# Acute respiratory tract infections in elderly patients increase systemic levels of hemostatic proteins

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**To cite this article:** Keller TT, van Wissen M, Mairuhu ATA, van Doornum GJJ, Brandjes DPM. Acute respiratory tract infections in elderly patients increase systemic levels of hemostatic proteins. *J Thromb Haemost* 2007; **5**: 1567–9.

Acute respiratory tract infections are associated with a temporarily increased risk of acute ischemic heart disease and venous thrombosis [1,2]. The precise underlying mechanism of this association is unclear, but infection-induced systemic inflammation with transient coagulation activation, and endothelial cell perturbation, may contribute to this [3,4]. Endothelial cell perturbation and coagulation activation are associated with an increased risk of future ischemic heart disease [5–8]. Several cross-sectional reports showed increased levels of hemostatic proteins during symptoms of acute respiratory tract infection [9–13]; however, these findings are difficult to interpret, as the diagnosis was not unequivocally established. Whether viruses infecting the respiratory tract indeed perturb endothelial cells and thereby increase the risk of acute ischemic heart disease remains to be proven. Therefore, we determined the effect of naturally occurring acute respiratory tract infection on hemostatic proteins in a prospective study.

We included 255 different subjects from a general practise, mean age 67 years (± 14 years). Eligible subjects were at risk for influenza, as defined by the criteria to participate in the national influenza vaccination campaign. The Institutional Review Board approved the study, and all subjects provided informed consent. At the time of inclusion, a medical history, including influenza vaccination status, was obtained and a blood sample was collected; immediately thereafter, the follow-up period started. Blood samples and nose swabs were obtained when the predefined criteria of flu-like symptoms were met. We defined flu-like disease as the acute onset of fever (body temperature > 37.8 °C) plus at least two of the following flu-like symptoms: headache, myalgia, sore throat, and cough. All specimens were obtained in the first 3 days of flu-like symptoms. In the convalescent phase, 14 days after the beginning of symptoms, the specimen collection was repeated. Blood was collected in empty vacuum tubes and in tubes containing 0.106 M trisodium citrate. All samples were taken in the morning and processed within 60 min: the tubes were centrifuged for 15 min at 2000 × *g*, and plasma was pooled and then centrifuged again at 2000 × *g* for 5 min. Subsequently, plasma was frozen and stored in aliquots of 0.5 mL at −80 °C. Prothrombin fragment 1 + 2 ($F_{1+2}$) and plasmin–$\alpha_2$-antiplasmin (PAP) complexes were measured by enzyme-linked immunosorbent assay (ELISA) from Dade Behring (Marburg, Germany), plasminogen activator inhibitor type I (PAI-1) antigen was measured by an ELISA from Hyphen BioMed (Andrésy, France), and von Willebrand factor (VWF) antigen was measured by an ELISA from Dako (Glostrup, Denmark). Results for VWF are presented as percentages of normal pool plasma. The presence of antibodies against respiratory pathogens was established using ELISAs for influenza A virus, influenza B virus, parainfluenza virus type 1 and type 2, respiratory syncytial virus (RSV), adenovirus, and specific IgM and IgG against Mycoplasma pneumoniae.

For the detection of IgA antibodies, in-house assays were used; for the detection of IgG or IgM antibodies, tests purchased from Serion/Viron (Würzburg, Germany) were used. We considered respiratory tract infection to be proven when there was a 4-fold elevation between the sample taken on the first day of flu-like symptoms and the sample taken in the convalescent phase 2 weeks later. In addition, samples were tested for RSV, influenza viruses type A and type B, parainfluenza viruses 1, 2, 3 and 4, adenovirus, enterovirus, rhinovirus and human metapneumovirus (HMPV) by routine diagnostic immunofluorescence assays 48 h after inoculation. Total RNA and DNA was isolated with the total nucleic acid isolation kit (Roche Applied Science, Almere, the Netherlands), and amplification of HMPV, human coronavirus NL63 and influenza virus was performed as described previously [14–17]. Amplification of RSV type A and type B, rhinovirus, coronavirus C43 and coronavirus 229E was performed using TaqMan EZ RT-PCR reagents (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). Detection was performed on an ABI7700 detection system. All materials were spiked with an internal control, consisting of a fixed amount of phocine distemper virus to monitor for inhibition and/or loss of material. Data are presented as median ± interquartile range (IQR). Levels of hemostatic proteins between baseline and the acute phase of the disease, and between

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Received 27 October 2006, accepted 8 March 2007

baseline and the convalescent phase, were compared using the Wilcoxon rank sum test. *P*-values of $< 0.05$ were considered to be statistically significant.

During the winter, a total of 53 subjects reported flu-like symptoms that met our predefined criteria of flu-like disease. Subjects with flu-like disease showed increased VWF levels (Table 1). Median VWF levels changed from 151% (IQR: 114–189) at baseline to 197% (IQR: 162–247) at the start of the symptoms ($P < 0.05$). Thrombin generation was not increased, as evidenced by comparable levels of $F_{1+2}$ at baseline and during the acute phase of disease. In subjects with flu-like disease fibrinolysis was activated, as demonstrated by an increase in median PAP levels from 343 μg $L^{-1}$ (IQR: 255–535) to 465 μg $L^{-1}$ (IQR: 283–638, $P < 0.05$). PAI-1 levels did not change. In the convalescent phase, 2 weeks after the start of symptoms, VWF and PAP levels returned to baseline. Causative pathogens, including influenza A virus, influenza B virus, parainfluenza virus, RSV and coronavirus, were found in 26% of the cases with flu-like disease. In subjects with proven viral infection, VWF antigen levels increased by 68% (IQR: 17–106) during the infection ($P < 0.05$). In the convalescent phase, 2 weeks after the start of symptoms, VWF levels returned to baseline. Median PAP levels increased by 136 μg $L^{-1}$ (IQR: – 18–287). However, this increase in PAP was not statistically significant. Finally, we compared the levels of hemostatic proteins between subjects with influenza infection, those with other proven viral infections, and those with clinical symptoms only. No significant differences were found in VWF, $F_{1+2}$, PAP and PAI-1 levels between these subgroups.

This study demonstrates that acute respiratory tract infections result in an increase of the hemostatic proteins VWF and PAP. The vascular point of impact seems to be the endothelium, as evidenced by an increase in VWF. Our study is the first to prospectively show that hemostatic proteins are increased in patients with proven acute respiratory tract infections. Although others have reported similar changes in patients with flu-like symptoms, the cross-sectional nature of these studies prohibited a definitive conclusion as to whether acute respiratory tract infection was in fact the cause of these changes [9–13]. These studies were limited, because only self-reported clinical symptoms were used to establish the diagnosis of the infection. This may have confounded the results, as non-infectious inflammatory diseases may also present with fever in combination with cough.

The prospective nature of our study renders the effect of seasonal variation on markers of coagulation and fibrinolysis quite unlikely. Although hemostatic proteins have a minimal seasonal variation, the fact that levels of hemostatic proteins returned to normal in the convalescent phase 2 weeks after the initiation of the disease episode further negates the option that the observed increase is the result of this variation. Also, as we had baseline measurements before the winter season, we were able to exclude the possibility that the increase in levels of hemostatic proteins was the result of pre-existing conditions.

From the results obtained, we conclude that naturally occurring respiratory tract infections in elderly human subjects result in endothelial cell perturbation, which is potentially associated with dysfunction. These hemostatic changes may form a link between acute respiratory tract infections and acute atherothrombotic disease. However, the precise increment in risk still needs to be established in a prospective study.

**Table 1** Hemostatic proteins in patients with respiratory tract infections

| | All subjects | Proven viral infection | Proven influenza |
|---|---|---|---|
| *n** | 54[†] | 14 | 9 |
| VWF (%)[‡] | | | |
| Baseline | 151 (114–189) | 175 (126–233) | 148 (125–217) |
| Acute | 197 (162–247)[§] | 220 (189–284)[§] | 208 (188–280)[§] |
| Convalescent | 147 (125–185)[¶] | 157 (137–185)[¶] | 149 (126–165)[¶] |
| $F_{1+2}$ (nmol $L^{-1}$)[‡] | | | |
| Baseline | 0.90 (0.66–1.11) | 0.77 (0.29–0.90) | 0.77 (0.32–1.09) |
| Acute | 0.92 (0.65–1.29) | 0.88 (0.47–0.94) | 0.90 (0.50–0.96) |
| Convalescent | 1.01 (0.80–1.19)[¶] | 0.99 (0.81–1.34)[¶] | 0.91 (0.40–1.13)[¶] |
| PAI-1 (μg $L^{-1}$)[‡] | | | |
| Baseline | 1.7 (0.6–3.4) | 2.2 (0.6–2.6) | 1.2 (0.5–2.6) |
| Acute | 1.6 (0.7–3.4) | 2.2 (0.6–3.4) | 2.8 (1.0–3.4) |
| Convalescent | 1.7 (0.6–3.4)[¶] | 0.8 (0.5–2.3)[¶] | 1.7 (0.8–2.3)[¶] |
| PAP (U $mL^{-1}$)[‡] | | | |
| Baseline | 343 (255–535) | 345 (260–612) | 344 (255–727) |
| Acute | 465 (283–638)[§] | 583 (342–719) | 583 (412–679) |
| Convalescent | 314 (237–559)[¶] | 549 (314–708)[¶] | 548 (343–743)[¶] |

VWF, von Willebrand factor; $F_{1+2}$, prothrombin fragment 1 + 2; PAI-1, plasminogen activator inhibitor type 1; PAP, plasmin–$\alpha_2$-antiplasmin.
*Number of subjects.
[†]Blood was collected in only 54 episodes, because of logistic reasons.
[‡]All data are presented as median ± interquartile range. Levels of hemostatic proteins were measured at baseline, and in the acute and convalescent phases of disease.
[§]Significantly different ($P < 0.05$) from baseline as calculated by the Wilcoxon rank sum test.
[¶]No statistical significant difference between the convalescent phase of infection and baseline was observed.

## Acknowledgements

We gratefully acknowledge the participants of the study, P. Sutorius and the staff of the General Practitioners Office Overveen. We are indebted to O. Ternede, V. Weeda, M. Vermeulen, T. van Hees and D. Wijffels for assistance in patient care.

## Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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