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Fibrin clot structure is affected by levels of particulate air pollution exposure in patients with venous thrombosis

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ABSTRACT

Background: Particulate air pollution is a risk factor for cardiovascular diseases and thrombosis. Long-term exposure to particulate matter with a diameter <10 µm (PM_{10}) has been associated with an increased risk of venous thrombosis.

Objectives: The aim of this study was to investigate whether or not particulate air pollution alters fibrin clot structure and thus modulates thrombosis risk.

Methods: We investigated fibrin polymerization by turbidity (maximum absorbance mOD), clot structure by confocal microscopy (fibre number per µm) and fibrin pore size by permeability (Ks $x10^{-10}$ cm^2) in 103 patients with deep vein thrombosis and 121 healthy controls, for whom levels of air pollution exposure had been recorded. Exposure groups were defined by mean PM_{10} concentrations over the 730 days before the event.

Results: We found a higher average number of fibres per clot area in patients than controls, but no difference in Ks or fibre thickness. When the two groups were divided into high or low exposure to PM_{10}, a significantly denser fibrin clot network structure with thicker fibres (higher maximum absorbance, $p<0.05$), decreased permeability (lower Ks value, $p<0.05$) and higher average fibre numbers per clot area ($p<0.05$) was observed in patients in the high exposure group compared to those with low exposure. There were no significant differences in fibrin clot structure between the two exposure levels in healthy subjects.

Conclusions: PM_{10} levels are associated with altered fibrin clot structure in patients with deep vein thrombosis but not in controls, suggesting that air pollution may trigger differences in fibrin clot structure only in patients predisposed to thrombotic disease.

HIGHTLIGHTS

- PM_{10} levels are associated with denser fibrin clot structure in patients with DVT.
- In the control group, high PM_{10} level did not contribute to denser fibrin clot structure formation.
- Air pollution may trigger differences in clot structure in patients predisposed to thrombosis.

KEY WORDS

Air Pollution, Blood Clotting, Fibrin, Particulate Matter, Venous Thrombosis

1. INTRODUCTION

Exposure to air pollution is associated with adverse effects on the pulmonary and cardiovascular systems (Franchini and Mannucci, 2012; Peters, 2005). Urban air

pollution is composed of both gaseous pollutants (e.g. ozone, nitrogen dioxide, and sulphur dioxide) and particulate matter (PM). The PM in air pollution is a mixture of particles of different sizes, shapes, surface area, chemical composition, solubility and origins that are suspended in the air (Pope 3rd, 2009). PM is categorized by aerodynamic diameter, with PM_{10} representing particles with a diameter of less than 10 µm (Brook, 2008; Polichetti et al., 2009). The smaller particles in $PM_{10,}$ which have a diameter of less than 2.5 µm ($PM_{2.5}$), are associated with combustion of fossil fuels and high temperature industrial processes (Brook et al., 2010; Newby et al., 2014; Pope 3rd, 2009). Ultrafine particulate matters with a diameter less than 100 nm ($PM_{0.1}$) are from fresh combustion and traffic–related pollution which can travel large distances (Brook et al., 2010; Newby et al., 2014). The PM in urban air pollution has been associated with cardiovascular mortality and morbidity in a number of studies (Morris, 2001; Polichetti et al., 2009; Shah et al., 2013).

A range of possible mechanisms by which PM may damage the cardiovascular system have been proposed, including atherogenesis, thrombosis and endothelial dysfunction (Mills et al., 2009). Observations that particulate air pollution exposure is associated with increased levels of circulating coagulation proteins (eg. factor VIII [FVIII], von Willebrand factor and fibrinogen) suggest that hypercoagulability due to PM exposure could be an important risk factor for thrombosis (Baccarelli et al., 2007b; Nemmar et al., 2006). A 70% increase in risk of deep vein thrombosis (DVT) for each 10 $\mu g/m^3$ elevation of PM_{10} was observed in a large cohort study in the Lombardy region (Baccarelli et al., 2008). However, the mechanisms underpinning the increased risk of thrombosis after exposure to ambient air pollution are still poorly understood.

Fibrin clot structure, mechanical properties and resistance to lysis, are emerging risk factors in cardiovascular disease and venous thrombosis (Undas and Ariens, 2011; Wolberg, 2007). Altered fibrin clot structure with compact, highly branched fibre networks, reduced permeability and prolonged lysis time has been associated with ischemic stroke and venous thrombosis (Undas and Ariens, 2011; Wolberg, 2007). In view of these associations between thrombosis and fibrin structure, we previously investigated the effects of particulate matter on fibrin clot structure. We found that particulate matter caused changes in fibrin clot structure and function in purified systems and human plasma (Metassan et al., 2010a). In contrast, we found no changes in fibrin clot structure in healthy individuals after 2 hours exposure to particulate matter while performing moderate exercise (Metassan et al., 2010b). However, the exposure in the latter study was transient and of short duration. Whether or not fibrin clot structure is affected by long-term exposure to high levels of air pollution, and particularly in patients with thrombosis exposed to air pollution is currently unknown.

In view of the association between exposure to particulate matter and thrombosis, and of the association between thrombosis and abnormal fibrin clot structure, we investigated the possible association between fibrin clot structure and PM_{10} levels in a well-characterized group of patients with DVT and healthy controls from the Lombardy region in Northern Italy.

2. METHODS

2.1 Study Population

The study population of patients and controls has been previously described in detail (Baccarelli et al., 2007; Baccarelli et al., 2007; Baccarelli et al., 2008; Baccarelli et al., 2009). Briefly, patients from the Lombardy region, Northern Italy were referred to the Angelo Bianchi Bonomi Thrombosis Center in Milan from January 1995 to September 2005 for a thrombophilia screening after a first episode of objectively confirmed lower-limb DVT with or without pulmonary embolism. Controls were healthy individuals, friends or partners of the patients, who were residents in the Lombardy region and volunteered to undergo thrombophilia screening. Thrombophilia was classified as being positive for at least one of the following: factor V Leiden, prothrombin G20210 mutation, antithrombin-, protein C-, protein S-deficiency, antiphospolipids antibodies and hyperhomocysteinemia. All patients and controls provided written consent and the study was approved by the local ethics committee. Methods for exposure assignment were previously described in detail (Baccarelli et al., 2007; Baccarelli et al., 2007; Baccarelli et al., 2008; Baccarelli et al., 2009). Hourly concentrations of PM_{10} were obtained from the Regional Environmental Protection Agency (ARPA Lombardia) which recorded the hourly air pollution data from January 1994 to September 2005 using monitors located at 53 different sites throughout the Lombardy region (Baccarelli et al., 2007). All patients and controls provided informal written consent and the study was approved by the local ethics committee.

2.2 Fibrin Polymerisation by Turbidity Analysis

For turbidimetric analysis of fibrin polymerisation, plasma was diluted 1:3 in 0.05 M Tris-HCl, 0.1 M NaCl, pH 7.5 in a 96-well plate, and 0.5 U/mL human thrombin (Sigma-Aldrich, St. Louis, Mo) and 10 mM Calcium Chloride (final concentrations) were added.

142 Immediately after the addition of thrombin and calcium, absorbency was read every
143 12 seconds at 340 nm for 60 minutes with a Kinetic Plate Reader (Spectramax Plus
144 384, Molecular Devices, UK). Lag time (defined as the time required for the OD to
145 increase >0.01) and maximum OD (ODmax) were measured. The lag phase of the
146 turbidity curve reflects the time required for lateral aggregation of fibrin fibres to start
147 from the addition of the activation mixture. Maximum absorbance at the plateau phase
148 reflects the fibre diameter and fibrinogen concentration. Polymerisation rate was
149 analysed by measuring the slope of the turbidity curve at its steepest or inflexion point
150 (Mills et al., 2002).

151

152 ***2.3 Laser Scanning Confocal Microscopy***

153 Laser scanning confocal microscopy provides detailed analysis of the fully hydrated
154 fibrin clot structure, allowing visualization of the 3D structure of the clot and direct
155 quantification of fibrin clot structure using image analysis tools. Human fibrinogen
156 labeled with fluorescein isothiocyanate (FITC) (final concentration 50 µg/ml) and 0.5
157 M $CaCl_2$ (final concentration 10 mM) were mixed with plasma samples (diluted 1:3 in
158 0.05 M Tris–HCl, 0.1M NaCl, pH 7.5). Next, human thrombin (final concentration 0.5
159 U/ml) was added to the mixture. Fibrin clots were prepared in a total volume of 60 µl,
160 and immediately upon the addition of thrombin, samples were briefly mixed and 30 µl
161 was transferred to a 6 channel µ-slide VI 0.4 (Ibidi, Martinsried, Germany), which was
162 placed in a humidity chamber for 30 minutes to prevent dehydration of the clot. The
163 3D structure of the clot was visualized by laser scanning confocal microscopy using a
164 LSM 700 T-PMT ZEISS microscope (ZEISS, Jena, Germany). Clot structure was
165 analysed using a 63x oil immersion lens with a 5W argon laser and 488 nm laser filter.

166 The images were collected in the format of 512x512 pixels. Fibre density was
167 calculated as the number of fibres crossing a straight line of fixed length across the
168 scan-field (Bhasin et al., 2008). All measurements were performed with Image J
169 version 1.25s software (Wayne Rasband, National Institutes of Health).

170

171 ***2.4 Analysis of Pore-structure by Permeation***

172 Permeability analysis was performed to study the average pore-structure of the fibrin
173 network. In this method, the flow rate of a liquid passing through the fibrin clot is
174 measured under a constant pressure drop. A total of 10 mM calcium chloride and 1
175 U/ml human thrombin (Sigma) (final concentrations) were added to 30 µL plasma
176 samples. After incubation in a wet chamber for 120 minutes at room temperature,
177 plastic tubes containing the clots were connected to a reservoir with buffer (0.05 M
178 Tris–HCl, 0.1 M NaCl, pH 7.5) and the buffer was allowed to flow through the fibrin
179 gels with a pressure drop of 4 cm. After washing for 120 minutes, the volume of the
180 buffer that flowed through the clot was measured in 4 sequential 30 minutes collections
181 over 120 minutes. The flow rate represents the pore size of fibrin clot structure,
182 expressed as Darcy constant or Ks. The Ks was calculated as previously described
183 (Mills et al., 2002).

184

185 ***2.5 Statistical Analysis***

186 All experiments were performed in triplicate. Fits of the Ks, ∆Abs340nm, and fibre
187 number distributions to the normal distribution were tested using the Kolmogorov-
188 Smirnov test. Pearson's correlation test was used to analyze the association between

clot parameters and patients' characteristics. The ANOVA and Chi Square tests were used for investigating the intergroup differences for continuous variables and categorical variables respectively. P-values less than 0.05 were considered to indicate statistical significance. Analysis was performed with SPSS version 20 (IBM, Portsmouth, UK).

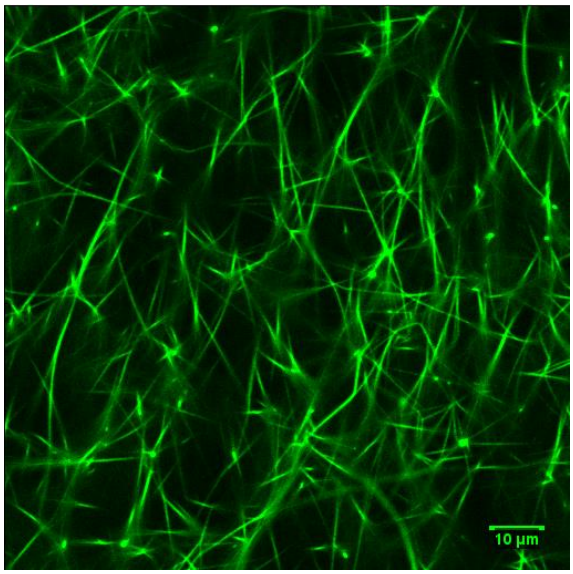
To analyze the data, we categorized several parameters from continued data to categorical data. As the concentrations of PM_{10} were variable between days, we decided to average all PM_{10} individual exposure data from the day of event (DVT) up to 730 days before the event. We chose 45.6 $\mu g/m^3$ as the cut-off point to divide patients and controls into two exposure groups because it represented the mean value of PM_{10} concentrations over the total period of 730 days. The participants with mean exposure PM_{10} levels below 45.6 $\mu g/m^3$ were classed as low exposure and those with PM_{10} exposure levels above 45.6 $\mu g/m^3$ were classed as high exposure. The number of subjects with positive thrombophilia in each group was represented by percentages.

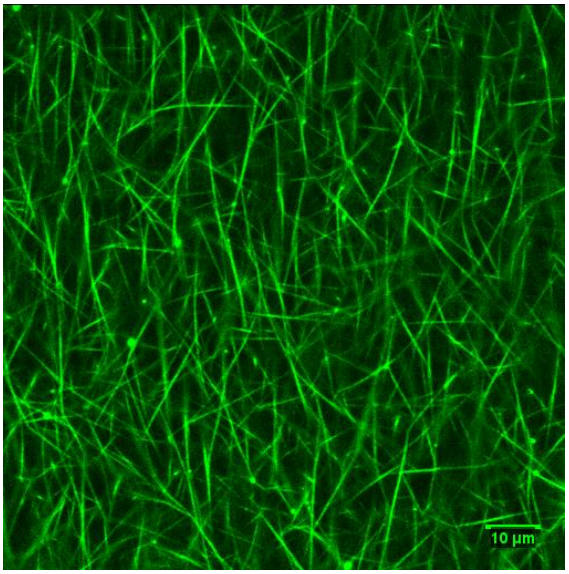
3. RESULTS

There were significant correlations between maximum absorbance and the number of fibres ($r = 0.4$, $p < 0.001$), maximum absorbance and Ks ($r = -0.5$, $p < 0.001$), and number of fibres and Ks ($r = -0.5$, $p < 0.001$). Maximum absorbance and fibre number were both positively correlated with age, body mass index (BMI), fibrinogen concentration and plasma level of FVIII, whereas Ks was negatively correlated, indicating that with increasing age, BMI, fibrinogen concentrations and FVIII levels, the fibrin fibres grew thicker, and were more compactly woven in the three-dimensional

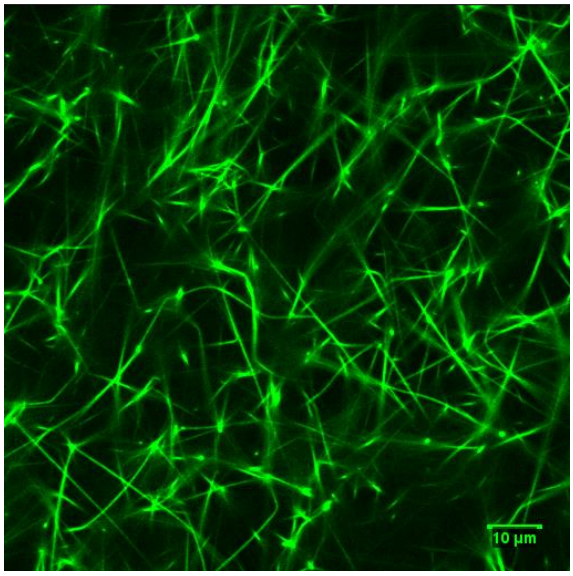
clot network, and that the clot was less permeable. Except for Ks, both fibre thickness ($r = 0.1$, $p = 0.042$) and fibre number ($r = 0.2$, $p = 0.001$) were associated with PM10 concentrations. Representative fibrin clot structures formed from plasma as imaged using laser scanning confocal microscopy are shown in Fig 1.

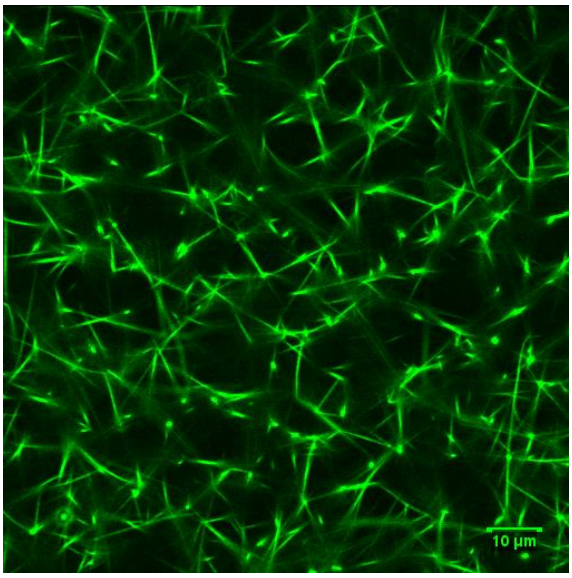
Figure 1. Representative fibrin clot structures formed from plasma of subjects exposed to high and low levels of PM_{10}





A. Patient A exposed to low PM10 levels B. Patient B exposed to high PM10 levels





C. Control A exposed to low PM10 levels D. Control B exposed to high PM10 levels

General characteristics, FVIII, fibrinogen, thrombophilia abnormalities and fibrin clot structure parameters of patients and controls are shown in Table 1. Patients and controls differed in sex distribution (more women among patients), BMI (higher in patients), and thrombophilia abnormalities (more frequent in patients). FVIII and fibrinogen were also higher in patients than controls. In terms of the clot structure, only fibre number was significantly different between patients and controls ($p = 0.018$).

241 However, there was a tendency for patients to show denser fibrin clot structure with
242 thicker fibres, more number of fibres per clot area and less permeable clots compared
243 to controls.

244 Patients exposed to high levels of air pollution showed higher concentrations of
245 fibrinogen compared to those exposed to low levels, whereas thrombophilia was
246 similarly distributed (Table 2). We also compared the fibrin clot structure parameters
247 by exposure levels in patients and controls. Patients in the high exposure group had
248 thicker fibres, more compactly arranged fibres and less permeable structures
249 compared to those in the low exposure levels. However, in controls only plasma levels
250 of coagulation FVIII were different between the two exposure groups ($p = 0.029$).

251 Table 3 shows logistic regression analysis of risk factors for DVT. The continuous data
252 age, BMI, fibrinogen concentration FVIII level were categorized into high and low
253 groups, the cut-off points were 51.9, 24.7, 299.7, and 125.3 respectively. The model
254 showed that increased age, BMI and fibrinogen concentrations did not contribute to
255 the development of DVT in this study. Male sex was a risk factor for DVT, as well as
256 FVIII, thrombophilia abnormalities and high level of PM_{10}.

257 Finally, we analyzed the relative contributions of age, sex, BMI, thrombophilia
258 abnormalities, PM_{10} and interaction of thrombophilia abnormalities and PM_{10} with the
259 variation in maximum absorbance, fibre number and Ks by linear regression in patients
260 and controls, respectively (Table 4). In the maximum absorbance model, BMI and
261 PM_{10} exposure both significantly contributed to the formation of thicker fibres in
262 patients only, whereas age was significantly correlated with maximum absorbance in
263 controls. In the fibre number model, PM_{10} and BMI were risk factors for more branched
264 fibre formation for both patients and controls. In the Ks model, exposure to PM_{10} did

not contribute to the alterations of clot structure in patients or controls. Permeability of the clot reduced with BMI increased in patients but not in controls. Neither thrombophilia abnormalities nor the interaction of thrombophilia abnormalities and PM_{10} were contributing to the alteration of fibrin clot structure in this study. Due to the relatively small sample size of patients who had thrombophilia, there was not enough power to investigate the relationship between fibrin clot structures and thrombophilia. Another reason for the absence of an effect of thrombophilia may be that there was a large degree of heterogeneity of the causes of thrombophilia in this small group, including defects of antithrombin, protein C, antiphospholipids, FV Leiden mutation or prothrombin mutation. Each of these could have differential effects on fibrin clot structure, and therefore overall, due to the small sample size, and heterogeneity of the group, there was no effect on clot structure in our study (figure shown in appendix).

278 **Table 1. Characteristics of patients with deep vein thrombosis and controls**

Variables	Patients Mean ±SD or percentage%	Controls Mean ±SD or percentage%	P-Value
Number of subjects	103	121	
Age (years)	53.7 ±14.7	50.4 ±13.9	0.085
BMI	25.5 ±4.2	24.0 ±4.3	0.014
Men %	48.5%	25.6%	<0.0001
Primary education or below %	70.9%	77.7%	0.187
Non-Smoking %	33.0%	76.9%	0.584
Thrombophilia^ %	40.8%	14%	<0.0001
Factor VIII (%)	141.6 ±43.1	108.1 ±27.4	<0.0001
Fibrinogen (mg/dl)	309.4 ±80.7	290.7 ±50.9	0.070
Maximum Absorbance (mOD)	719.2 ±175.2	679.3 ±156.9	0.073
Fibre Number (per µm)	22.5 ±3.5	21.3 ±3.8	0.018
Ks ($x10^{-10}$ cm^2)	28.8 ±8.8	31.4 ±12.2	0.248

279 **^** Thrombophilia was classified as being positive for at least one of the following: factor V Leiden, prothrombin G20210 mutation,
280 antithrombin-, protein C-, protein S-deficiency, antiphospolipids antibodies and hyperhomocysteinemia.
281

Table 2. Clotting parameters (mean ± SD) in patients and controls of high and low PM_{10} exposure

Variables	Patients			Control		
	Low Exp (n=23)	High Exp (n=80)	P-Value	Low Exp (n=72)	High Exp (n=49)	P-Value
PM_{10} Levels ($\mu g/m^3$)	39.3 ±8.5	48.9 ±2.6	<0.001	41.61 ±4.8	49.20 ±3.0	<0.001
Thrombophilia≠	47.8%	38.8%	0.435	12.5%	16.3%	0.552
Factor VIII (%)	132.2 ±38.0	144.2 ±44.3	0.260	114.2 ±30.2	101.7 ±22.9	0.029
Fibrinogen (mg/dl)	277.2 ±81.3	320.4 ±78.1	0.026	283.8 ±64.9	300.4 ±45.3	0.168
Maximum Absorbance (mOD)	626.4 ±155.5	745.8 ±172.2	0.003	675.9 ±171.2	684.2 ±134.7	0.776
Fibre Number (per µm)	20.4 ±3.9	23 ±3.1	0.001	21 ±4	21.7 ±3.4	0.307
Ks ($x10^{-10}$ cm^2)	33.7 ±11.2	26.3 ±6.1	0.006	30.1 ±11.4	35.2 ±14.5	0.236

≠The percentage of subjects with positive thrombophilia in each group

Table 3. Logistic regression analysis of risk factors for deep vein thrombosis

Determinants or Variables	OR	95% CI	p-value
Age > 51.9 years	0.70	0.32-1.52	0.368
Men	3.02	1.36-6.74	0.007
BMI > 24.73	0.88	0.39-1.95	0.748
Thrombophilia	2.65	1.16-6.05	0.020
FVIII > 125.27%	5.52	2.52-12.10	<0.0001
Fibrinogen > 299.73 mg/dl	1.44	0.65-3.17	0.371
PM_{10} Exposure Level > 45.6 µg/m^3	3.85	1.79-8.28	0.001

Table 4. Linear regression analysis of risk factors for Maximum Absorbance, Fibre Number and Ks (cases/controls)

Determinants	Maximum Absorbance Correlation Coefficient (95% CI)		Fibre Number Correlation Coefficient (95% CI)		Ks Correlation Coefficient (95% CI)	
	Case	Control	Case	Control	Case	Control
Age (years)	0.47 (-1.75-2.70)	2.30* (0.10-4.49)	-0.02 (-0.07-0.03)	0.09 *** (0.04-0.14)	-0.15 (-0.33-0.02)	-0.26 (-0.52-0.01)
Men	9.01 (-56.58-74.60)	-47.2 (-112.65-18.24)	-0.18 (-1.58-1.22)	-0. 42 (-1.84-1.00)	-1.16 (-6.64-4.33)	3.03 (-5.68-11.74)
BMI	18.12 *** (10.34-25.91)	5.93 (-1.26-13.12)	0.22 * (0.06-0.39)	0.22 ** (0.06-0.37)	-0.65 * (-1.30-(-0.01))	-0.53 (-1.30-0.24)
Thrombophilia	358.56 (-128.83-845.96)	-227.89 (-886.78-431.00)	8.03 (-2.67-18.74)	7.78 (-6.48-22.04)	-16.08 (-48.96-16.81)	-70.42 (-153.83-12.99)
PM_{10} Exposure Level ($\mu g/m^3$)	10.07 ** (3.58-16.56)	-1.44 (-6.90-4.03)	0.22 ** (0.08-0.35)	0.12* (0.01-0.24)	-0.32 (-0.75-0.12)	0.41 (-0.48-1.31)
Interaction (Thrombophilia and PM_{10} exposure)	-7.62 (-17.95-2.71)	4.99 (-9.29-19.26)	-0.157 (-0.38-0.07)	-0.21 (-0.52-0.10)	0.34 (-0.39-1.08)	1.85 (-0.10-3.80)

The correlation coefficient is of statistical significance *p<0.05; **p<0.01; ***p<0.001.

4. DISCUSSION

We found that after long-term and high-level exposure to air pollution (PM_{10} concentrations over 45.6 $\mu g/m^3$), patients with DVT had significantly denser fibrin clot structures compared to those living in areas with lower levels of exposure (PM_{10} less than 45.6 $\mu g/m^3$). In the high exposure group, clots from patients contained thicker fibres, more compact fibre arrangements and less permeable fibrin structures. There were no significant differences in fibrin clot structure between the two exposure levels in healthy subjects.

The mechanisms underpinning this difference between patients and healthy controls are unknown but may be related to the differences in susceptibility of fibrin clot structure to air pollution PM exposure. In a previous *in vitro* study we found that at least part of the effect of particulate matter on fibrin structure could be attributed to oxidative stress induced by the particles (Metassan et al., 2010). It is possible that healthy subjects are more resistant to oxidative stress than patients with venous thrombosis, since the latter may have an enhanced inflammatory state (Franchini and Mannucci, 2011), that increases oxidative stress. Alternatively, due to increased levels of inflammatory proteins and coagulation activation in patients with venous thrombosis, any additional oxidative effects caused by air pollution on fibrin clot structure could be more pronounced, perhaps due to a threshold effect, or a minimum level of oxidative stress needed for effects on clot structure to become apparent. Finally, due to the inflammatory state, pulmonary function may be impaired, leading to translocation of ultrafine PM into the circulation. However, these considerations remain speculative as there currently are no reliable methods to analyze PM in the blood, nor do we have detailed information regarding the pulmonary function in our patients.

We previously investigated the effects of transient exposure (2 hours) to diesel particle air pollution in a controlled environment in healthy, young individuals (Metassan et al., 2010), finding that fibrin clot structure in plasma from subjects after short-term diesel exhaust exposure was not significantly different compared to those who were exposed to filtered air (Metassan et al., 2010). Our current findings extend these findings and further indicate that even after chronic, long-term and high level exposure to particulate matter, the fibrin clot properties in healthy controls remain similar to those who were exposed to lower levels of PM exposure.

Consistent with a larger previous study on the association between air pollution and venous thrombosis (Baccarelli et al., 2008), PM_{10} exposure in the current study was a strong risk factor for DVT and men had higher risk of DVT than women. Baccarelli et al. (2008) showed that DVT risk was associated with the concentrations of PM_{10} measured during the year before diagnosis (Baccarelli et al., 2008). In the current study, sex, levels of factor VIII, thrombophilia abnormalities, and PM_{10} exposure level were all significantly associated with the risk of DVT. Increased levels of coagulation factors, such as factor VIII, have previously been associated with increased risk of thrombosis (A Undas et al., 2009). Thrombophilia abnormalities are also contributing factors that modulate fibrin clot structure. The prothrombin G20210 mutation leads to the increase plasma level of prothrombin which triggers the formation of denser clot structure composed of more branched thinner fibres (Wolberg and Campbell, 2008). However, age, BMI and fibrinogen concentrations were not significantly associated with DVT.

We also observed some differences in clot structure between patients and controls. Clots formed from plasma of patients had denser, less permeable fibrin clot structures

containing more, thicker fibres compared to controls, although the differences did not reach statistical significance, possibly due to the relatively small number of subjects studied. These data provide some support to previous studies by Undas *et al.*(2009), in which plasma from patients with DVT and pulmonary embolism (PE) formed clots with lower clot permeability and higher maximum absorbency than controls (A Undas et al., 2009). But there were still some different findings that in our study in the patient group, there were 93 patients with deep vein thrombosis (DVT) and 10 patients with pulmonary embolism (PE). By comparing the fibrin clot structures, there were no significant difference between patients with DVT and patients with PE (shown in the table in the appendix). However, Undas *et al.* previously found differences in that patients with DVT only (n=66) had denser fibrin clot structure and prolonged clot lysis time compared to patients with DVT and PE (n=34) (Anetta Undas et al., 2009). The finding that there were no differences in our study may due to the relatively small sample size of patients with PE, since only 10 patients had DVT and PE compared with 93 patients with DVT only.

One possible mechanism by which air pollution may contribute to the development of thrombosis could involve local pulmonary inflammatory and oxidative responses with the release of prothrombotic factors and inflammatory cytokines into the circulation after the inhalation of particles (Emmerechts and Hoylaerts, 2012; Mills et al., 2009; Newby et al., 2014). Previous animal studies showed that PM_{10} caused lung inflammation following intrapulmonary instillation of PM and inhalation of concentrated ambient particles (Donaldson et al., 2005; Elder et al., 2004; Mills et al., 2009). In clinical studies, pulmonary inflammation occurred after inhalation of both concentrated ambient particulate matters and dilute diesel particles (Ghio et al., 2000; Mills et al., 2009; Salvi et al., 1999). After exposure, plasma concentrations of pro-inflammatory

375 cytokines such as interleukin (IL) - 1β, IL-6 and tumor necrosis factor-α increased
376 (Donaldson et al., 2005; Fujii et al., 2002; Mills et al., 2009). In both animal and clinical
377 studies, exposure of PM also led to the elevation of fibrinogen concentrations (Mills et
378 al., 2009; Schwartz, 2001). High concentrations of fibrinogen shorten the lag phase of
379 polymerization, increased branch point densities, fibre thickness and clot rigidity, with
380 concurrent increases in the resistance of the clot to fibrinolysis (Scott et al., 2004;
381 Weisel, 2007).

382 A second possible mechanism may involve direct translocation of particulate matter
383 from the pulmonary alveoli into the blood circulation, crossing the pulmonary
384 epithelium and vascular endothelium barrier (Emmerechts and Hoylaerts, 2012; Mills
385 et al., 2009; Newby et al., 2014). Particles with diameters less than 10 μm can be
386 inhaled deeply into the lungs (Mills et al., 2009). A number of other factors may
387 influence the possible translocation of PM, including charge, chemical composition,
388 and propensity to form aggregates (Mills et al., 2009). The size and shape of the
389 particles could affect the region of deposition in the respiratory system, with smaller
390 sized particles penetrating deeper into the lung. Macrophages may not be able to
391 recognize particles with a diameter less than 500nm, and for this reason, ultrafine
392 particulate matters may enter the blood or lymphatic systems more easily and transfer
393 to different organs (Teow et al., 2011). Once in the circulation, the particles could
394 interact with vascular endothelial cells and have direct effects on the atherosclerotic
395 plaque (Mills et al., 2009), platelets (Lauer et al., 2009) and fibrin clot formation,
396 structure and stability.

397 As Mills et al. mentioned that diesel exhaust inhalation causes vascular dysfunction
398 and impaired endogenous fibrinolysis (Mills et al., 2005). Furthermore, previous

studies have shown that denser fibrin clot structures were associated with prolonged lysis time (Ajjan and Grant, 2006; Ariens, 2013; Scott et al., 2004; Undas and Ariens, 2011). Therefore, as patients exposed to high level of air pollution had denser fibrin clot structures, the lysis time compared to those patients only exposed to low level of air pollution is likely longer. Future studies will be needed to further evaluate the effects of air pollution exposure on fibrinolysis in patients with venous thrombosis.

Possible limitations of our study include the relatively small study sample size (due to the time-consuming nature of fibrin structure analysis), and that we had no information regarding personal levels of air pollution exposure for the participants. The concentrations of PM_{10} in this study were measured according to the area of residence for the subjects, which were different for each subject and spanned several residential areas in Lombardy. Therefore, although exposure to air pollution was not measured with personal monitors, the data obtained did provide average daily, specific and long-term individual exposure to air pollution.

5. CONCLUSION

In conclusion, this study showed patients with venous thrombosis exposed to high level of air pollution had denser fibrin clot structures with thicker fibres (higher maximum absorbance), decreased permeability (lower Ks value) and higher fibre numbers compared to those in the low exposure group, indicative of a prothrombotic clot structure. There were no differences in fibrin clot structure measurements between the two exposure groups in controls, suggesting that air pollution may trigger differences in fibrin clot structure only in patients predisposed to thrombotic disease.

6. LIST OF ABBREVIATIONS

PM -- Particulate Matter

FVIII -- Factor VIII

DVT -- Deep Vein Thrombosis

7. ACKNOWLEDGEMENT

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9. APPENDIX

Table 5. Clotting parameters (mean ± SD) in patients with DVT only and patients with DVT and PE

Patients with DVT Only Clotting Parameters	Mean (±SD)	Patients with DVT and PE Clotting Parameters	Mean (±SD)	P Value
Maximum Absorbance	68.799 (±18.839)	Maximum Absorbance	59.806 (±11.109)	0.167
Fibre Number	21.915 (±3.844)	Fibre Number	20.677 (±3.523)	0.206
Ks	2.868 (±0.884)	Ks	2.913 (±0.899)	0.890

577 Table 6. Clotting parameters (mean ± SD) in patients with and without thrombophilia abnormalities

Patients with Thrombophilia Abnormalities (n=61) Clotting Parameters	**Mean (±SD)**	**Patients without Thrombophilia Abnormalities (n=42) Clotting Parameters**	**Mean (±SD)**	**P Value**
Maximum Absorbance	71.815 (±18.606)	Maximum Absorbance	71.989 (±16.889)	0.568
Fibre Number	22.922 (±3.043)	Fibre Number	22.182 (±3.718)	0.365
Ks	2.840 (±0.738)	Ks	2.901 (±0.978)	0.205

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