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Diesel exhaust inhalation increases thrombus formation in man†

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Aims Although the mechanism is unclear, exposure to traffic-derived air pollution is a trigger for acute myocardial infarction (MI). The aim of this study is to investigate the effect of diesel exhaust inhalation on platelet activation and thrombus formation in men.

Methods and results In a double-blind randomized crossover study, 20 healthy volunteers were exposed to dilute diesel exhaust (350 $\mu g/m^3$) and filtered air. Thrombus formation, coagulation, platelet activation, and inflammatory markers were measured at 2 and 6 h following exposure. Thrombus formation was measured using the Badimon *ex vivo* perfusion chamber. Platelet activation was assessed by flow cytometry. Compared with filtered air, diesel exhaust inhalation increased thrombus formation under low- and high-shear conditions by 24% [change in thrombus area 2229 μm^2, 95% confidence interval (CI) 1143–3315 μm^2, $P = 0.0002$] and 19% (change in thrombus area 2451 μm^2, 95% CI 1190–3712 μm^2, $P = 0.0005$), respectively. This increased thrombogenicity was seen at 2 and 6 h, using two different diesel engines and fuels. Diesel exhaust also increased platelet–neutrophil and platelet–monocyte aggregates by 52% (absolute change 6%, 95% CI 2–10%, $P = 0.01$) and 30% (absolute change 3%, 95% CI 0.2–7%, $P = 0.03$), respectively, at 2 h following exposure compared with filtered air.

Conclusion Inhalation of diesel exhaust increases *ex vivo* thrombus formation and causes *in vivo* platelet activation in man. These findings provide a potential mechanism linking exposure to combustion-derived air pollution with the triggering of acute MI.

Keywords Air pollution • Particulate matter • Thrombosis • Platelet activation

Introduction

Chronic exposure to air pollution is a major cause of cardiovascular morbidity and mortality worldwide.[1] Recently, exposure to traffic-derived air pollution has been associated with the triggering of acute myocardial infarction (MI).[2,3] Although air pollution consists of a heterogeneous mixture of gaseous and particulate matter, adverse cardiovascular events are most strongly associated with exposure to fine particulate matter (diameter <2.5 μm, $PM_{2.5}$).[4,5] An important component of $PM_{2.5}$ is nanoparticulate matter generated during the combustion of diesel fuel.[6] These particles, with an aerodynamic diameter ≤ 100 nm, readily deposit within human alveoli and possess a considerable surface area that may contribute to their biological toxicity.[7]

Despite the strength and consistency of observational data, the pathophysiological mechanisms linking air pollution with adverse cardiovascular events remain unclear. They have been proposed to include endothelial dysfunction,[8–10] myocardial ischaemia,[10]

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altered autonomic function,[11] systemic inflammation,[9] and platelet activation.[12] Thrombosis plays a central role in the pathogenesis of atherosclerosis. As well as contributing to atherogenesis, thrombosis at the site of a disrupted coronary arterial plaque may cause acute vessel occlusion, resulting in an acute coronary syndrome (ACS). In pre-clinical studies employing a thrombotic vascular injury model, tracheal instillation of diesel exhaust particles caused platelet activation and increased arterial and venous thrombus formation.[12] Given that exposure to combustion-derived pollutants appears to act as a trigger for MI and that the majority of such events are due to thrombus formation at the site of an atheromatous plaque, we hypothesized that exposure to diesel exhaust would increase *in vivo* platelet activation and enhance thrombus formation in an *ex vivo* clinical model of arterial injury.

Methods

Subjects

Twenty healthy non-smokers aged between 21 and 44 years were enrolled into the study (*Table 1*). The study was performed with the approval of local research Ethics Committees, in accordance with the Declaration of Helsinki and the written informed consent of all volunteers. Volunteers were recruited using advertisements and from local healthy volunteer databases. Exclusion criteria were the use of regular medication or clinical evidence of atherosclerosis, arrhythmias, diabetes mellitus, hypertension, renal or hepatic impairment, asthma, occupational exposure to air pollution, intercurrent infective disease, or any other clinically significant illness. Subjects had normal lung function and reported no symptoms of respiratory tract infection within the 6-week period preceding the study.

Study design

Subjects attended on two occasions at least one week apart and received either filtered air or dilute diesel exhaust in a double-blind randomized crossover design. Exposures were performed at separate dedicated exposure facilities by technical staff with no involvement in the clinical studies. The order of the exposures was randomized based on an independently determined exposure protocol. Subjects remained indoors following exposures to minimize confounding effects of ambient air pollution. The primary endpoint was *ex vivo* thrombus formation. Secondary endpoints were *in vivo* platelet activation assessed by flow cytometry and changes in haematological and coagulation variables and soluble markers of inflammation.

Clinical studies were performed in dedicated clinical research facilities by clinical staff blinded to exposure allocation. Based on previous vascular and inflammatory studies,[8] initial thrombosis studies were performed 6 h after exposure in eight subjects (protocol 1). In light of the findings, further thrombosis and flow cytometric studies were performed at 2 and 6 h in a separate cohort of 12 subjects (protocol 2). The second protocol was designed to confirm the initial findings, to assess temporal effects, to investigate potential mechanisms, and to determine whether the initial findings were reproducible with a different type of diesel exposure.

Table 1 Baseline volunteer characteristics

	Value
Age (years)	26 ± 5
Height (m)	1.8 ± 0.1
Weight (kg)	73 ± 3
Body mass index (kg/m^2)	22 ± 1
Pulse (bpm)	58 ± 2
Systolic blood pressure (mmHg)	120 ± 3
Diastolic blood pressure (mmHg)	71 ± 2
Forced expiratory volume in 1 s (FEV1, L)	4.9 ± 0.1
% Predicted FEV1	108 ± 3
Forced vital capacity (FVC, L)	5.8 ± 0.2
% Predicted FVC	103 ± 3

Data are presented as mean ± standard deviation.
Pooled data from protocols 1 and 2 ($n = 20$).

Protocol 1

Exposures were performed for 2 h in a mobile ambient particle concentrator exposure laboratory in Edinburgh, UK. During exposures, subjects performed moderate exercise (minute ventilation 25 L/min/m^2) on a bicycle ergometer for 15 min alternated with 15 min rest periods. Temperature and humidity in the chamber were controlled at 22°C and 50%, respectively. Diesel exhaust was generated by an idling engine (type F3M2011, 2.2 L, 500 rpm; Deutz, Germany) using gas oil (Petroplus Refining, UK). Over 90% of the exhaust fumes were shunted away, with the remainder being diluted with air and fed into the exposure chamber at a steady-state concentration. Air in the chamber was continuously monitored with exposures standardized using continuous measurement of nitrogen oxide (NO_x) concentrations to deliver a particulate concentration of 350 μg/m^3. There was little variation in particle mass (348 ± 68 μg/m^3), particle number (1.2 ± 0.1 × 10^6/cm^3), NO_x (0.58 ± 0.03 ppm), NO_2 (0.23 ± 0.02 ppm), NO (0.36 ± 0.02 ppm), CO (3.54 ± 0.76 ppm), and total hydrocarbon (2.8 ± 0.1 μg/m^3) concentrations between exposures.

Protocol 2

In Umeå, Sweden, subjects were exposed for 1 h in a purpose-built diesel exposure chamber according to a standard protocol, as described previously.[8,13] Diesel exhaust was generated by an idling Volvo engine (TD45, 4.5 L, 680 rpm) using Gasoil E10 (Preem, Sweden), as described previously.[8,13] During exposures, subjects performed periods of exercise as described earlier. Exposures were standardized using continuous measurement of NO_x to deliver a particulate concentration of 350 μg/m^3. There was little variation in particle mass (330 ± 12 μg/m^3), particle number (1.26 ± 0.01 × 10^6/cm^3), NO_x (2.78 ± 0.03 ppm), NO_2 (0.62 ± 0.01 ppm), NO (2.15 ± 0.03 ppm), CO (3.08 ± 0.12 ppm), and total hydrocarbon (1.58 ± 0.16 μg/m^3) concentrations between exposures.

Ex vivo thrombosis studies

Thrombus formation was measured using the Badimon chamber. This technique has principally been used previously to assess the efficacy of novel antithrombotic agents.[14–16] In brief, a pump was used to draw blood from an antecubital vein through a series of three cylindrical perfusion chambers maintained at 37°C in a water bath. Carefully prepared strips of porcine aorta, from which the intima and a thin layer of media had been removed, acted as the thrombogenic substrate. The rheological conditions in the first chamber simulate those of patent coronary arteries (low-shear rate, ~212 s^{-1}), whereas those in the

second and third chambers simulate those of mildly stenosed coronary arteries (high-shear rate, $\sim$1690 s^{-1}). The model thus acts as one of the deep coronary arterial injury. Each study lasted for 5 min during which flow was maintained at a constant rate of 10 mL/min. All studies were performed using the same perfusion chamber and by the same operator.

Immediately after each study, porcine strips with thrombus attached were removed and fixed in 4% paraformaldehyde. Strips were wax-embedded, sectioned, and stained with Masson's Trichrome. Images were acquired at $\times$20 magnification, and the thrombus area was measured using an Ariol image acquisition system (Applied Imaging, USA) and Image-Pro Plus software (Media Cybernetics, USA) by a blinded operator. Results from at least six sections were averaged to determine thrombus area for each chamber, as described previously.[14–16]

Flow cytometry

Samples were obtained at 2 and 6 h, immediately prior to each thrombosis study, and processed according to previously described protocols.[17] In brief, blood was taken from an antecubital vein using a 21-gauge cannula and anticoagulated with D-phenylalanyl-L-prolyl-L-arginine chloromethylketone (75 μm; Cambridge Biosciences, UK). Samples were not analysed unless venesection achieved rapid and uninterrupted blood flow. Five minutes after sample collection, samples were stained with the following conjugated monoclonal antibodies: phycoerythrin (PE)-conjugated CD14 (Dako, Denmark), PE-conjugated CD62P, and PE-conjugated CD154 (Becton-Dickinson, UK); PE-conjugated CD11b, PE-conjugated CD40, fluorescein isothiocyanate (FITC)-conjugated CD42a, and FITC-conjugated CD14 (Serotec, USA); and appropriate control isotypes. All antibodies were diluted 1:20. Once stained, samples were incubated for 20 min at room temperature to identify P-selectin and CD40L on the platelet surface and CD40 on the monocyte surface. Monocyte and platelet–leucocyte samples were fixed with FACS-Lyse (Becton-Dickinson). Platelet samples were fixed with 1% paraformaldehyde. Samples were analysed within 24 h using a FACScan flow cytometer (Becton-Dickinson). Platelet–monocyte and platelet–neutrophil aggregates were defined as monocytes or neutrophils positive for CD42a. Data analysis was performed using FlowJo (Treestar, USA).

Blood sampling

Samples were obtained before exposure and at 2 and 6 h. Samples were analysed for total white cell count, differential cell count, and platelets by an autoanalyser. Plasma interleukin-6 (IL-6), tumour necrosis factor-α (TNF-α), soluble CD40 ligand (sCD40L), soluble P-selectin, intercellular adhesion molecule-1 (ICAM-1), and C-reactive protein were measured with commercially available ELISAs (R&D Systems, UK). Prothrombin time (PT, reagents from Medirox, Sweden) and activated partial thromboplastin time (aPTT, reagents from Dade Behring, USA) were measured using a CA-7000 analyser (Sysmex, Japan).

Statistical analysis

Data presented are pooled from protocols 1 and 2 unless otherwise stated. Continuous variables are reported as mean $\pm$ standard deviation. Statistical analysis was performed in Excel (Microsoft Corporation, USA), using a modified *t*-test (two-sided) to account for potential period effects.[18] Statistical significance was taken at *P*-value less than 0.05.

Results

Exposures and clinical studies were well tolerated with no adverse symptoms reported. All volunteers completed both study visits.

Total leucocyte, monocyte and platelet counts, PT, and aPTT were unaltered following dilute diesel exhaust and filtered air (*Table 2*). Although neutrophil count appeared to increase and lymphocyte count appeared to decrease following both exposures, there were no differences in the magnitude of these changes following dilute diesel exhaust compared with filtered air (*Table 2*).

Markers of inflammation and platelet activation

There was a heterogeneous cytokine response following both dilute diesel exhaust and filtered air exposures (*Table 3*, data from protocol 2). Changes in plasma TNF-α, IL-6, C-reactive protein, and soluble ICAM-1 concentrations were similar following both exposures. Following dilute diesel exhaust exposure, plasma sCD40L concentrations were increased at 2 h ($P = 0.003$ vs. filtered air), and the fall at 6 h following filtered air exposure was attenuated ($P = 0.011$ vs. filtered air). Similarly, the fall in plasma soluble P-selectin concentration at 6 h following filtered air exposure was attenuated following diesel exhaust exposure ($P = 0.003$).

Flow cytometry

Monocyte surface expression of CD40 and platelet surface expression of CD40L and P-selectin were similar following dilute diesel exhaust and filtered air exposure (data on file). Compared with filtered air, diesel exhaust exposure increased platelet–neutrophil and platelet–monocyte aggregates at 2 h by 52% [absolute change 6%, 95% confidence interval (CI) 2–10%, $P = 0.01$] and 30% (absolute change 3%, 95% CI 0.2–7%, $P = 0.03$), respectively (*Figure 1*, data from protocol 2). There was a trend towards similar increases at 6 h, although these were not statistically significant.

Thrombus formation

Thrombus formation increased following dilute diesel exhaust by 23% in the low-shear chamber (change in thrombus area 1941 μm^2, 95% CI 873–3008 μm^2, $P = 0.002$) and by 21% in the high-shear chamber (change in thrombus area 2916 μm^2, 95% CI 1365–4466 μm^2, $P = 0.001$), compared with filtered air at 6 h (*Figure 2*).

In protocol 2, thrombus formation at 2 h increased by 27% in the low-shear chamber and 21% in the high-shear chamber following dilute diesel compared with filtered air (change in thrombus area 2772 μm^2, 95% CI 879–4721 μm^2, $P = 0.04$ and change in thrombus area 2312 μm^2, 95% CI 597–4026 μm^2, $P = 0.014$, respectively; *Figure 3*). Likewise, thrombus formation at 6 h was increased by 22% (change in thrombus area 2254 μm^2, 95% CI 244–3924 μm^2, $P = 0.033$) in the low-shear chamber and appeared to increase (13%; change in thrombus area 1467 μm^2, 95% CI −230–3163 μm^2, $P = 0.083$) in the high-shear chamber (*Figure 3*, data from protocol 2).

Table 2 Effects of dilute diesel exhaust on haematological and coagulation variables

	Before exposure	2 h	6 h	Δ 2 h	Δ 6 h	*P*-values	
						2 h	6 h
Filtered air							
Leucocytes ($\times 10^9$ cells/L)	5.44 ± 1.42	5.27 ± 1.16	5.75 ± 1.07	−0.18 ± 1.08	0.26 ± 1.19	—	—
Lymphocytes ($\times 10^9$ cells/L)	2.17 ± 0.67	1.92 ± 0.52	1.68 ± 0.43	−0.48 ± 0.46	−0.51 ± 0.53	—	—
Neutrophils ($\times 10^9$ cells/L)	2.62 ± 0.69	3.00 ± 0.75	3.51 ± 0.91	0.42 ± 0.68	0.89 ± 0.86	—	—
Monocytes ($\times 10^9$ cells/L)	0.47 ± 0.16	0.42 ± 0.14	0.41 ± 0.16	−0.06 ± 0.11	−0.06 ± 0.10	—	—
Platelets ($\times 10^9$ cells/L)	223 ± 38	221 ± 35	223 ± 30	−1.80 ± 18	1.79 ± 22	—	—
INR[a]	1.00 ± 0.06	1.03 ± 0.07	1.04 ± 0.07	0.02 ± 0.04	0.03 ± 0.05	—	—
aPTT[a] (sec)	28.9 ± 0.91	29.1 ± 0.89	28.7 ± 0.90	0.28 ± 0.47	−0.20 ± 0.58	—	—
Diesel exhaust							
Leucocytes ($\times 10^9$ cells/L)	5.45 ± 1.22	5.51 ± 1.54	5.53 ± 1.31	0.06 ± 1.34	0.07 ± 1.41	0.54	0.52
Lymphocytes ($\times 10^9$ cells/L)	2.20 ± 0.73	1.67 ± 0.55	1.61 ± 0.42	−0.53 ± 0.36	−0.59 ± 0.58	0.60	0.30
Neutrophils ($\times 10^9$ cells/L)	2.60 ± 0.64	3.26 ± 1.42	3.38 ± 1.17	0.68 ± 1.27	0.81 ± 1.17	0.90	0.90
Monocytes ($\times 10^9$ cells/L)	0.49 ± 0.11	0.45 ± 0.12	0.45 ± 0.13	−0.07 ± 0.09	−0.08 ± 0.08	0.69	0.60
Platelets ($\times 10^9$ cells/L)	227 ± 38	223 ± 47	218 ± 31	−4.15 ± 21	−3.74 ± 16	0.72	0.32
INR[a]	1.02 ± 0.06	1.02 ± 0.08	1.03 ± 0.08	0.01 ± 0.03	0.01 ± 0.03	0.55	0.26
aPTT[a] (s)	29.3 ± 1.39	29.22 ± 0.87	29.2 ± 0.89	−0.07 ± 0.86	−0.06 ± 0.74	0.22	0.83

Data are presented as mean ± standard deviation.
P-values are for comparison of diesel exhaust vs. filtered air.
aPTT, activated partial thromboplastin time; INR, international normalized ratio of prothrombin time.
Pooled data from protocols 1 and 2, ($n = 20$, except [a]unavailable for protocol 1).

Table 3 Effects of dilute diesel exposure on markers of inflammation and platelet activation

	Before exposure	2 h	6 h	Δ 2 h	Δ 6 h	*P*-values	
						Δ 2 h	Δ 6 h
Filtered air							
TNF-α (pg/mL)	0.62 ± 0.73	—	0.36 ± 0.32	—	−0.26 ± 0.30	—	—
IL-6 (pg/mL)	0.60 ± 0.76	—	0.88 ± 0.80	—	0.28 ± 1.1	—	—
C-reactive protein (mg/L)	1.02 ± 0.66	1.09 ± 1.42	1.13 ± 1.64	0.07 ± 0.17	0.12 ± 0.16	—	—
Soluble CD40L (pg/mL)	66 ± 29	75 ± 8	41 ± 14	9.00 ± 29	−20 ± 26	—	—
Soluble P-selectin (mg/mL)	61 ± 27	37 ± 8	44 ± 18	−25 ± 22	−18 ± 26	—	—
Soluble ICAM-1 (mg/mL)	278 ± 65	170 ± 25	271 ± 69	−109 ± 56	−6.40 ± 66	—	—
Diesel exhaust							
TNF-α (pg/mL)	0.55 ± 0.41	—	0.57 ± 0.44	—	0.06 ± 0.44	—	0.11
IL-6 (pg/mL)	0.27 ± 0.19	—	0.78 ± 0.61	—	0.52 ± 0.66	—	0.51
C-reactive protein (mg/L)	0.67 ± 0.65	0.66 ± 0.94	0.65 ± 0.85	−0.01 ± 0.10	−0.02 ± 0.04	0.48	0.30
Soluble CD40L (pg/mL)	48 ± 16	78 ± 8	42 ± 10	30 ± 15	−0.85 ± 18	0.01	0.01
Soluble P-selectin (mg/mL)	51 ± 18	37 ± 10	54 ± 14	−14 ± 13	2.89 ± 16	0.11	0.003
Soluble ICAM-1 (mg/mL)	263 ± 56	181 ± 33	280 ± 58	−82 ± 37	17 ± 59	0.49	0.64

Data are presented as mean ± standard deviation.
Data from protocol 2 ($n = 12$).
P-values are for the comparison of diesel exhaust vs. filtered air.
TNF-α, tumour necrosis factor-α; IL-6, interleukin-6; CD40L, CD40 ligand; ICAM-1, intercellular adhesion molecule-1.

Discussion

Short-term exposure to traffic-derived air pollution is associated with acute cardiovascular events.[2,3] This is the first study to demonstrate that inhalation of diesel exhaust, a common urban air pollutant, causes platelet activation and enhances thrombus formation in men. This provides a plausible mechanism linking exposure to particulate air pollution with acute cardiovascular events including MI.

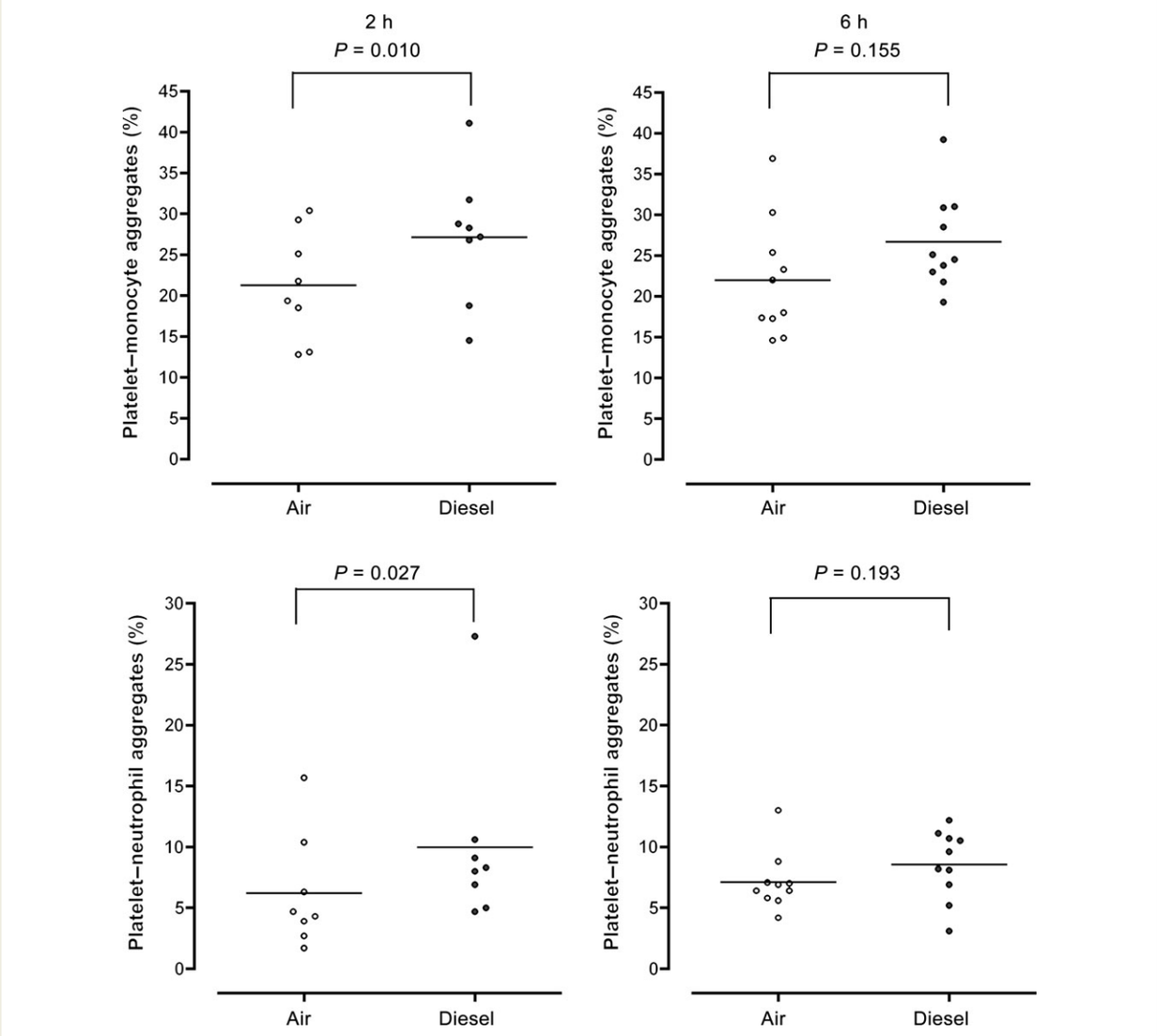

Figure 1 Platelet–leucocyte aggregates 2 and 6 h following dilute diesel exhaust (●) and filtered air (○) exposures (protocol 2, $n = 12$).

Effect of diesel exhaust on thrombosis

Despite the suggestion from observational studies that exposure to combustion-derived air pollution is associated with MI, few studies have examined whether controlled exposure alters thrombotic potential. Developing a reproducible *in vivo* model of thrombosis for use in human studies is challenging. We therefore used the Badimon chamber as a validated *ex vivo* model of arterial injury and thrombosis.[14] It has previously been used to evaluate the effects of novel antithrombotic regimens and has a number of advantages over other techniques.[14–16] It allows the measurement of thrombus formation in native (non-anticoagulated) whole blood triggered by exposure to a physiologically relevant substrate and under flow conditions mimicking those in diseased coronary arteries. Thus, this is a particularly relevant model as it broadly simulates the intra-arterial conditions following spontaneous or iatrogenic plaque disruption within the coronary vasculature.

Taken together with our previous finding that dilute diesel exhaust exposure impairs endothelial t-PA release,[8] we suggest that enhanced thrombus formation is an important mechanism that may explain the association of MI shortly after traffic pollution exposure.

Effect of diesel exhaust on platelet activation

Platelets are key components of arterial thrombosis. Shortening of closure times in a platelet function analyser have been observed following tracheal instillation of diesel exhaust particles in hamsters[12] and their addition to human blood enhances platelet

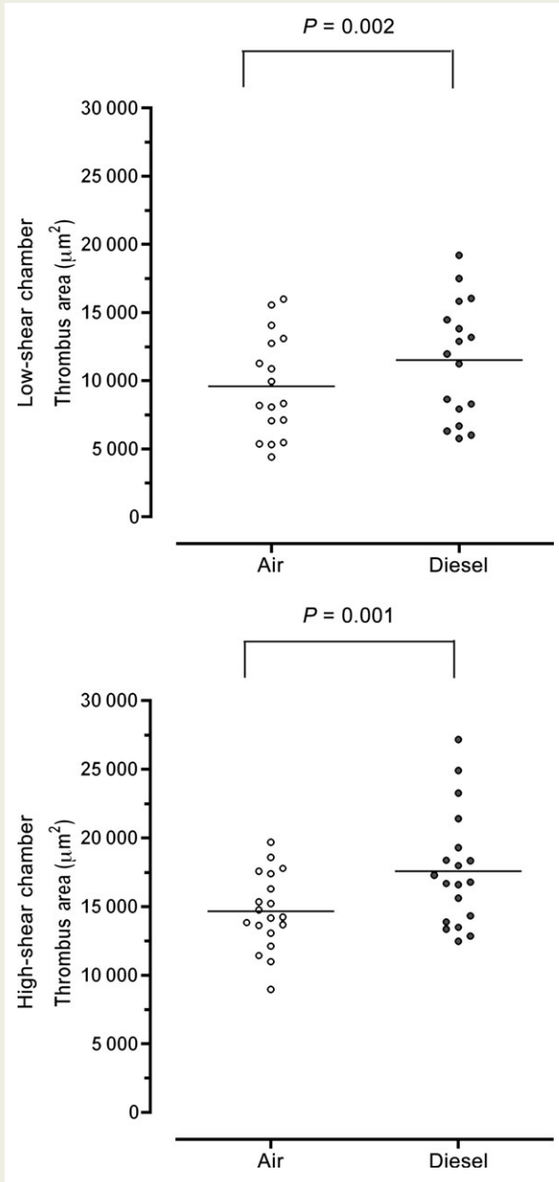

Figure 2 Thrombus formation 6 h following dilute diesel exhaust (●) and filtered air (○) exposures (protocols 1 and 2, $n = 20$).

aggregation.[19] Here, we used flow cytometry to measure the surface expression of platelet and leucocyte activation markers as well as platelet–leucocyte aggregates, a technique increasingly recognized as the gold standard measure of *in vivo* platelet activation, including in patients with ACS.[20]

We observed an increase in platelet–neutrophil and platelet–monocyte aggregates after dilute diesel exhaust exposure, suggesting that enhanced thrombus formation was mediated through platelet activation. These findings are consistent with an increase in circulating platelet–leucocyte aggregates observed in women exposed to biomass smoke.[21] In addition, tracheal instillation of carbon nanotubes increased platelet–leucocyte aggregates and thrombus formation in a murine model of vascular injury.[22] Interestingly, blockade of P-selectin abrogated platelet–leucocyte aggregation and thrombus formation, suggesting that P-selectin serves as a link between pulmonary inflammation, systemic inflammation, and enhanced thrombogenicity. Although platelet–monocyte binding is principally dependent on P-selectin, we did not observe an increase in the platelet surface expression of P-selectin. However, in patients with MI, platelet–monocyte aggregates have been shown to be a more sensitive marker of platelet activation than P-selectin,[23] as P-selectin is rapidly shed from the platelet surface.[24] Exposure to dilute diesel exhaust also increased plasma sCD40L levels. This is in keeping with studies that demonstrated upregulation of the CD40/CD40L pathway in cigarette smokers[25] and following exposure to ultrafine particles.[26,27] As platelets contain large amounts of CD40L that is released following activation,[28] the increase in sCD40L we observed further strengthens the argument that the enhanced thrombus formation observed was driven principally by platelet activation.

It is not possible from our study to determine the mechanism of platelet activation. Debate remains as to whether inhaled components of diesel exhaust can translocate into the systemic circulation[29,30] to mediate direct effects on blood and vascular components. A substantial body of evidence supports a role for oxidative stress and inflammation in mediating the adverse effects of air pollution.[31] Although we did not observe an increase in cellular or soluble inflammatory markers, this does not preclude a role for factors not assessed here. The ability of diesel exhaust exposure to cause pulmonary inflammation is not in doubt,[32] and we have demonstrated previously that diesel particles are capable of generating free radicals,[33] which may activate platelets by reducing endothelial and platelet-derived nitric oxide and antioxidants.

Effect of diesel exhaust on coagulation

A number of previous ambient and controlled exposure studies have evaluated the association between plasma concentrations of coagulation factors and particulate air pollution with mixed results. Although some have demonstrated increased levels of fibrinogen[34–36] and von Willebrand factor,[37] other studies measuring the same factors have failed to show any association with particulate exposure.[37–39] This apparent disparity may well be explained by variations in study design and perhaps, more importantly, the type of exposure investigated. Two previous studies have investigated the effect of controlled diesel exhaust exposure on coagulation factors in men, with neither demonstrating a significant effect.[40,41] Although a recent observational study reported a small reduction in PT associated with ambient exposure to PM_{10}, [42] we found no effect on PT or aPTT following exposure to diesel exhaust. Despite higher effective particulate matter concentrations, our findings are in keeping with previous controlled diesel exposure studies that failed to demonstrate changes in fibrinogen, von Willebrand factor, D-dimer, pro-thrombin fragments 1 and 2, tissue-plasminogen activator, and plasminogen activator inhibitor-1.[40,41]

Population risk and diesel exposure

Diesel exhaust is an important source of combustion-derived air pollution. We have now performed a large number of inhalation

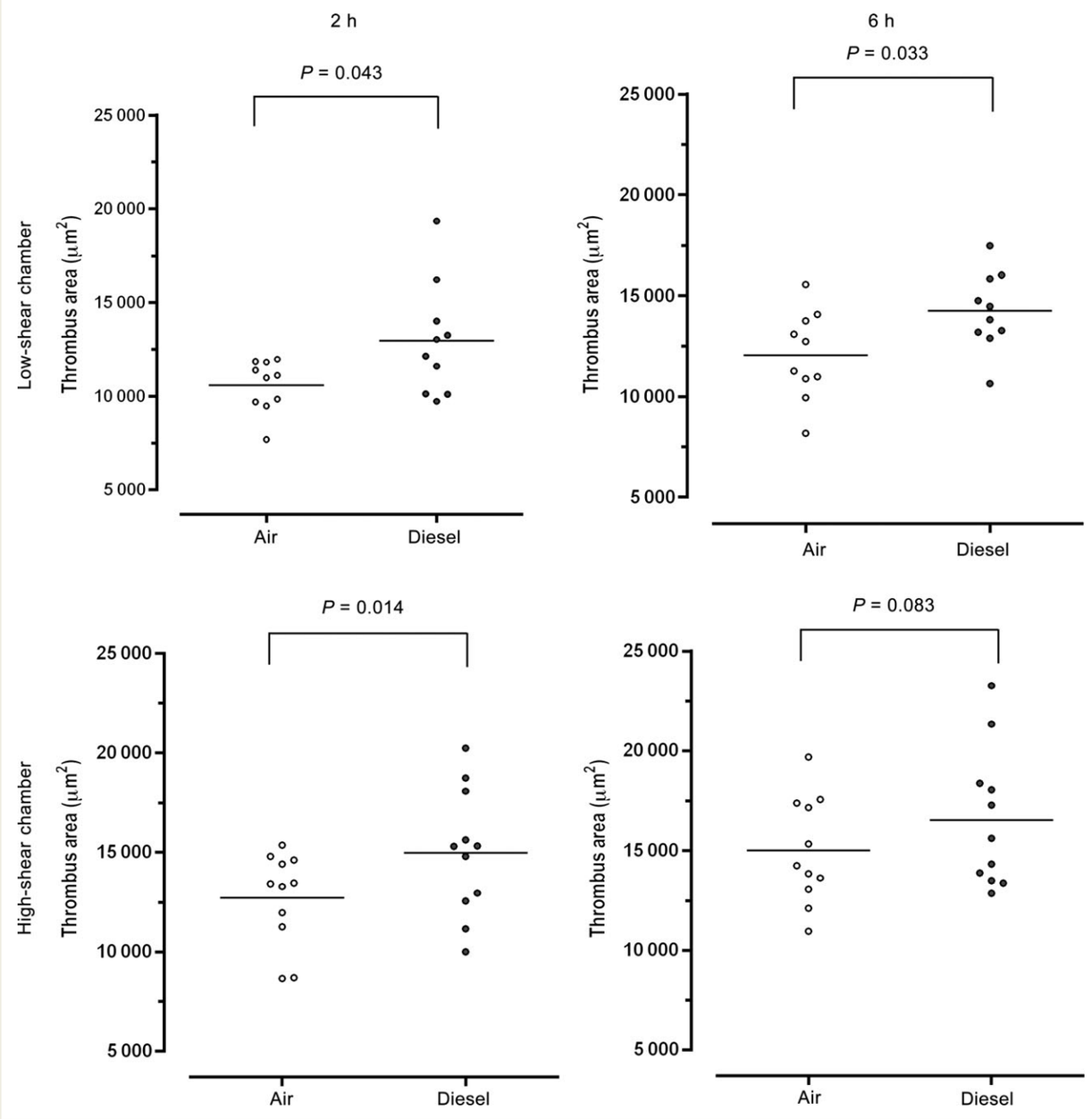

Figure 3 Thrombus formation 2 and 6 h following dilute diesel exhaust (●) and filtered air (○) exposures (protocol 2, $n = 12$).

exposures in healthy subjects and patients with cardiovascular disease using well-characterized systems. Particulate levels during these exposures are comparable with those in heavy traffic and occupational settings in large cities. Here, we used two types of diesel engines and two different commercially available fuels. The particulate component was similar in both protocols. Despite differences in the method of diesel exhaust generation and the gaseous component, prothrombotic effects were consistent.

Although the overall implications of exposure to traffic-derived pollution are significant from a population perspective, individual risk is modest. Observational data support the notion that risk is greatest in those with pre-existing cardiovascular disease. Indeed, we have recently demonstrated that dilute diesel exhaust inhalation has pro-ischaemic effects in patients with prior MI.[10] In the present study, we have extended these findings using a clinical model of severe arterial injury that is reflective of the intravascular conditions in a patient with a ruptured or denuded atheromatous plaque. Our findings of enhanced platelet activation and thrombus formation further highlight the potential-increased propensity of 'at-risk' populations to suffer adverse cardiovascular consequences following exposure to air pollution, although it is not possible from this study to determine

whether diesel exhaust exposure enhances thrombogenicity in patients on antiplatelet therapies.

Conclusions

Inhalation of dilute diesel exhaust causes platelet activation and increased thrombus formation in men. This study provides a plausible pathophysiological link that may explain the association between combustion-derived air pollution and acute cardiovascular events. Further work is required to clarify more precisely the mechanism of enhanced thrombogenicity and to investigate how this potentially harmful effect may be abrogated.

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References

1. Brunekreef B, Holgate ST. Air pollution and health. *Lancet* 2002;**360**:1233–1242.
2. Peters A, Dockery DW, Muller JE, Mittleman MA. Increased particulate air pollution and the triggering of myocardial infarction. *Circulation* 2001;**103**:2810–2815.
3. Peters A, von Klot S, Heier M, Trentinaglia I, Hormann A, Wichmann HE, Lowel H. Exposure to traffic and the onset of myocardial infarction. *N Engl J Med* 2004;**351**:1721–1730.
4. Dockery DW, Pope CA III, Xu X, Spengler JD, Ware JH, Fay ME, Ferris BG Jr, Speizer FE. An association between air pollution and mortality in six US cities. *N Engl J Med* 1993;**329**:1753–1759.
5. Miller KA, Siscovick DS, Sheppard L, Shepherd K, Sullivan JH, Anderson GL, Kaufman JD. Long-term exposure to air pollution and incidence of cardiovascular events in women. *N Engl J Med* 2007;**356**:447–458.
6. Charron A, Harrison RM. Fine (PM2.5) and coarse (PM2.5-10) particulate matter on a heavily trafficked London highway: sources and processes. *Environ Sci Technol* 2005;**39**:7768–7776.
7. Donaldson K, Brown D, Clouter A, Duffin R, MacNee W, Renwick L, Tran L, Stone V. The pulmonary toxicology of ultrafine particles. *J Aerosol Med* 2002;**15**:213–220.
8. Mills NL, Tornqvist H, Robinson SD, Gonzalez M, Darnley K, MacNee W, Boon NA, Donaldson K, Blomberg A, Sandstrom T, Newby DE. Diesel exhaust inhalation causes vascular dysfunction and impaired endogenous fibrinolysis. *Circulation* 2005;**112**:3930–3936.
9. Tornqvist H, Mills NL, Gonzalez M, Miller MR, Robinson SD, Megson IL, Macnee W, Donaldson K, Soderberg S, Newby DE, Sandstrom T, Blomberg A. Persistent endothelial dysfunction following diesel exhaust inhalation in man. *Am J Respir Crit Care Med* 2007;**176**:325–326.
10. Mills N, Tornqvist H, Gonzalez M, Vink E, Robinson SD, Soderberg S, Boon NA, Donaldson K, Sandstrom T, Blomberg A, Newby DE. Ischemic and thrombotic effects of dilute diesel exhaust inhalation in men with coronary heart disease. *N Engl J Med* 2007;**357**:1075–1082.
11. Gold DR, Litonjua A, Schwartz J, Lovett E, Larson A, Nearing B, Allen G, Verrier M, Cherry R, Verrier R. Ambient pollution and heart rate variability. *Circulation* 2000;**101**:1267–1273.
12. Nemmar A, Hoet PH, Dinsdale D, Vermylen J, Hoylaerts MF, Nemery B. Diesel exhaust particles in lung acutely enhance experimental peripheral thrombosis. *Circulation* 2003;**107**:1202–1208.
13. Rudell B, Sandstrom T, Hammarstrom U, Ledin ML, Horstedt P, Stjernberg N. Evaluation of an exposure setup for studying effects of diesel exhaust in humans. *Int Arch Occup Environ Health* 1994;**66**:77–83.
14. Badimon L, Turitto V, Rosemark JA, Badimon JJ, Fuster V. Characterization of a tubular flow chamber for studying platelet interaction with biologic and prosthetic materials: deposition of indium 111-labeled platelets on collagen, subendothelium, and expanded polytetrafluoroethylene. *J Lab Clin Med* 1987;**110**:706–718.
15. Osende JI, Badimon JJ, Fuster V, Herson P, Rabito P, Vidhun R, Zaman A, Rodriguez OJ, Lev EI, Rauch U, Heflt G, Fallon JT, Crandall JP. Blood thrombogenicity in type 2 diabetes mellitus patients is associated with glycemic control. *J Am Coll Cardiol* 2001;**38**:1307–1312.
16. Wahlander K, Eriksson-Lepkowska M, Nystrom P, Eriksson UG, Sarich TC, Badimon JJ, Kalies I, Elg M, Bylock A. Antithrombotic effects of ximelagatran plus acetylsalicylic acid (ASA) and clopidogrel plus ASA in a human *ex vivo* arterial thrombosis model. *Thromb Haemost* 2006;**95**:447–453.
17. Harding SA, Sarma J, Din JN, Maciocia PM, Newby DE, Fox KA. Clopidogrel reduces platelet–leucocyte aggregation, monocyte activation and RANTES secretion in type 2 diabetes mellitus. *Heart* 2006;**92**:1335–1337.
18. Pocock SJ. *Clinical Trials: A Practical Approach*. John Wiley & Sons; 1983.
19. Radomski A, Jurasz P, Alonso-Escolano D, Drews M, Morandi M, Malinski T, Radomski MW. Nanoparticle-induced platelet aggregation and vascular thrombosis. *Br J Pharmacol* 2005;**146**:882–893.
20. Freedman JE, Loscalzo J. Platelet–monocyte aggregates: bridging thrombosis and inflammation. *Circulation* 2002;**105**:2130–2132.
21. Ray MR, Mukherjee S, Roychoudhury S, Bhattacharya P, Banerjee M, Siddique S, Chakraborty S, Lahiri T. Platelet activation, upregulation of CD11b/ CD18 expression on leukocytes and increase in circulating leukocyte–platelet aggregates in Indian women chronically exposed to biomass smoke. *Hum Exp Toxicol* 2006;**25**:627–635.
22. Nemmar A, Hoet PH, Vandervoort P, Dinsdale D, Nemery B, Hoylaerts MF. Enhanced peripheral thrombogenicity after lung inflammation is mediated by platelet–leukocyte activation: role of P-selectin. *J Thromb Haemost* 2007;**5**:1217–1226.
23. Michelson AD, Barnard MR, Krueger LA, Valeri CR, Furman MI. Circulating monocyte–platelet aggregates are a more sensitive marker of *in vivo* platelet activation than platelet surface P-selectin: studies in baboons, human coronary intervention, and human acute myocardial infarction. *Circulation* 2001;**104**:1533–1537.
24. Michelson AD, Barnard MR, Hechtman HB, MacGregor H, Connolly RJ, Loscalzo J, Valeri CR. *In vivo* tracking of platelets: circulating degranulated platelets rapidly lose surface P-selectin but continue to circulate and function. *Proc Natl Acad Sci USA* 1996;**93**:11877–11882.
25. Harding SA, Sarma J, Josephs DH, Cruden NL, Din JN, Twomey PJ, Fox KA, Newby DE. Upregulation of the CD40/CD40 ligand dyad and platelet–monocyte aggregation in cigarette smokers. *Circulation* 2004;**109**:1926–1929.
26. Becker S, Soukup J. Coarse(PM(2.5-10)), fine(PM(2.5)), and ultrafine air pollution particles induce/increase immune costimulatory receptors on human blood-derived monocytes but not on alveolar macrophages. *J Toxicol Environ Health A* 2003;**66**:847–859.
27. Ruckerl R, Phipps RP, Schneider A, Frampton M, Cyrys J, Oberdorster G, Wichmann HE, Peters A. Ultrafine particles and platelet activation in patients with coronary heart disease—results from a prospective panel study. *Part Fibre Toxicol* 2007;**4**:1.
28. Phipps RP, Kaufman J, Blumberg N. Platelet derived CD154 (CD40 ligand) and febrile responses to transfusion. *Lancet* 2001;**357**:2023–2024.
29. Mills NL, Amin N, Robinson SD, Anand A, Davies J, Patel D, de la Fuente JM, Cassee FR, Boon NA, Macnee W, Millar AM, Donaldson K, Newby DE. Do inhaled carbon nanoparticles translocate directly into the circulation in humans? *Am J Respir Crit Care Med* 2006;**173**:426–431.
30. Nemmar A, Hoet PH, Vanquickenborne B, Dinsdale D, Thomeer M, Hoylaerts MF, Vanbilloen H, Mortelmans L, Nemery B. Passage of inhaled particles into the blood circulation in humans. *Circulation* 2002;**105**:411–414.
31. Donaldson K, Tran L, Jimenez LA, Duffin R, Newby DE, Mills N, MacNee W, Stone V. Combustion-derived nanoparticles: a review of their toxicology following inhalation exposure. *Part Fibre Toxicol* 2005;**2**:10.
32. Salvi S, Blomberg A, Rudell B, Kelly F, Sandstrom T, Holgate ST, Frew A. Acute inflammatory responses in the airways and peripheral blood after short-term exposure to diesel exhaust in healthy human volunteers. *Am J Respir Crit Care Med* 1999;**159**:702–709.
33. Tornqvist H, Mills NL, Gonzalez M, Miller MR, Robinson SD, Megson IL, Macnee W, Donaldson K, Soderberg S, Newby DE, Sandstrom T, Blomberg A. Persistent endothelial dysfunction in humans after diesel exhaust inhalation. *Am J Respir Crit Care Med* 2007;**176**:395–400.

34. Ghio AJ, Kim C, Devlin RB. Concentrated ambient air particles induce mild pulmonary inflammation in healthy human volunteers. *Am J Respir Crit Care Med* 2000; **162**:981–988.
35. Pekkanen J, Brunner EJ, Anderson HR, Tiittanen P, Atkinson RW. Daily concentrations of air pollution and plasma fibrinogen in London. *Occup Environ Med* 2000;**57**:818–822.
36. Mutlu GM, Green D, Bellmeyer A, Baker CM, Burgess Z, Rajamannan N, Christman JW, Foiles N, Kamp DW, Ghio AJ, Chandel NS, Dean DA, Sznajder JI, Budinger GR. Ambient particulate matter accelerates coagulation via an IL-6-dependent pathway. *J Clin Invest* 2007;**117**:2952–2961.
37. Ruckerl R, Ibald-Mulli A, Koenig W, Schneider A, Woelke G, Cyrys J, Heinrich J, Marder V, Frampton M, Wichmann HE, Peters A. Air pollution and markers of inflammation and coagulation in patients with coronary heart disease. *Am J Respir Crit Care Med* 2006;**173**:432–441.
38. Gong H Jr, Sioutas C, Linn WS. Controlled exposures of healthy and asthmatic volunteers to concentrated ambient particles in metropolitan Los Angeles. *Res Rep Health Eff Inst* 2003;1–36. discussion 37–47.
39. Elder AC, Gelein R, Azadniv M, Frampton M, Finkelstein J, Oberdorster G. Systemic effects of inhaled ultrafine particles in two compromised, aged rat strains. *Inhal Toxicol* 2004;**16**:461–471.
40. Blomberg A, Tornqvist H, Desmyter L, Deneys V, Hermans C. Exposure to diesel exhaust nanoparticles does not induce blood hypercoagulability in an at-risk population. *J Thromb Haemost* 2005;**3**:2103–2105.
41. Carlsten C, Kaufman JD, Peretz A, Trenga CA, Sheppard L, Sullivan JH. Coagulation markers in healthy human subjects exposed to diesel exhaust. *Thromb Res* 2007;**120**:849–855.
42. Baccarelli A, Zanobetti A, Martinelli I, Grillo P, Hou L, Giacomini S, Bonzini M, Lanzani G, Mannucci PM, Bertazzi PA, Schwartz J. Effects of exposure to air pollution on blood coagulation. *J Thromb Haemost* 2007;**5**:252–260.

CLINICAL VIGNETTE

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A left atrial mobile cystic thrombus

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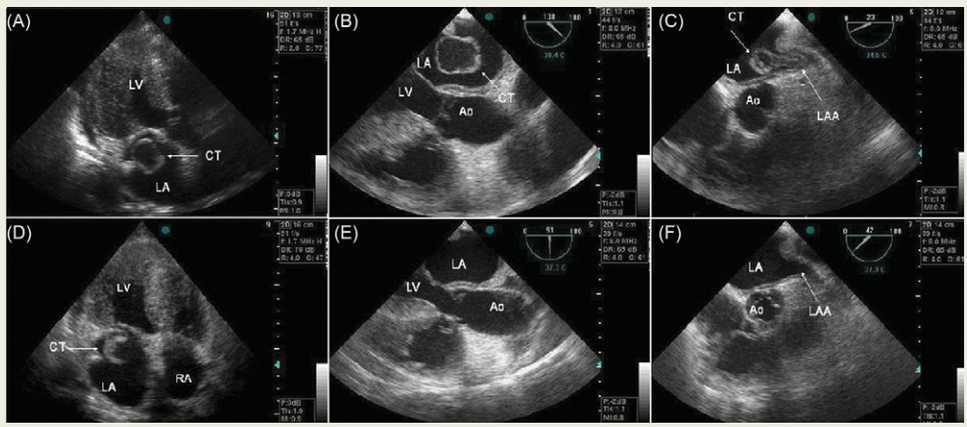

A 58-year-old-woman was admitted to our clinic for worsening dyspnoea. She had a history of uncontrolled hypertension. Physical examination was unremarkable. Electrocardiogram revealed normal sinus rhythm. Transthoracic echocardiography showed a large, mobile cystic mass with smooth contours (20 × 27 mm) in the body of enlarged left atrium (LA) (Panel A). Left ventricular chamber size was in normal limits but there was moderate hypertrophy accompanied with mild impairment of systolic function (ejection fraction was 45%). Transoesophageal echocardiography revealed mobile cystic thrombus in the LA originating from left atrial appendage (LAA) (Panels B and C). We started to treat the patient with intravenous unfractionated heparin with a target activated partial thromboplastin time of 50–70 s. The following day, cystic thrombus decreased in size; however, the very mobile behaviour of the thrombus persisted (Panel D). Transoesophageal echocardiographic examinations revealed complete disappearence of thrombus in both LA and LAA 3 days after starting intravenous heparin treatment (Panels E and F). During follow-up, the patient was free of any neurological complication.

LA thrombus formation in the setting of sinus rhythm is rare. Blood screening work-up for conditions predisposing thrombosis formation was negative. The probable predisposing factors for thrombus formation in the present case were mild left ventricular systolic dysfunction, moderate left ventricular hyperthrophy, and LA enlargement. The cystic character of thrombus is unusual and differential diagnosis for blood cyst, hydatid cyst, and myxoma should be kept in mind.

Panels A–F. Transthoracic and transoesophageal echocardiographic images revealing a large, mobile cystic thrombus with smooth contours (20 × 27 mm) in the body of enlarged LA originating from LAA (A–C). Transthoracic echocardiographic view of the thrombus 1 day after starting heparin demonstrating that the thrombus decreased in size (D). Transoesophageal echocardiographic examinations revealed complete disappearence of thrombus in both of LA and LAA 3 days after starting intravenous heparin treatment (Panels E and F). LA, left atrium; CT, cystic thrombus; LA, left atrium; LAA, left atrial appendage; LV, left ventricule; Ao, aorta; RA, right atrium.