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Effect of Experimental Endotoxemia on Thrombelastography Parameters, Secondary and Tertiary Hemostasis in Dogs

O. Eralp, Z. Yilmaz, K. Failing, A. Moritz, and N. Bauer

Background: Thrombelastography (TEG) and indicators of secondary and tertiary hemostasis might be altered in dogs with endotoxemia.

Hypothesis: Endotoxemia influences measures of coagulation in dogs.

Animals: Ten healthy cross-bred dogs.

Material and Methods: Prospective laboratory study between controls (n = 5) receiving 0.9% saline IV and the study group (n = 5) treated with low-dose lipopolysaccharide (0.02 mg/kg IV). Physical examination and sampling for measurement of leukocytes, platelets, and coagulation variables were performed at time points 0, 1, 4, and 24 hours. Coagulation variables included kaolin-activated TEG, 1-stage prothrombin time (OSPT), activated partial thromboplastin time (aPTT), fibrinogen, factor VIII, antithrombin, protein C, protein S, activated protein C (APC)-ratio calculated from aPTT with and without presence of APC), and D-Dimers.

Results: Endotoxemia-induced clinical signs included lethargy (n = 5/5), diarrhea (n = 4/5), emesis (n = 4/5), and abdominal pain (2/5). After 1 hour there was severe leukopenia ($2.5 \pm 0.7 \times 10^9$/L; mean ± SD, $P < .0001$) and a 2.2-fold increase in D-Dimers (0.81 ± 0.64 mg/L, $P < .0001$). After 4 hours there was hyperthermia (40.3 ± 0.4°C, $P < .0001$) and increases in OSPT (10.5 ± 2.7 seconds, $P < .0001$), aPTT (16.7 ± 5.2 seconds, $P = 0.002$). A significant decrease in fibrinogen (1.5 ± 1.0 g/L, $P = 0.001$), protein C ($31 \pm 33\%$, $P < .0001$), protein S ($63 \pm 47\%$, $P < .0001$), TEG α (58 ± 19, $P = .007$), and TEG maximal amplitude (50 ± 19 mm, $P = .003$) was seen compared with the controls. APC-ratio rose significantly (2.5 ± 0.2, $P < .0001$) without exceeding the reference interval (n = 4/5).

Conclusion and Clinical Importance: D-Dimers are the earliest indicator for endotoxemia-associated coagulation abnormalities followed by decreased protein C concentration. APC-ratio and TEG were not good screening variables.

Key words: Acute phase reaction; Coagulation; Disseminated intravascular coagulation; Protein C; Protein S; Systemic inflammatory response syndrome; Thromboembolism.

The systemic inflammatory response syndrome (SIRS) resulting from bacterial infection remains a major health threat in human and veterinary medicine. Endotoxin, a unique lipopolysaccharide (LPS) found on the outer membrane of gram-negative bacteria, is one of the most potent initiating factors of SIRS.[1] The inflammatory process results in tissue factor expression and generation of proinflammatory mediators and thus in an activation of coagulation, which is subsequently followed by decreased activity of anticoagulant mechanisms because of consumption and depletion of anticoagulant proteins, cytokine-induced downregulation of anticoagulant proteins, and impaired fibrinolysis.[2–4]

Studies in people demonstrated that severe sepsis was associated with decreased activities of protein C and antithrombin (AT).[5–8] Similar findings have been observed in dogs with naturally occurring sepsis.[9–12]

In the majority of studies, the regulation of coagulation after initiation of a septic process has not been investigated. Although studying the early dynamic changes of coagulation after infection would be ideally carried out in canine patients with the naturally developing syndrome, there are ethical limitations to this approach. An alternative method is the use of LPS administration in research animals. However, the overwhelming induction of endotoxemia used in high-dose models of endotoxemia is unlike the smoldering presentation of the septic process typically seen in clinical patients.[13] Thus, the application of information gained from a rapidly fatal, severe insult to a clinical patient with more smoldering disease has been considered to be difficult.[14]

It can be hypothesized that low-dose endotoxemia results in rapid changes of coagulation system possibly characterized by an initially hypercoaguable reaction followed by a hypocoagulatory state.

Thus, it was the aim of our study to characterize the response of the coagulation system to low-dose LPS

Abbreviations:

APC	activated protein C
aPTT	activated partial thromboplastin time
AT	antithrombin
DIC	disseminated intravascular coagulation
FVIII	factor VIII
LPS	lipopolyscaccharide
OSPT	1-stage prothrombin time
PLT	platelet
SIRS	systemic inflammatory response syndrome
TEG	thrombelastography
WBC	white blood cells

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application in healthy dogs compared with a placebo (saline) as reflected by dynamic alterations of traditional and novel coagulation parameters, which have been recently introduced[9,10,15] for this species. Measurements included a point of care test (thrombelastography [TEG]), platelet (PLT) count, white blood cell count (WBC), secondary hemostasis (1-stage prothrombin time [OSPT], activated partial thromboplastin time [aPTT], fibrinogen, factor VIII [FVIII]) as well as physiological anticoagulants (AT, protein C, protein S, APC ratio, ie, the ratio of aPTT measured in the presence and absence of activated protein C [APC] indicating the anticoagulant response of plasma to added APC and thus a resistance against APC in case of a decreased APC ratio), and variables reflecting fibrinolysis (D-Dimers; TEG LY30).

Materials and Methods

Study Design

The prospective investigation was ethically approved by the Animal Care and Use Committee of Uludag University, Bursa, Turkey.

The impact of endotoxemia was assessed prospectively in healthy cross-bred dogs administered LPS. Results were compared with a control group receiving saline as well as with laboratory intern reference intervals. For TEG parameters and variables reflecting secondary and tertiary hemostasis, reference intervals were obtained from 56 healthy adult dogs.[15,16] Briefly, the reference intervals for kaolin-activated TEG variables were as follows: *R*: 1.8–8.6 minutes; *K*: 1.3–5.7 minutes; angle α: 36.9–74.6°; MA: 42.9–67.9 mm, and *G*: 3.2–9.6 kdyn/cm^2.[16]

Diagnosis of disseminated intravascular coagulation (DIC) was made when ≥3 of the traditional coagulation tests were altered and consistent with a coagulation consumption disorder (thrombocytopenia and prolonged clotting times of OSPT, APTT, thrombin time), together with an increment in plasma concentrations of D-Dimers >0.67 μg/L or consumption of the main coagulation inhibitor AT as published previously.[17]

Dogs

Ten adult cross-bred dogs (4 males and 6 females) with a median age 3 years (range 2–5 years) and a median body weight of 18 kg (range 15–22 kg), housed in the Animal Husbandry and Diseases Research and Application Centre of Uludag University, were included in the study. They were healthy based on a normal physical, hematological, and clinical chemical examination. Water was provided ad libitum, and the dogs were fed with standardized, pelleted diet twice daily before the experiment.

The dogs were assigned into 2 groups, the study group (n = 5; 2 males, 3 females) and the control group (n = 5; 2 males, 3 females). Dogs in the study group received endotoxin (LPS)[a] dissolved in sterile saline 0.9%[b] IV over a time period of 5 minutes at a dosage of 0.02 mg/kg. Dogs in the control group received 0.2 mL/kg sterile 0.9% saline solution IV. Dogs were provided water 3 times a day, and food (the pelleted diet) twice a day during the experiments, which were performed October 18–24, 2008. Dogs were monitored clinically and hematologically for 24 hours.

Physical Examination

Physical examination was performed before treatment (0 hour) as well as 1, 4, and 24 hours after administration of LPS or the placebo and body temperature, heart rate, and respiratory rate were recorded.

Sampling

Venous blood samples were collected by venipuncture before application of LPS or the placebo (0 hour) and 1, 4, and 24 hours after treatment from the brachiocephalic vein.

Specimens for hematology were collected into vacutainer tubes containing potassium ethylendiamine-tetraacetate. Hematological examination included measurement of WBCs and PLT count. The anticoagulant tubes were inverted several times immediately after sample acquisition to allow for adequate mixing. Hematological analysis was performed directly after the blood collection with an automatic analyzer with optical scatter and impedance methods.[c] Blood smears were not available to evaluate leukocyte morphology and the number of band neutrophils.

For measurement of coagulation parameters and TEG analysis, sampling and sample preparation were performed as published previously.[15,16] Citrated plasma for coagulation analysis was stored at −80°C until analysis. Analysis was performed within 3 weeks after sampling. Sample stability was proven by the authors to be >12 months (unpublished data) and 7–59 months, respectively, for human specimens.[18]

Test Methods Applied at the STA Compact

Coagulation parameters other than TEG variables were assayed in the Central Laboratory, Faculty of Veterinary Medicine, Justus-Liebig-University, Giessen, Germany with the automated coagulation analyzer STA Compact.[d] The frozen samples were shipped by overnight express (TNT) to the Central Laboratory. Directly after arrival, they were transferred to a −80°C freezer and were analyzed within the next 14 days. Before the analysis, plasma samples were thawed at 37°C in a water bath and centrifuged at 850 × *g* for 10 minutes. Test methods and internal quality control were performed as reported previously.[15]

TEG Analysis

Kaolin-activated TEG analysis was performed as a single test at Uludag University, Bursa, Turkey, with recalcified citrated whole blood according to the manufacturer's recommendations with a TEG5000 analyzer.[e,16]

Statistical Analysis

Results were analyzed with the statistical software packages Graph Pad Prism[f] and BMDP.[g,19] A Shapiro-Wilk test was used to assess the assumption of normality. In case of nonnormal distribution (PLTs, WBC, *R*, and *K* values), logarithmic transformation of data was performed before the analysis was done. The differences of the time course between the 2 groups were assessed by a 2-way analysis of variance with repeated measures regarding the factor "time." In case of a statistical significant interaction between the factors "group" and "time" differences between both groups at different time points were assessed with a *t*-test with pooled variances and pooled degrees of freedom. For the *t*-tests, the level of significance was set at α = 0.013 after Bonferroni's correction. The relation between the frequency of DIC in the control group and the study group was assessed with a 2-tailed Fisher's exact-test. In general, level of significance was set at α = 0.05.

Results

There was a significant impact of endotoxemia on body temperature, WBCs, PLT, MA-value, *G*-value, OSPT, aPTT, fibrinogen, D-Dimer concentration, AT, protein C, and protein S (Figs 1–3). Dogs in the control group did not develop any significant clinical abnormalities after application of the placebo. In the LPS group,

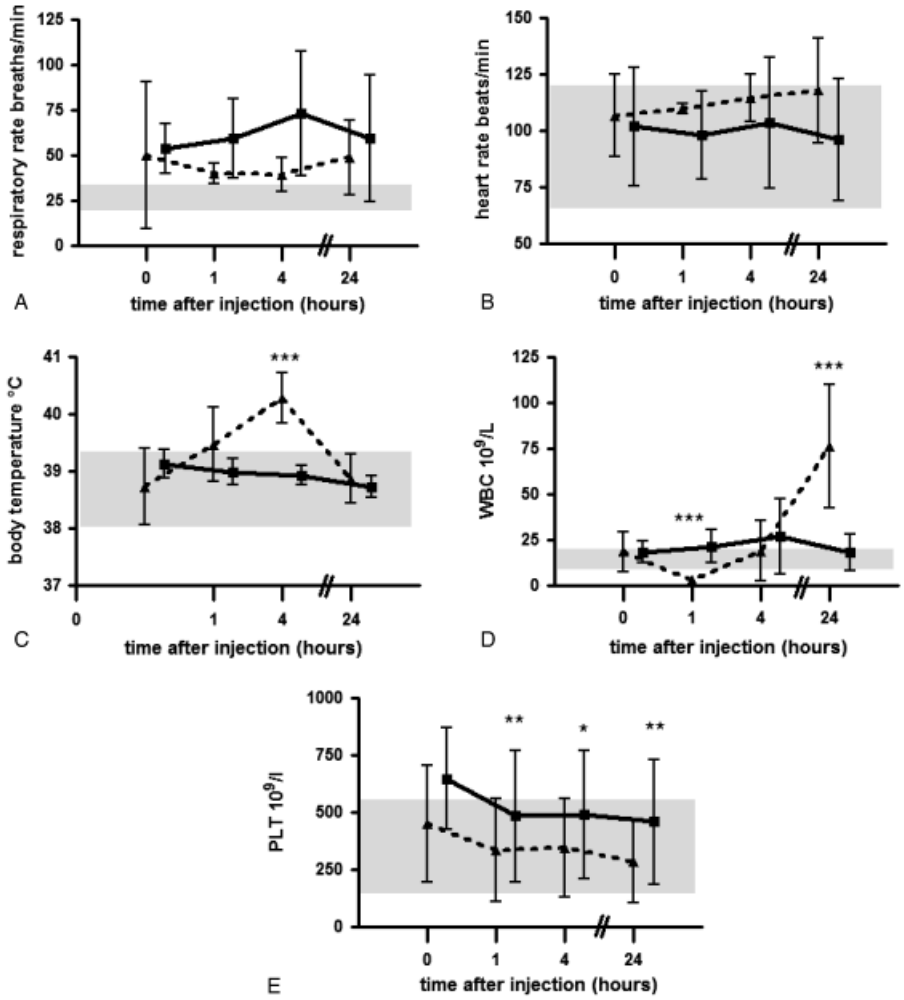

Fig 1. Dynamic alterations in clinical parameters and hematology after LPS injection (dotted line) compared with the control group (solid line). The gray markings are consistent with the reference interval. Data are expressed as group mean ± standard deviation (n = 5 dogs in each group). Level of significance was set at $\alpha = 0.05$ (leading to $P < .013$ after Bonferroni's correction). $^{*}P < .013$; $^{**}P < .005$; $^{***}P < .0001$ compared with the controls at the respective time points. LPS, lipopolyscaccharide; PLT, platelets; WBC, white blood cells; min, minute.

all dogs had clinical signs including lethargy (n = 5/5), diarrhea (n = 4/5), emesis (n = 4/5), and abdominal pain during palpation (2/5) as well as a weak pulse and mildly (n = 2/5) to moderately (n = 1/5) reduced skin turgor and dry mucus membranes.

The respiratory rate of both groups was above the upper limit of the reference interval (34 breaths/min) in the majority of dogs (Fig 1). Respiratory rate and heart rate were not significantly affected by the induction of endotoxemia.

Injection of LPS resulted in a significant increase in body temperature above the upper limit of the reference interval (38.0–39.2°C) when compared with the changes in the control group, reaching a peak of 40.3 ± 0.4°C at time point 4 hours ($P < .0001$, *t*-test).

After induction of endotoxemia, 5/5 dogs developed leukopenia with mean leukocyte counts of $2.5 \pm 0.7 \times 10^9$/L at time point 1 hour (Fig 1D, $P < .0001$, *t*-test). In all dogs, leukopenia was followed by a significant increase in WBCs compared with the mean results in the control group 24 hours after LPS administration with mean WBCs of 76.0 ± 33.7×10^9/L ($P < .0001$, *t*-test). In 2/5 dogs of the control group, a transient increase in leukocyte count (35×10^9/L and 58×10^9/L, respectively) was seen at time point 4 hours, which, however, was not accompanied by fever or any other clinical sign of an inflammatory process.

At time points 1, 4, and 24 hours, PLT count decreased significantly in the endotoxemia group when compared with the means in the placebo group ($P < .005$, *t*-test) and a marked thrombocytopenia was present in 1/5 dogs with PLT counts ranging from 16 to 31×10^9/L (reference interval $150–500 \times 10^9$/L).

Compared with the mean results in the controls, endotoxemia resulted in a mild decrease in angle α (from 71 ± 6° to 65 ± 9°, $P = .007$, *t*-test), MA (from 64 ± 8 to 58 ± 19 mm, $P = .003$, *t*-test), and *G* (9.7 ± 3.3 to 6.1 ± 3.6 kdyn/cm^2, $P = .002$, *t*-test) at time point 4 hours, with

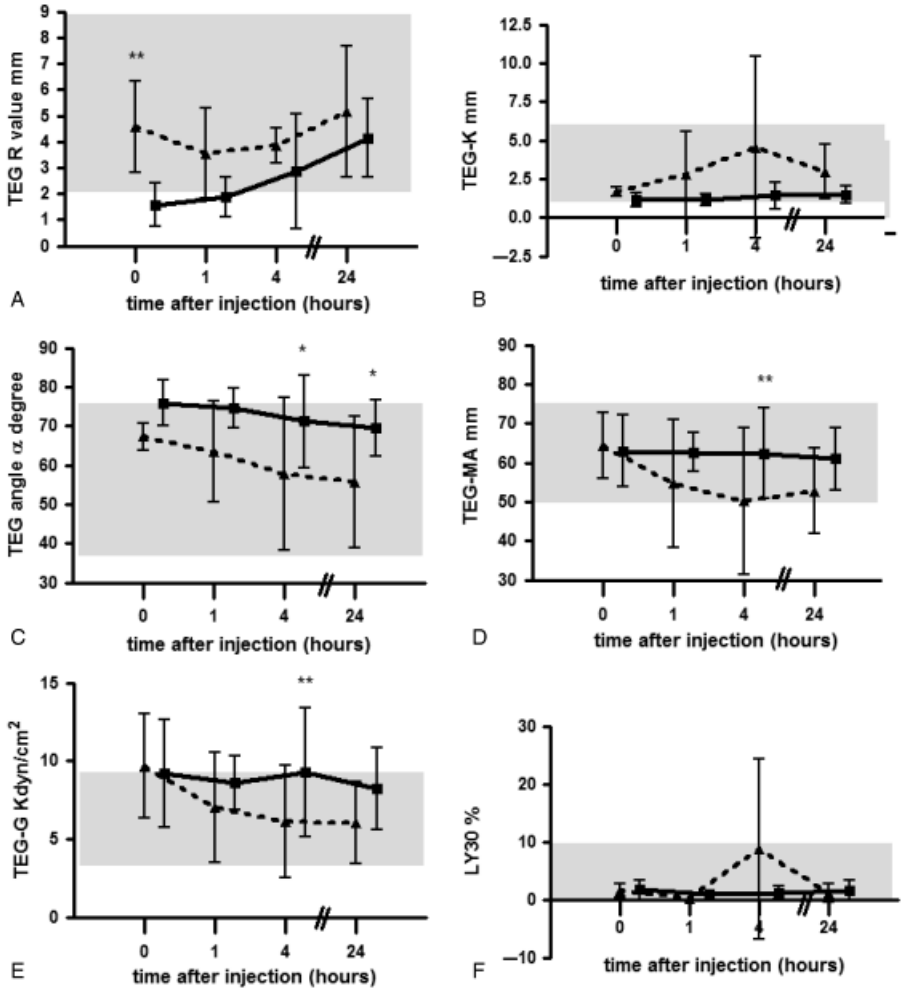

Fig 2. Dynamic changes in thrombelastography parameters after LPS administration (dotted line) compared with the control group (solid line). See Figure 1 for remainder of key. kdyn, kilo dyn; LPS, lipopolyscaccharide; G = overall clot strength; K = kinetic time; LY30 = lysis 30 minutes after the maximal amplitude; mm = millimeter; MA = maximal amplitude; R = reaction time; TEG = thrombelastography.

a broad overlap between both groups (Fig 2). Lowest MA and *G*-values were observed in the dog developing severe thrombocytopenia after LPS administration.

There was a significant increase in OSPT (from 7.2 ± 0.2 to 10.5 ± 2.7 seconds, $P < .0001$, *t*-test) and aPTT (from 12.6 ± 0.9 to 16.7 ± 5.2 seconds, $P = .002$, *t*-test) and a significant decrease in fibrinogen (from 3.0 ± 0.8 to 1.5 ± 1.0 g/L, $P = .001$, *t*-test) 4 hours after LPS administration, which was followed by a significant increase in fibrinogen at time point 24 hours ($P = .007$, *t*-test) (Fig 3).

Within 1 hour after induction of endotoxemia, there was a 2.2-fold increase of D-Dimers from a mean baseline concentration 0.37 ± 0.07 to 0.81 ± 0.64 mg/L ($P < .0001$, *t*-test). A 2nd significant peak was observed at time point 24 hours ($P < .0001$, *t*-test). Overall, mean D-Dimer plasma concentrations were significantly higher than in the control group ($P < .0001$) and remained >0.67 μg/mL for the duration of the experiment.

Endotoxemia was associated with a significant decrease in AT activity at time points 4 (from 146 ± 11 to 127 ± 12%, $P = .009$, *t*-test) and 24 hours (122 ± 8%, $P = .006$, *t*-test) compared with the mean values in the control group

After LPS treatment, protein C activity decreased by 68% from a mean baseline value of 81 ± 15% to lowest measurements at time point 4 hours (mean 31 ± 33%). At time points 4 and 24 hours, 4/5 dogs demonstrated protein C plasma concentrations below the lower limit of the reference interval of 75% and there was a significant difference compared with the means in the control group ($P < .0001$ and $P < .001$, respectively, *t*-test).

A similar effect was seen for protein S activity, decreasing by 34% from a mean baseline value of 108 ± 27% to mean results of 63 ± 47%. Protein S activity was significantly different from the means in the control group at time point 4 hours ($P < .0001$, *t*-test). Endotoxemia induced an increase of APC-ratio from a mean baseline value 2.2 ± 0.1 2.5 ± 0.2. Although the endotoxin-induced raise of APC-ratio was significantly higher than the mean results obtained at the time points 4 and 24 hours in the control group ($P < .0001$, *t*-test), the majority of dogs in the study group demonstrated

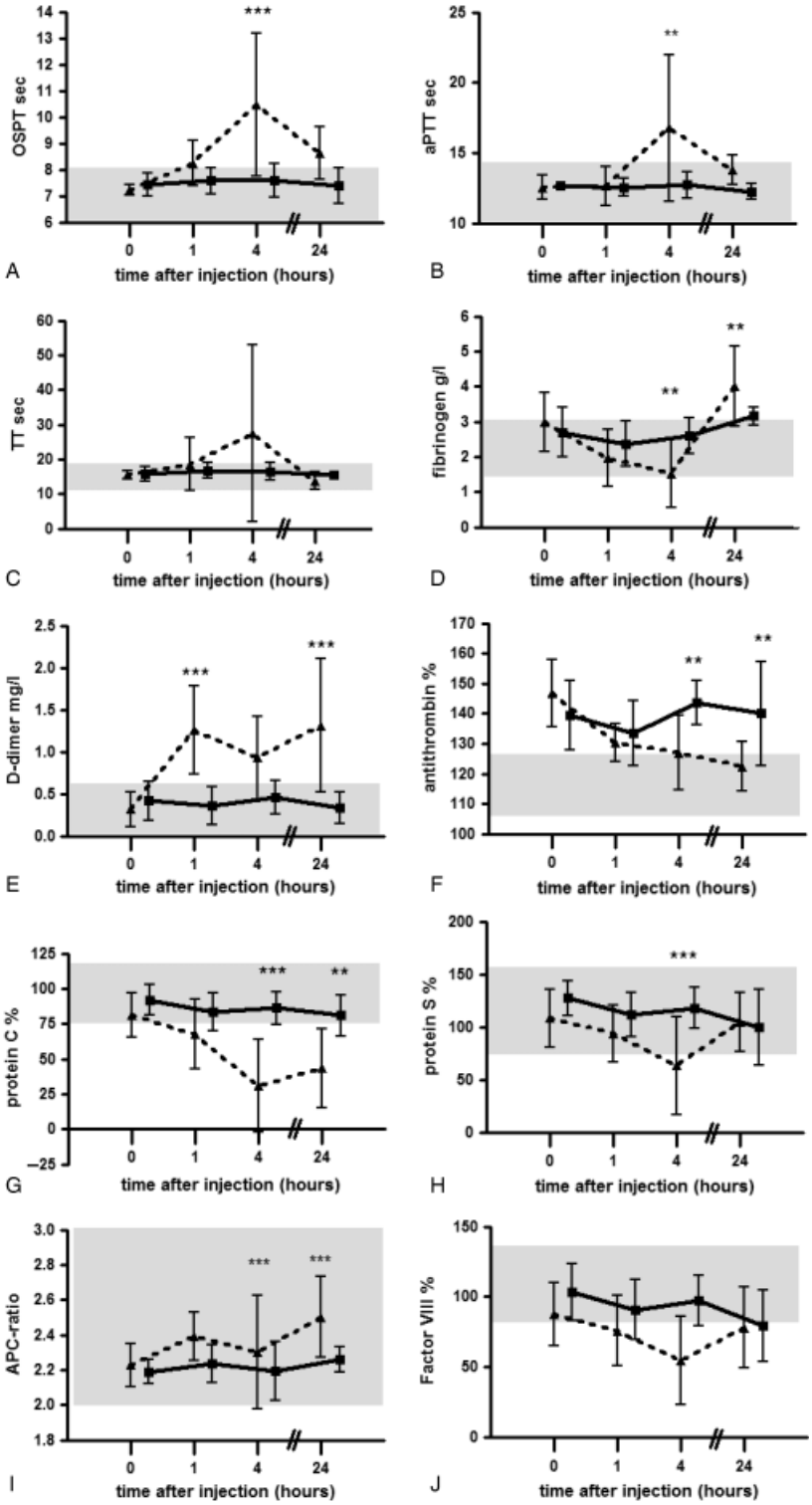

Fig 3. Dynamic alterations in plasma coagulation parameters after LPS injection (dotted line) compared with the control group (solid line). See Figure 1 for remainder of key. APC, activated protein C; aPTT, activated partial thromboplastin time; LPS, lipopolyscaccharide; OSPT, 1-stage prothrombin time; sec, seconds; TT, thrombin time.

an APC-ratio within the reference interval (2.0–3.0). Only in 1/5 dogs of the endotoxemia group, a decreased APC-ratio of 1.71 was recognized 4 hours after application of LPS.

FVIII activity was not significantly affected by injection of LPS.

Three of 5 dogs in the LPS group and none in the control group developed coagulation abnormalities

consistent with DIC after endotoxin injection. Based on the Fisher's exact-test, the frequency of DIC was not significantly different in both groups ($P = .17$)

Discussion

This study documents the response of the coagulation system to LPS administration in otherwise healthy dogs.

The peak of most severe coagulation abnormalities was accompanied by mild to moderate clinical and hematological signs of SIRS (fever and leukopenia). Given the results of the current study, respiratory rate and heart rate are not sensitive parameters to detect the onset of endotoxemia. The respiratory rate was exceeding the reference interval in both groups, which might have been caused by high ambient temperatures. However, maximal ambient temperatures ranged between 18.8 and 23.6°C[h] during the study period so that excitement of the dogs during the physical examination appears to be the most likely cause.

Comparable to the results in the current study in dogs, transient leukopenia was observed 4 hours after application of LPS in dogs[20] and in a feline model of low-dose endotoxemia.[14] Others reported a nadir of leukocyte count 2 hours after the application of 1 μg LPS in dogs.[21] Like in the current study, leukopenia was followed by a leukocytosis 24 hours after administration of endotoxin in the previous study in dogs,[20] whereas in the other experimental investigations in dogs and cats, the study period was <5[21] and 6 hours,[14] respectively, after administration of LPS so that a probable increase in leukocyte counts might have been missed. The transient leukocytosis recognized in 2 dogs of the control group was unexpected as this finding was not associated with any clinical signs or fever indicative of an acute inflammatory process. As the dogs did not show any clinical abnormalities, the etiology of the transient leukocytosis was not detected here. Transient leukocytosis, however, was not associated with coagulation abnormalities so that its impact of the results of the study was considered to be not significant. In contrast to other coagulation parameters, TEG-variables did not differ significantly between controls and study group or results showed a marked overlap between the groups even in case of significance. A probable reason for that might be that only 3/5 dogs developed a DIC. It has been suggested that septic human patients without DIC show a trend toward thrombelastometry (ROTEM) tracings indicative of hypercoagulation, whereas tracings of patients with overt DIC were indicative of hypocoagulation.[22] As individual reaction toward endotoxemia are different, the TEGs of the dogs at a given time point might be hypercoaguable, normocoaguable, or hypocoaguable, which is a possible explanation for the absence of significant TEG findings and the marked overlap between study group and control dogs observed in the current study. The current investigation is the 1st study in dogs evaluating the impact of endotoxemia on fibrinolysis as assessed by TEG. In accordance with the results reported here, evidence of fibrinolysis was also not observed in pigs[i] and in people.[23] It is well known that fibrinolysis is strongly activated and sequentially inhibited in septic processes, which can be detected as an increase in tissue plasminogen activator followed by a subsequent increase in plasminogen activator inhibitor 1 levels. In severe sepsis and septic shock, this activation is followed by an overload and exhaustion of fibrinolytic capacity, and depletion of plasminogen and anticoagulant factors develop.[24] Thus, it was hypothesized previously that the absence of fibrinolysis during TEG in severely septic human patients was caused by the lack of lysing factors.[23] This hypothesis is also a probable explanation for the absence of fibrinolysis found in the current study in dogs and in the investigations in pigs.

A probable reason for discrepant findings in various studies might be different concentrations of endotoxin present in experimental animals or patients. It can be hypothesized that presence of higher amounts of endotoxin results in more inflammation and thus a more severe coagulopathy and prothrombotic state.

This hypothesis is substantiated by previous investigations in dogs and rats, which have shown that the severity of LPS-induced change of variables is often dose-dependent, ie more severe changes were seen if a higher LPS dose was administered.[25,26]

Regarding the results of the current study, the diagnostic use of TEG as early marker of coagulation abnormalities in patients appears to be problematic as the majority of TEG parameters remained within the reference interval and changes are only detected if follow-up examinations are available. A similar finding was obtained in septic human patients without bleeding complications showing thrombelastometry (ROTEM) variables within the reference interval; however, an improvement was seen after treatment.[27]

The response to LPS administration has been reported previously in dogs for several coagulation parameters; however, in the previous study, a markedly higher LPS dose (6 mg/kg) was applied so that the results are not entirely comparable to the findings reported here.[20] In accordance to the current investigation, a transient hypofibringenemia followed by a hyperfibrinogenemia was noted in the previous study as well as a decrease in protein C activity, AT, FVIII, PLT, and the leukocyte count.[20] Activation and subsequent consumption of protein C have been also observed in septic human patients.[6,7,28]

In the current study, the most significant endotoxin-induced change of coagulation parameters was demonstrated for protein C activity and D-Dimers. A similar result was recognized in people with naturally occurring sepsis.[24] In accordance with the current study in dogs, a less pronounced decrease in protein S compared with the AT and protein C plasma activities was seen in people.[5] In contrast to this, others reported that protein S activity was not significantly different even in patients with severe sepsis when compared with the controls.[24,29] The initial decrease in fibrinogen plasma concentration with subsequent rise above the reference interval at time point 24 hours observed in the study group can be explained by a consumption because of DIC, which was followed by an acute phase reaction.[30]

In people, a poor in vitro anticoagulant response of plasma to added APC is termed APC resistance and results in thrombophilia and hypercoagulatory state.[31,32] APC resistance may be because of a mutation of factor V or acquired.[33] As reviewed elsewhere, acquired APC resistance in patients may be associated with acute phase reaction,[34] cancer, or the use of oral contraceptives or increased FVIII activity.[32] APC resistance in the absence of factor V mutation shows a high prevalence of 10–15% in the general human population[35,36] and is associated with a risk of thromboembolism. The original APC resistance assay, which has also been performed in the current study, is based on the ability of APC to prolong the aPTT by inactivating factor Va and factor VIIIa.[32] The test result is expressed as the unitless ratio of aPTT determined in the presence and absence of human APC, ie, the APC-ratio.[32] An APC ratio >2.1 has been considered normal in man[37] and a similar lower reference interval for the APC ratio of 2.0 (confidence interval 1.9–2.1) was recently found in dogs,[15] however, the diagnostic use of this parameter is still unknown for dogs. Based on the results of the current study it can be concluded that the behavior of the APC-ratio is not comparable with its alterations in people observed during the course of inflammation.

In the previous experimental study in dogs evaluating coagulation variables, a markedly higher LPS dose (6 mg/kg) was applied, resulting in the death of 2/5 patients.[20] Sublethal LPS injection was chosen for the current investigation to avoid several pitfalls reported for high-dose bolus endotoxemia models, including acute overwhelming inflammation, induction of an immediate severe hypodynamic circulatory response, atypical massive cytokine production, and rapid mortality.[13] Unlike models of high-dose endotoxemia resulting in rapid mortality, this model is more likely to provide clinically useful data for the evaluation of novel coagulation parameters in canine patients. The reaction to LPS appears to be species-specific as both low-dose (2 μg/kg/h IV for 4 hours)[14] and high-dose (3–10 mg/kg) LPS infusion[38] did not produce significant coagulation abnormalities in cats.

A potential downside of IV endotoxin sepsis models—and also the current study—is that they are not necessarily representative for all situations of clinical disease as septic foci in patients generally seed the body with bacteria continuously rather than over a short period of time.[13] Nevertheless, coagulation parameters were not changed at this time point so that there was no apparent influence on the coagulation process.

In this study, platelet aggregates or clumps could not be excluded as a contributing factor to the thrombocytopenia, as peripheral blood smears were not reviewed. Mild to moderate platelet clumping was reported to contribute to a decrease in platelet count when measured by the impedance method.[39] However, in a recent study, PLTs in canine blood collected in EDTA were minimally clumped in most blood smears and the interpretation of platelet count and platelet indices was not significantly affected, as compared with blood collected in citrate.[40]

Overall, the current study clearly demonstrated that a rise in D-Dimers is the earliest indicator for endotoxemia-associated coagulation abnormalities followed by a marked decrease in protein C, protein S, and AT, whereas alteration in FVIII was not significantly different from the controls in the 1st 24 hours of a systemic inflammatory response caused by endotoxemia. In contrast to humans, APC ratio did not appear to be a good screening parameter in canine septic patients, but further studies are required to elucidate the diagnostic use of this parameter in dogs. TEG variables are often within the reference interval after initiation of endotoxemia, so that follow-up examinations of the dogs are essential to detect abnormalities of the coagulation system.

Footnotes

[a] Lipopolysaccharide, Escherichia coli serotype 055:B5, purity 497%; Sigma, St Louis, MO

[b] NaCl solution; Baxter, Istanbul, Turkey

[c] CELL-DYN 3500; Abbott, Wiesbaden, Germany

[d] STA Compact, Roche Diagnostics GmbH, Mannheim, Germany

[e] TEG 5000 Thrombelastograph, Haemonetics Corporation (formerly Haemoscope Corporation), Braintree, MA

[f] Graph Pad Software, San Diego, CA

[g] BMDP Statistical Software Inc, Los Angeles, CA

[h] Historical weather, Bursa, Turkey: http://www.tutiempo.net/en/Climate/Bursa/10-2008/171160.htm

[i] Nates JL, Doursout M-F, Weavind LM, Chelly J. EA thrombelastographic study of lipopolysaccharide induced coagulation abnormalities in a pig endotoxemic shock model. Critical Care Med 27(12) (Supplement):A102, December 1999 (Abstract poster presentation)

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