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 pneumoniae were established using ELISAs (Serion/Viron, Würzburg, Germany). Throat swabs were tested by means of previously described real-time PCR for respiratory syncytial
Numbers 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-α2-antiplasmin complex; PAI-1, 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).

1Tendency towards significant difference (P = 0.051–0.099).

One patient using oral anticoagulation was excluded from the analysis.

1Markers 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.

References
1 Smeeht L, Thomas SL, Hall AJ, Hubbard R, Farrington P, Vallance P. Risk of myocardial infarction and stroke after acute infection or vaccination. N Engl J Med 2004; 351: 2611–8.
2 Zurru MC, Alonzo C, Brescacin L, Romano M, Camera LA, Waisman G, Cristiano E, Ovbiegele B. Recent respiratory infection predicts atherothrombotic stroke. Case-control study in a buenes aires healthcare system. Stroke 2009; 40: 1986–90.
3 Smeeht L, Cook C, Thomas S, Hall AJ, Hubbard R, Vallance P. Risk of deep vein thrombosis and pulmonary embolism after acute infection in a community setting. Lancet 2006; 367: 1075–9.
4 Libby P. Inflammation in atherosclerosis. Nature 2002; 420: 868–74.
5 Esmon CT. The impact of the inflammatory response on coagulation. Thromb Res 2004; 114: 321–7.
6 Keller TT, van der Sluijs KF, de Kruif MD, Gerdes VE, Meijers JC, Florquin S, van der Poll T, van Gorp EC, Brandjes DP, Buller HR, Levi M. Effects on coagulation and fibrinolysis induced by influenza in mice with a reduced capacity to generate activated protein C and a deficiency in plasminogen activator inhibitor type 1. Circ Res 2006; 99: 1261–9.
7 Visseren FL, Bousman JJ, Bouter KP, Diepersloot RJ, de Groot PH, Erkelenz DW. Procoagulant activity of endothelial cells after infection with respiratory viruses. Thromb Haemost 2000; 84: 319–24.
8 Horan JT, Francis CW, Falsey AR, Kolassa J, Smith BH, Hall WJ. Prothrombotic changes in hemostatic parameters and C-reactive protein in the elderly with winter acute respiratory tract infections. Thromb Haemost 2001; 85: 245–9.
9 Kabu NK, Francis CW, Hall WJ, Falsey AR, Smith BH. Protein S declines during winter respiratory infections. J Thromb Haemost 2003; 1: 729–34.
10 Cushman M, Lemaire RN, Kuller LH, Psaty BM, Macy EM, Sharrett AR, Tracy RP. Fibrinolytic activation markers predict myocardial infarction in the elderly. The Cardiovascular Health Study. Arterioscler Thromb Vasc Biol 1999; 19: 493–8.
11 Morange PE, Simon C, Alessi MC, Luc G, Arveiler D, Ferrieres J, Amouyel P, Evans A, Ducimetiere P, Juhan-Vague I. Endothelial cell markers and the risk of coronary heart disease; the Prospective Epidemiological Study of Myocardial Infarction (PRIME) study. Circulation 2004; 109: 1343–8.
12 Kohler HP, Grant PJ. Plasminogen-activator inhibitor type 1 and coronary artery disease. N Engl J Med 2000; 342: 1792–801.
13 Lee KW, Lip GY, Tayebjee M, Foster W, Blann AD. Circulating endothelial cells, von Willebrand factor, interleukin-6, and prognosis in patients with acute coronary syndromes. Blood 2005; 105: 526–32.
14 Keller TT, van Wissen M, Mairuhu AT, van Doornum GJ, Brandjes DP. Acute respiratory tract infections in elderly patients increase systemic levels of hemostatic proteins. J Thromb Haemost 2007; 5: 1567–9.
15 Hemker HC, Giesen P, Al Dieri R, Regnault V, de Smedt E, Wagenhoef R, Lecompte T, Beguin S. Calibrated automated thrombin generation measurement in clotting plasma. Pathophysiol Haemost Thromb 2003; 33: 4–15.
16 Bosia S, Esposito S, Niesters HG, Zucotti GV, Marsiglia G, Lanari M, Zuin G, Pelucchi C, Osterhaus AD, Principi N. Role of respiratory pathogens in infants hospitalized for a first episode of wheezing and their impact on recurrences. Clin Microbiol Infect 2008; 14: 677–84.
17 Orbe J, Zudaire M, Serrano R, Canas-Canella I, Martinez DS, Rodriguez JA, Paramo JA. Increased thrombin generation after acute versus chronic coronary disease as assessed by the thrombin generation test. Thromb Haemost 2008; 99: 382–7.
18 Makris TK, Krespi PG, Hatzizacharias AN, Gialeraki AE, Anastasiadis G, Triposkiadis FK, Mandalaki T, Kyriakidis MK. Resistance to activated protein C and FV leiden mutation in patients with a history of acute myocardial infarction or primary hypertension. Am J Hypertens 2000; 13: 61–5.