2.7
24 h	12.1 ± 1.0	12.2 ± 1.1	12.0 ± 1.3	11.8 ± 1.1	10.6 ± 0.1
36 h	11.7 ± 1.1	11.6 ± 0.9	11.6 ± 0.9	11.5 ± 1.0	11.1 ± 1.2
Generalized linear model analysis	—	—	—	—	—
Percent shortening, %					
Baseline	14.4 ± 1.0	14.8 ± 0.8	14.4 ± 1.3	14.4 ± 1.0	14.1 ± 1.4
30 min	11.6 ± 0.9	13.9 ± 1.0	13.7 ± 0.7	13.7 ± 1.0	13.7 ± 1.2
6 h	14.7 ± 1.5	13.6 ± 2.2	13.0 ± 1.9	13.2 ± 3.1	14.2 ± 1.4
12 h	13.7 ± 2.4	11.7 ± 2.1	11.2 ± 2.1	10.9 ± 2.0	15.2 ± 1.7
24 h	13.8 ± 1.0	14.1 ± 0.8	14.0 ± 1.2	14.0 ± 0.9	12.9 ± 0.9
36 h	14.1 ± 1.2	14.0 ± 0.7	14.1 ± 1.2	14.2 ± 1.0	13.4 ± 3.0
Generalized linear model analysis	—	—	—	—	—

Thrombin Identification. The proteomic analysis of the plasma protein obtained from the AAA patient group revealed that the spot corresponding to thrombin (No. 17) was detectable between 6 h and 24 h after declamping and disappeared at 36 h. This spot was identified by comparison of the two-dimensional electrophoresis gels of the patients studied with the corresponding spot present in the SWISS-2D database plasma map. Probably because of the low amount of thrombin in plasma, it was not possible to confirm this identification by matrix-assisted laser desorption/ionization-time of flight mass spectrometry. Western blot analysis was then performed to assay thrombin on the plasma of all patients investigated. Densitometric analysis (fig. 2) revealed a marked increase in thrombin 6 h after declamping ($P < 0.0001$ *vs.* baseline), followed by a progressive reduction with a return to baseline values at 36 h (fig. 2).

Proteomic Profiling of Plasma Samples in AS Subjects

Analysis with ImageMaster 2D Software of the 2DE gels obtained from AS patients after induction of anesthesia (baseline) and at 6, 12, 24, and 36 h after surgery revealed great differences as compared to the AAA group. More than 900 spots were detected in each gel, ranging from 10 to 200 kDa with a pH between 3 and 10; however, of the 18 spots constantly changing in AAA patients, only 6 of these spots were detectable (fig. 1). Moreover, these six spots showed no changes in expression levels after AS surgery as compared to baseline. Western blot analysis for thrombin also performed on plasma samples of the AS patients (fig. 2), showed no changes in expression level after AS surgery as compared to baseline.

Effect of Plasma Samples on Isolated Rat Myocytes

Mechanical properties of control myocyte were significantly affected by the group of patients who gave plasma, the time of withdrawal, and the concentration of plasma added to myocytes. Generalized linear model analysis indeed revealed a three-way interaction among group, time, and concentration of plasma added to myocytes for sarcomere peak velocity of shortening ($P = 0.003$) and relengthening ($P = 0.002$) and percent shortening ($P < 0.0001$). The interactions of group of patients who gave plasma and time ($P < 0.0001$ for all contractility variables) and concentration of plasma added and time (at least $P < 0.006$ for all contractility variables) were also significant as the main effects of time ($P < 0.0001$ for all). The effect of AAA plasma on myocyte contractility was concentration-dependent (fig. 3).

Table 4. Continued

+AAA Plasma				
5%	10%	20%	20% + Heparin	20% + Melagatran
−12.5 ± 2.5	−12.7 ± 2.6	−12.2 ± 2.2	−12.7 ± 1.1	−12.0 ± 0.6
−11.2 ± 1.2	−11.4 ± 0.8	−11.2 ± 1.2*	−11.0 ± 0.8	−11.2 ± 1.1
−9.5 ± 1.6	−5.3 ± 1.0*†‡§‖	0*†‡§‖	−10.5 ± 1.5	−10.3 ± 1.7
−7.8 ± 0.7*†	−4.3 ± 0.4*†‡§‖	0*†‡§‖	−10.3 ± 1.3	−10.2 ± 0.9
−8.7 ± 0.7	−6.8 ± 0.3*†‡§‖	−3.9 ± 0.3*†‡§‖	−11.3 ± 0.9	−11.5 ± 1.4
−10.9 ± 2.4	−11.0 ± 2.1	−10.8 ± 2.4	−11.1 ± 0.5	−11.2 ± 0.4
†‡	*†‡§‖	*†‡§‖	—	—
11.7 ± 2.0	11.6 ± 2.1	11.9 ± 2.2	12.7 ± 1.1	12.1 ± 1.1
11.2 ± 0.9	11.4 ± 1.0	11.3 ± 0.9	11.0 ± 1.1	11.5 ± 1.2
8.7 ± 1.8	5.3 ± 1.0*†‡§‖	0*†‡§‖	10.4 ± 1.4	10.2 ± 1.4
7.7 ± 1.1*†	4.4 ± 0.5*†‡§‖	0*†‡§‖	10.3 ± 1.5	10.1 ± 1.4
8.2 ± 0.5‡	6.1 ± 0.4*†‡§‖	3.7 ± 0.3*†‡§‖	10.7 ± 0.8	11.4 ± 1.8
10.7 ± 1.3†‡	10.9 ± 1.4	10.9 ± 1.3	10.9 ± 0.4	11.1 ± 1.4
†‡	*†‡§‖	*†‡§‖	—	—
13.8 ± 1.2	13.9 ± 1.4	13.9 ± 2.2	14.0 ± 1.3	13.4 ± 1.8
13.6 ± 1.1	13.6 ± 1.1	13.6 ± 1.1	13.2 ± 0.7	12.9 ± 0.8
11.2 ± 1.8	6.3 ± 1.2*†‡§‖	0*†‡§‖	13.0 ± 1.6	12.6 ± 1.4
9.2 ± 0.3*†	5.3 ± 0.5*†‡§‖	0*†‡§‖	11.9 ± 1.4	11.7 ± 1.2
9.4 ± 1.0‡	7.7 ± 0.7*†‡§‖	4.6 ± 0.3*†‡§‖	12.8 ± 1.1	12.7 ± 0.9
13.7 ± 3.4	13.6 ± 3.0	13.6 ± 3.2	13.0 ± 1.4	12.5 ± 0.6
†‡	*†‡§‖	*†‡§‖	—	—

Values are presented as mean ± SD. Each experiment was performed at least in triplicate in all patients.

Generalized linear model (group × time) and *post hoc* analyses (Tukey test): * $P < 0.01$ *vs.* baseline; † $P < 0.01$ *vs.* buffer; ‡ $P < 0.01$ *vs.* control (AS plasma); § $P < 0.01$ *vs*. heparin; ‖ $P < 0.01$ *vs*. melagatran.

AAA plasma = plasma obtained from patients undergoing abdominal aortic aneurysm repair (n = 10); AS plasma = plasma obtained from patients undergoing abdominal surgery (n = 8).

Post hoc analyses revealed that myocyte response to AAA plasma significantly differed from the response to AS plasma and buffer ($P < 0.0001$ for all contractility variables). Conversely, the response to AS plasma did not differ from the myocyte response to buffer (NS) (table 4). In particular, preincubation of isolated rat ventricular myocytes with 20% plasma obtained at 6, 12, and 24 h after aortic declamping resulted in a marked impairment of myocyte contractility. The inhibitory effect was not observed when myocytes were preincubated with plasma obtained either before surgery or 36 h after declamping. Likewise, plasma obtained from AS patients did not affect control myocyte contractility.

Inhibitory effects on myocyte contractility were abolished in the presence of heparin (0.5 U/ml) or melagatran (0.5 μmol/l) (table 4).

Discussion

The present findings indicate that increased thrombin generation may play a role in cryptic hemodynamic instability in patients undergoing AAA surgery. The assay of proteomic profiling in the current study depicted complex changes occurring only after AAA surgery, with marked activation of thrombin and Complement factor B 6 h after aortic declamping. This observation has important clinical implications because it indicates a time window during which the heparin dosage used in AAA surgery may be insufficient. Large-scale thrombin activation is known to occur in patients undergoing AAA surgery,[9–11] and an increase in thrombin generation markers (thrombin-antithrombin complex) was observed between 1 and 24 h after declamping.[12,28] This procoagulant state makes the use of perioperative anticoagulation management in AAA surgery compulsory to reduce the risk of thrombotic complications that predominantly occur in the early postoperative period, including myocardial infarction, lower extremity and intestinal ischemia, stroke, and venous thrombosis.[29,30] In extreme cases, consumptive coagulopathy may also cause postoperative bleeding.[12] However, no standardized heparin treatment exists,[9,12,31] and the use of heparin on the first postoperative day is usually avoided. Therefore, the first clinical implication of the current results is that failure to use heparin treatment in the early postoperative period or at least the low heparin dose (30 IU/kg) used during surgery in the current study, may contribute to significant thrombin activation.

According to the current findings, thrombin activation seems also to exert functional effects independently of thrombosis. Plasma concentrations of both pro- and antiinflammatory cytokines increase within 24 h after AAA surgery.[4–6,31–33] The release of proinflammatory cytokines, which may depend on the extent of ischemia and reperfusion (I/R),[4] seems to contribute to transient organ dysfunction.[5,8,34,35] The early thrombin activation is considered to participate in the reaction by inducing cytokine release.[36,37] However, our study would seems to suggest a different interpretation. AAA plasma obtained 6 h after surgery significantly inhibited the systolic functional properties of isolated control myocytes, an effect completely abolished by thrombin antagonism. At the same time, the significant reduction of CCE (the ratio between hemodynamic work performed and energetic expenditure),[22] with unchanged indexes of cardiac function, might indicate an increased energy expenditure of the cardiovascular system to maintain homeostasis. These observations suggest a direct inhibitory effect of thrombin on myocyte contractility. In the current study, we did not assess the effect of thrombin on control myocytes without blood components. This issue was investigated in previous studies with conflicting results. Thrombin (1 U/ml) was reported to induce a rapid increase in contraction strength, beat frequency, and action potential duration in both chick[17] and rat[18] embryonic or neonatal myocytes. These effects are associated with activation of phospholipase C,[17] mitogen-activated protein kinase activity,[18] and hypertrophic response.[38] However, an opposite response has been observed in adult cells, where the same thrombin concentration induced a significant reduction in percent shortening as well as in shortening and relengthening velocity.[19] Although the enzymatic dissociation used to isolate myocytes may alter thrombin receptor structure,[20] it should be borne in mind that this dissociation was also used to obtain neonatal cells.[17,18] Moreover, our study reveals that the effect of plasma is reversed by heparin and the thrombin antagonist, thus suggesting the functional integrity of the thrombin receptor. On the other hand, an important discrepancy between the responses of neonatal and of adult myocytes has also been reported after angiotensin II receptor stimulation.[26] The blunted activity of intracellular phosphatase in neonatal cells[39] enhances mitogen-activated protein kinase activity, with increased formation of factors (endothelin-1), which are finally active on cell contractility. Conversely, the inhibitory effects of thrombin on adult myocytes have been found to be related to increased sarcolemmal Na+/H+ exchanger activity.[40,41] Three different fibrinogen chains also increased in plasma at 6 and 12 h after aortic declamping, and this could be associated with increased thrombotic risk. Fibrinogen contains two sets of three chains (α, β, and γ) linked by disulfide bonds, and it is essential for fibrin formation under the influence of thrombin.[42] Activated platelets bind to fibrin *via* the N-terminal of the β-chain and the extreme C-terminal of the γ chain. The fibrinogen γ chain was also found to decrease cardiomyocyte contractility through binding Intercellular Adhesion Molecule-1.[43] However, the ability of thrombin antagonism to reverse the effect of

plasma seems to rule out a major role for fibrinogen. The second potential clinical implication of our study is thus that the enhanced thrombin generation after AAA surgery may contribute to impairing left ventricular function in the early postoperative period.

The enhanced thrombin generation observed at 6 h after declamping is then followed (at 24 and 36 h) by an antiproteolytic response, with expression of protease inhibitors (α-1-antichymotripsin and α-1 antitrypsin), which are not present at basal time. Although apolipoprotein A-IV function in the atherosclerotic process is not well characterized, apolipoprotein A-IV overexpression was shown to produce significant protection against atherosclerosis.[44,45] This protein was significantly increased in our patients between 6 h and 36 h after AAA surgery.

There are also limitations to the current study that must be recognized. Although the 2DE approach is extensively used for the separation of proteins,[46] many proteins with low concentrations may not be visible for detection in the presence of other and high-abundance proteins.[46] In addition, more than one protein may be present in a single gel spot.[46] Finally, intergel variations may also constitute an important limitation of the technique. To overcome these ambiguities (limitations), we used other methods to confirm changes observed in AAA patients. In addition, our preliminary observational study may require following further investigation to assess the hemodynamic effects of an early thrombin inhibition after AAA repair, although these studies may be limited by the perioperative hemorrhagic risk. We did not investigate the relationship between quantitative measurements of plasma thrombin and hemodynamic function. This can be considered a further limitation of the current study. The overall numbers of open procedure has been drastically decreasing in recent years as a result of the advent of endovascular procedures, so that the confinement of our observation to open AAA surgery might be considered a possible limitation of the study. However, this selection allowed us to accurately measure the recognized main source of thrombin activation in open AAA surgery, the time of ischemia. Thrombin activation in patients undergoing endovascular aneurysm repair was indeed found to be even greater than in conventional surgery,[12] probably as a result of the participation of less standardizable factors such as (1) the use of endovascular tools, (2) periprocedural release of prothrombotic substances from the aneurysmatic thrombus, (3) contrast media,[47] and (4) blood contact with the foreign materials.[12]

In conclusion, regardless of its prothrombotic properties, thrombin may contribute to determining hemodynamic instability and organ dysfunction in the early perioperative period after AAA surgery.

References

1. Mangano DT, Goldman L: Preoperative assessment of patients with known or suspected coronary disease. N Engl J Med 1995; 333:1750–6
2. Lee TH, Marcantonio ER, Mangione CM, Thomas EJ, Polanczyk CA, Cook EF, Sugarbaker DJ, Donaldson MC, Poss R, Ho KK, Ludwig LE, Pedan A, Goldman L: Derivation and prospective validation of a simple index for prediction of cardiac risk of major noncardiac surgery. Circulation 1999; 100:1043–9
3. McFalls EO, Ward HB, Moritz TE, Apple FS, Goldman S, Pierpont G, Larsen GC, Hattler B, Shunk K, Littooy F, Santilli S, Rapp J, Thottapurathu L, Krupski W, Reda DJ, Henderson WG: Predictors and outcomes of a perioperative myocardial infarction following elective vascular surgery in patients with documented coronary artery disease: Results of the CARP trial. Eur Heart J 2008; 29:394–401
4. Welborn MB, Oldenburg HS, Hess PJ, Huber TS, Martin TD, Rauwerda JA, Wesdorp RI, Espat NJ, Copeland EM 3rd, Moldawer LL, Seeger JM: The relationship between visceral ischemia, proinflammatory cytokines, and organ injury in patients undergoing thoracoabdominal aortic aneurysm repair. Crit Care Med 2000; 28:3191–7
5. Adembri C, Kastamoniti E, Bertolozzi I, Vanni S, Dorigo W, Coppo M, Pratesi C, De Gaudio AR, Gensini GF, Modesti PA: Pulmonary injury follows systemic inflammatory reaction in infrarenal aortic surgery. Crit Care Med 2004; 32:1170–7
6. Galle C, De Maertelaer V, Motte S, Zhou L, Stordeur P, Delville JP, Li R, Ferreira J, Goldman M, Capel P, Wautrecht JC, Pradier O, Dereume JP: Early inflammatory response after elective abdominal aortic aneurysm repair: A comparison between endovascular procedure and conventional surgery. J Vasc Surg 2000; 32:234–46
7. Norwood MG, Bown MJ, Sayers RD: Ischaemia-reperfusion injury and regional inflammatory responses in abdominal aortic aneurysm repair. Eur J Vasc Endovasc Surg 2004; 28:234–45
8. Gray E, Thomas S, Mistry Y, Poole S: Inhibition of tissue factor and cytokine release. Haemostasis 1996; 26:92–5
9. Holmberg A, Siegbahn A, Westman B, Bergqvist D: Ischaemia and reperfusion during open abdominal aortic aneurysm surgery induce extensive thrombin generation and activity. Eur J Vasc Endovasc Surg 1999; 18:11–6
10. Adam DJ, Ludlam CA, Ruckley CV, Bradbury AW: Coagulation and fibrinolysis in patients undergoing operation for ruptured and nonruptured infrarenal abdominal aortic aneurysms. J Vasc Surg 1999; 30:641–50
11. Holmberg A, Bergqvist D, Siegbahn A: Coagulation and fibrinolysis after open infrarenal abdominal aortic aneurysm repair in a long-term perspective. Thromb Res 1999; 96:99–105
12. Englberger L, Savolainen H, Jandus P, Widmer M, Do do D, Haeberli A, Baumgartner I, Carrel TP, Schmidli J: Activated coagulation during open and endovascular abdominal aortic aneurysm repair. J Vasc Surg 2006; 43:1124–9
13. Nimjee SM, Keys JR, Pitoc GA, Quick G, Rusconi CP, Sullenger BA: A novel antidote-controlled anticoagulant reduces thrombin generation and inflammation and improves cardiac function in cardiopulmonary bypass surgery. Mol Ther 2006; 14:408–15
14. Carr JA, Silverman N: The heparin-protamine interaction. A review. J Cardiovasc Surg 1999; 40:659–66
15. Shigeta O, Kojima H, Hiramatsu Y, Jikuya T, Terada Y, Atsumi N, Sakakibara Y, Nagasawa T, Mitsui T: Low-dose protamine based on heparin-protamine titration method reduces platelet dysfunction after cardiopulmonary bypass. J Thorac Cardiovasc Surg 1999; 118:354–60
16. Hird RB, Wakefield TW, Mukherjee R, Jones BU, Crawford FA, Andrews PC, Stanley JC, Spinale FG: Direct effects of protamine sulfate on myocyte contractile processe: Cellular and molecular mechanisms. Circulation 1995; 92: 433–46
17. Chien WW, Mohabir R, Clusin WT: Effect of thrombin on calcium homeostasis in chick embryonic heart cells. Receptor-operated calcium entry with inositol trisphosphate and a pertussis toxin-sensitive G protein as second messengers. J Clin Invest 1990; 85:1436–43
18. Jiang T, Kuznetsov V, Pak E, Zhang H, Robinson RB, Steinberg SF: Thrombin receptor actions in neonatal rat ventricular myocytes. Circ Res 1996; 78: 553–63
19. Hird RB, Crawford FA Jr, Mukherjee R, Spinale FG: Direct effects of thrombin on myocyte contractile function. Ann Thorac Surg 1995; 59:288–93
20. Jiang T, Danilo P Jr, Steinberg SF: The thrombin receptor elevates intracellular calcium in adult rat ventricular myocytes. J Mol Cell Cardiol 1998; 30:2193–9
21. Romano SM, Pistolesi M: Assessment of cardiac output from systemic arterial pressure in humans. Crit Care Med 2002; 30:1834–41
22. Balderi T, Forfori F, Marra V, Di Salvo C, Dorigo M, Anselmino M, Romano SM, Giunta F: Continuous hemodynamic monitoring during laparoscopic gastric bypass in superobese patients by pressure recording analytical method. Obes Surg 2008; 18:1007–14
23. Hughes GJ, Frutiger S, Paquet N, Ravier F, Pasquali C, Sanchez JC, James R, Tissot JD, Bjellqvist B, Hochstrasser DF: Plasma protein map: An update by microsequencing. Electrophoresis 1992; 13:707–14
24. Hochstrasser DF, Patchornik A, Merril CR: Development of polyacrylamide gels that improve the separation of proteins and their detection by silver staining. Anal Biochem 1988; 173:412–23

25. Shevchenko A, Jensen ON, Podtelejnikov AV, Sagliocco F, Wilm M, Vorm O, Mortensen P, Shevchenko A, Boucherie H, Mann M: Linking genome and proteome by mass spectrometry: Large-scale identification of yeast proteins from two dimensional gels. Proc Natl Acad Sci U S A 1996; 93:14440–5

26. Modesti A, Bertolozzi I, Gamberi T, Marchetta M, Lumachi C, Coppo M, Moroni F, Toscano T, Lucchese G, Gensini GF, Modesti PA: Hyperglycemia activates JAK2 signaling pathway in human failing myocytes *via* angiotensin II-mediated oxidative stress. Diabetes 2005; 54:394–401

27. Gustafsson D, Antonsson T, Bylund R, Eriksson U, Gyzander E, Nilsson I, Elg M, Mattsson C, Deinum J, Pehrsson S, Karlsson O, Nilsson A, Sörensen H: Effects of melagatran, a new low-molecular-weight thrombin inhibitor, on thrombin and fibrinolytic enzymes. Thromb Haemost 1998; 79:110–8

28. Samama CM, Thiry D, Elalamy I, Diaby M, Guillosson JJ, Kieffer E, Coriat P: Perioperative activation of hemostasis in vascular surgery patients. ANESTHESIOLOGY 2001; 94:74–8

29. Cuypers PW, Gardien M, Buth J, Charbon J, Peels CH, Hop W, Laheij RJ: Cardiac response and complications during endovascular repair of abdominal aortic aneurysms: A concurrent comparison with open surgery. J Vasc Surg 2001; 33:353–60

30. Becquemin JP, Chemla E, Chatellier G, Allaire E, Mellière D, Desgranges P: Perioperative factors influencing the outcome of elective abdominal aorta aneurysm repair. Eur J Vasc Endovasc Surg 2000; 20:84–9

31. Fiane AE, Videm V, Lingaas PS, Heggelund L, Nielsen EW, Geiran OR, Fung M, Mollnes TE: Mechanism of complement activation and its role in the inflammatory response after thoracoabdominal aortic aneurysm repair. Circulation 2003; 108:849–56

32. Roumen RM, Hendriks T, van der Ven-Jongekrijg J, Nieuwenhuijzen GA, Sauerwein RW, van der Meer JW, Goris RJ: Cytokine patterns in patients after major vascular surgery, hemorrhagic shock, and severe blunt trauma. Relation with subsequent adult respiratory distress syndrome and multiple organ failure. Ann Surg 1993; 218:769–76

33. Oldenburg HS, Burress Welborn M, Pruitt JH, Boelens PG, Seeger JM, Martin TD, Wesdorp RI, Rauwerda JA, van Leeuwen PA, Moldawer LL: Interleukin-10 appearance following thoraco-abdominal and abdominal aortic aneurysm repair is associated with the duration of visceral ischaemia. Eur J Vasc Endovasc Surg 2000; 20:169–72

34. Groeneveld AB, Raijmakers PG, Rauwerda JA, Hack CE: The inflammatory response to vascular surgery-associated ischaemia and reperfusion in man: Effect on postoperative pulmonary function. Eur J Vasc Endovasc Surg 1997; 14:351–9

35. Fleischmann KE, Goldman L, Young B, Lee TH: Association between cardiac and noncardiac complications in patients undergoing noncardiac surgery: Outcomes and effects on length of stay. Am J Med 2003; 115:515–20

36. Steinberg SF, Robinson RB, Lieberman HB, Stern DM, Rosen MR: Thrombin modulates phosphoinositide metabolism, cytosolic calcium, and impulse initiation in the heart. Circ Res 1991; 68:1216–29

37. Tapparelli C, Metternich R, Ehrhardt C, Cook NS: Synthetic low-molecular weight thrombin inhibitors: Molecular design and pharmacological profile. Trends Pharmacol Sci 1993; 14:366–76

38. Glembotski CC, Irons CE, Krown KA, Murray SF, Sprenkle AB, Sei CA: Myocardial alpha-thrombin receptor activation induces hypertrophy and increases atrial natriuretic factor gene expression. J Biol Chem 1999; 268:20646–52

39. Hefti MA, Harder BA, Eppenberger HM, Schaub MC: Signaling pathways in cardiac myocyte hypertrophy. J Mol Cell Cardiol 1997; 29:2873–92

40. Yasutake M, Haworth RS, King A, Avkiran M: Thrombin activates the sarcolemmal Na(+)-H+ exchanger. Evidence for a receptor-mediated mechanism involving protein kinase C. Circ Res 1996; 79:705–15

41. Avkiran M, Haworth RS: Regulatory effects of G protein-coupled receptors on cardiac sarcolemmal Na+/H+ exchanger activity: Signalling and significance. Cardiovasc Res 2003; 57:942–52

42. Marshall J, Kupchak P, Zhu W, Yantha J, Vrees T, Furesz S: Processing of serum proteins underlies the mass spectral fingerprinting of myocardial infarction. J Proteome Res 2003; 2:361–72

43. Boyd JH, Chau EH, Tokunanga C, Bateman RM, Haljan G, Davani EY, Wang Y, Walley KR: Fibrinogen decreases cardiomyocyte contractility through an ICAM-1-dependent mechanism. Crit Care 2008; 12:R2

44. Ostos MA, Conconi M, Vergnes L, Baroukh N, Ribalta J, Girona J, Caillaud JM, Ochoa A, Zakin MM: Antioxidative and antiatherosclerotic effects of human apolipoprotein A-IV in apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol 2001; 21:1023–8

45. Navab M, Anantharamaiah GM, Reddy ST, Van Lenten BJ, Fogelman AM: Apo A-1 mimetic peptides as atheroprotective agents in murine models. Curr Drug Targets 2008; 9:204–9

46. Nair KS, Jaleel A, Asmann YW, Short KR, Raghavakaimal S: Proteomic research: Potential opportunities for clinical and physiological investigators. Am J Physiol Endocrinol Metab 2004; 286:863–74

47. Stormorken H, Sakariassen KS: Contrast media effects on hemostatic and thrombotic parameters. Possible consequences for practical techniques and prophylactic measures. Acta Radiol Suppl 1995; 399:173–81