Acute respiratory tract infection leads to procoagulant changes in human subjects

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Acute respiratory tract infections are associated with an increased risk of acute ischemic heart disease, stroke and venous thromboembolism [1–3]. A transient change in local hemodynamic factors, coagulation activation, reduced generation of anticoagulant activated protein C (APC), inhibition of fibrinolysis and endothelial cell perturbation as a result of systemic inflammation might be underlying mechanisms [4,5]. Indeed, it has been shown that respiratory viruses are able to activate coagulation, causing a reduction in clotting time and an increase in the expression of tissue factor and thrombin generation, the latter by reduced levels of protein C [6,7]. Also, increased levels of hemostatic proteins during symptoms of acute respiratory tract infection have been shown [8,9]. Endothelial cell perturbation and increased levels of hemostatic markers, such as von Willebrand factor (VWF), D-dimer, plasmin-α2-antiplasmin complexes (PAP) and plasminogen activator inhibitor-1 (PAI-1), are risk factors for ischemic heart disease [10–12]. High VWF levels are also related to a short-term increased risk of plaque rupture and subsequent thrombus formation [13].

Recently, we have shown that respiratory tract infections in elderly human subjects result in increased levels of VWF and PAP complexes, and thus a procoagulant state [14]. In the present study, we examined the effect of a naturally occurring acute respiratory tract infection on hemostatic proteins in more detail in a prospective cohort study.

We included 372 men and women of 55 years and older from a general practitioners' office before the winter of 2005–2006. Patients with an infection or an influenza-like illness (ILI; definition as described previously [14]) within 3 weeks before recruitment were excluded. The Institutional Review Board approved the study and all subjects provided informed consent. A detailed medical history and blood samples were obtained at entry. In case of ILI blood samples and throat swabs were obtained within 1 day. This was repeated 2–3 days later and 14 days after the acute phase. Blood was collected and processed as described previously [14].

High-sensitive CRP (hs-CRP) was measured using the Synchron LX system (Beckman Coulter, Fullerton, USA), prothrombin fragment 1 + 2 (F1 + 2) by ELISA from Siemens Healthcare Diagnostics (Marburg, Germany) and PAP complexes by ELISA from DRG (Marburg, Germany). PAI-1 activity was assayed on a Behring Coagulation System (Siemens Healthcare Diagnostics). D-dimer was measured with a particle-enhanced immunoturbidimetric assay (Innovance D-dimer; Siemens Healthcare Diagnostics) and VWF using a homemade immunoassay employing antibodies from Dako (Glostrup, Denmark). Results of VWF are presented as percentages of normal pooled plasma. The generation of thrombin in clotting plasma was assayed by Calibrated Automated Thrombogram as described by Hemker *et al.* [15].

Coagulation was triggered by recalcification in the presence of 5 pM recombinant human tissue factor (Innovin; Siemens Healthcare Diagnostics), 4 μM phospholipids and 417 μM fluorogenic substrate Z-Gly-Gly-Arg-AMC (Bachem, Bubendorf, Switzerland). Fluorescence was monitored using the Fluoroskan Ascent fluorometer (ThermoLabsystems, Helsinki, Finland), and the endogenous thrombin potential (ETP), peak thrombin, time to peak, lag time and velocity index were calculated using the Thrombinoscope software (Thrombinoscope BV, Maastricht, the Netherlands).

The sensitivity to activated protein C (APC; Enzyme Research Laboratories, South Bend, IN, USA) of each plasma sample was determined in both the presence and absence of approximately 4 nM APC. The APC concentrations used were adjusted to maintain a residual thrombin generation activity of approximately 10% in normal pooled plasma. Normal pooled plasma was run in parallel on each plate. The normalized ratio (APC-sr) was determined by dividing the APC-sr of an individual by the APC-sr of the pooled plasma. A normalized APC-sr > 1.0 reflects an APC-resistant phenotype.

The presence of IgA and IgG antibodies against influenza A and B, parainfluenza, respiratory syncytial virus (RSV), adenovirus and specific IgM and IgG against mycoplasma pneumonia were established using ELISAs (Serion/Viron, Würzburg, Germany). Throat swabs were tested by means of previously described real-time PCR for respiratory syncytial

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virus, influenza A and B, adenovirus, parainfluenza, rhinovirus and human coronavirus [16]. We considered recent respiratory tract proven when there was a positive specific IgA or IgM or a four-fold elevation of specific IgG between the results of the samples taken on the first day of illness and 2 weeks later, or when results from the PCR on throat swab samples were positive.

Data are presented as medians ± interquartile range (IQR). Variables were compared using Wilcoxon's signed ranks test. *P*-values of < 0.05 were considered statistically significant.

Fifteen out of the 372 subjects contacted us with an ILI. Eleven infections were found in 10 subjects: influenza A (5), influenza B (1), adenovirus (1), coronavirus NL (1), coronavirus OC43 (2) and mycoplasma pneumoniae (1). One subject was simultaneously infected with influenza A and coronavirus OC43. In five subjects no causative pathogen could be found. All 15 subjects fulfilled the clinical criteria for ILI and had significantly increased levels of hs-CRP during the acute phase compared with baseline (Table 1). During the acute phase, we observed significant increases in levels of VWF, PAP and D-dimer compared with baseline, whereas F1 + 2 and PAI-1 did not change. D-dimer level was also statistically increased in the convalescent phase compared with baseline, whereas VWF and PAP levels had almost returned to baseline levels.

Parameters of thrombin generation (ETP, peak thrombin and velocity index) showed significant procoagulant changes during the infection period that remained even in the convalescent phase. Also, the infections resulted in an acquired APC resistance that persisted in the convalescent phase, most obvious in men. We can not explain why the APC resistance is more obvious in men. The persistent APC resistance might be explained by elevated levels of factor (F)VIII or decreased levels of protein S. However, as VWF returns to baseline, FVIII would be expected to normalize as well. Why these levels remain different in the convalescent phase is not clear.

The present findings show that the hemostatic system is affected in a number of ways during the acute phase of a respiratory tract infection. First, significantly elevated levels of VWF indicated that there was an activation of the endothelium. Second, significantly elevated levels of PAP and D-dimer indicated that there was increased fibrinolysis. And third, the changes in increased *ex vivo* thrombin generation (ETP) and enhanced resistance to APC were suggestive of a procoagulant state after the infection. Our findings correlate with those of a similar study performed earlier by our group [14]. Others have reported similar changes of hemostatic markers in patients with flu-like symptoms [8,9], but in these studies the infection was not confirmed with objective analysis.

The non-elevated levels of prothrombin fragment F1 + 2 suggest that there was no enhanced *in vivo* thrombin formation. However, the significantly elevated ETP suggests the potential for increased thrombin formation and therefore a procoagulant state during the acute phase of a respiratory tract infection. The increased levels of D-dimer do indicate enhanced *in vivo* thrombin formation, but this may in part be as a result of the enhanced fibrinolysis that was observed. The apparent paradox between D-dimer and F1 + 2 may also be explained by the shorter half-life of F1 + 2 compared with D-dimer.

As subjects were their own controls, factors that could influence hemostatic proteins, such as pre-existing conditions

Table 1 hs-CRP and hemostatic markers in subjects with a respiratory tract Infection

	Median levels of hs-CRP and hemostatic proteins			
	$T = 0$	$T = 1$	$T = 2$	$T = 3$
hs-CRP (mg L^{-1})	2.6 (1.3–3.8)	46.8 (21.2–114)*	13.9 (5.0–47.7)*	2.6 (2.2–8.0)
vWF antigen (%)	123 (82–166)	211 (157–247)*	196 (148–237)*	129 (102–195)†
F1 + 2 (pmol L^{-1})‡	247 (206–326)	200 (170–317)	235 (186–273)	218 (187–300)
PAP (μg L^{-1})	458 (347–605)	673 (438–1041)*	778 (602–1029)*	516 (404–647)
D-Dimer (mg L^{-1} FEU)‡	0.32 (0.24–0.56)	0.53 (0.37–0.70)*	0.59 (0.45–0.80)*	0.55 (0.44–0.98)*
PAI-1 (IU mL^{-1})	4.4 (3.1–5.2)	4.2 (3.6–5.5)	4.5 (4.0–5.3)	5.2 (4.4–5.9)†
Peak thrombin (nM)‡§	311 (269–356)	376 (335–414)*	401 (367–447)*	404 (359–432)*
Vel. Index (nM min^{-1})‡§	121 (73–138)	168 (141–187)*	180 (161–212)*	160 (141–202)*
ETP (nm min^{-1})‡§	1803 (1660–2067)	2009 (1661–2298)*	2041 (1851–2485)*	2155 (1846–2456)*
APC-sr‡				
Men ($n = 6$)	0.73 (0.59–1.78)	1.62 (0.99–3.65)	1.70 (1.21–3.95)	2.07 (1.40–4.60)†
Women ($n = 8$)	0.37 (0.05–0.67)	0.90 (0.35–1.32)*	1.26 (0.46–2.39)*	0.93 (0.43–1.60)*
Men + women	0.65 (0.26–0.78)	1.05 (0.63–2.12)*	1.60 (0.97–2.63)*	1.29 (0.74–1.98)*

Number of subjects: 15, with 11 confirmed infections in 10 subjects; infection could not be proven in 5 subjects. None of the female subjects were using hormone replacement therapy.

Numbers are medians with interquartile range (IQR) in parentheses. $T = 0$: baseline; $T = 1$: acute phase (influenza-like illness); $T = 2$: 2–3 days after $T = 1$; $T = 3$: 14 days after $T = 1$.

hs-CRP, high sensitive C-reactive protein; VWF, von Willebrand factor; F1 + 2, prothrombin fragment 1 + 2; PAP, plasmin-α_2-antiplasmin complex; PAI, plasminogen activator inhibitor-1; Vel. Index, velocity index; ETP, endogenous thrombin potential; APC-sr, activated protein C-sensitivity ratio.

*Significantly different from baseline ($P < 0.05$).

†Tendency towards significant difference ($P = 0.051$–0.099).

‡One patient using oral anticoagulation was excluded from the analysis.

§Markers of thrombin generation.

and medication, can be excluded. The fact that levels of most hemostatic proteins and CRP almost returned to baseline 2 weeks after the initiation of the disease episode, indicates that it is unlikely that the observed increase is the result of seasonal variation. However, as a result of the design of the study, some effect of seasonal variation can not be excluded.

In conclusion, the present study shows that naturally occurring respiratory tract infections in human subjects result in endothelial cell perturbation (VWF) and an increased fibrinolytic state (PAP, D-dimer) with the potential for increased coagulation (ETP and APC-sr). Because VWF, PAP complexes, ETP and resistance to APC have been suggested to increase the risk of ischemic heart disease and venous thromboembolism [10,11,17,18], we suggest that the induced hemostatic changes may form a link between acute respiratory tract infections and acute atherothrombotic disease. The precise relation to risk still needs to be established in (large) prospective studies.

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Disclosure of Conflict of Interest

The authors state that they have no conflict of interest.

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