SUPPLEMENTARY APPENDIX

C-reactive protein and risk of venous thromboembolism: results from a population-based case-crossover study

Gro Grimnes,[1,2] Trond Isaksen,[1,2] Ynse Ieuwe Gerardus Vladimir Tichelaar,[1,3] Jan Brox,[1,2] Sigrid Kufaas Brækkan[1,2] and John-Bjarne Hansen[1,2]

[1]K.G. Jebsen Thrombosis Research and Expertise Center (TREC), Department of Clinical Medicine, UiT The Arctic University of Norway, Tromsø, Norway; [2]Division of Internal Medicine, University Hospital of North Norway, Tromsø, Norway and [3]Department of Vascular Medicine, Academic Medical Center, University of Amsterdam, the Netherlands

 doi:10.3324/haematol.2017.186957

Received: December 21, 2017.

Accepted: April 18, 2018.

Pre-published: April 19, 2018.

Correspondence: gro.grimnes@uit.no

Supplementary appendix for:

C-reactive protein and risk of venous thromboembolism: Results from a population-based case-crossover study

Gro Grimnes,[1,2] Trond Isaksen,[1,2] Ynse Ieuwe Gerardus Vladimir Tichelaar,[1,3] Jan Brox,[1,2] Sigrid Kufaas Brækkan,[1,2] and John-Bjarne Hansen[1,2]

1. K.G. Jebsen Thrombosis Research and Expertise Center (TREC), Department of Clinical Medicine, UiT The Arctic University of Norway, Tromsø, Norway

2. Division of Internal Medicine, University Hospital of North Norway, Tromsø, Norway

3. Department of Vascular Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Methods:

Study population

VTE-cases were recruited from the fourth survey of the Tromsø Study. The fourth survey of this single-center, population-based cohort study was conducted in 1994/95, and 27158 (77%) inhabitants in the municipality of Tromsø, aged ≥25 years, participated. The Tromsø Study cohort has been described in detail elsewhere.[1] All incident VTE events among the study participants were recorded from the date of enrollment (1994-95) until December 31, 2012. For each potential VTE case, the medical records were reviewed by trained personnel, and VTE events were recorded only when clinical signs and symptoms of DVT or PE were combined with objective confirmation by radiological procedures, and resulted in a VTE diagnosis requiring treatment, as previously described.[2] The University Hospital of North Norway is the only hospital serving the Tromsø region, and all relevant diagnostics and hospital care are provided by this hospital. The study was approved by the regional ethics committee, and all participants provided informed written consent.

Study design

We conducted a case-crossover study including all incident VTE cases (n=707) diagnosed among the participants of the fourth Tromsø Study during 1994-2012. In the case-crossover, each participant serves as his/her own control, and potential confounding by patient characteristics and chronic conditions and diseases is largely controlled for by this design.[3] The case-crossover design is therefore well suited for studying transient risk factors or triggers. In this study, a hazard period of 90 days preceding the incident VTE was compared to four preceding 90 day control periods. The length of these hazards and control periods was pre-defined based on the definition of provoking factors, as described by Kearon et al.[4] To avoid carry-over effects, we included a 90 day washout period between the hazard and control periods (Figure 1). For every VTE case, trained medical personnel searched the hospital medical records for relevant risk factors, diagnostic procedures, surgical and medical treatment, laboratory test results and diagnoses during hospital admissions, day care and outpatient clinic visits in any of the hazard or control periods. We did not have access to medical records from general practice.

Definition of transient risk factors

A transient risk factor, or trigger, was defined by its presence during the defined 90-days period. If an exposure occurred over several days, it was considered to have occurred if any of the days of exposure fell within the specified 90-day time period.

CRP was analyzed at the Department of Clinical Biochemistry, University Hospital of North Norway, when requested by a clinician. CRP was analyzed in serum with a particle-enhanced immunoturbidimetric assay on a Modular P (1992-2001), Hitachi 917 (2001-2008) or Cobas 8000 (2008-2012) autoanalyzer (Roche Hitachi, Mannheim, Germany), with reagents from Roche Diagnostics (Mannheim, Germany). The lower cut-off level of the reported CRP value was 5 mg/L, and measurements of CRP lower than 5 mg/L were set to this value. The analytical coefficient of variation for CRP was 3%.

CRP measurements from the last two days before the date of VTE were not included in the analyses to avoid reverse causation, as CRP in these cases could be caused by an inflammatory response to the VTE itself. We additionally conducted sensitivity analyses where this time-frame was extended to the last seven days before the VTE. If a participant had several CRP-measurements during a control or hazard period, the maximum CRP value for each period was used in the study.

Immobilization was defined as the presence of one of the following: bedrest for three days or more, ECOG (*Eastern Cooperative Oncology Group)* score of four, or other immobilizing factors specified in the patient's medical record (e.g. confinement to wheelchair, cast immobilization etc.). Infection was recorded if an acute infection was noted by a physician in the patient's medical record, and this definition included both community-acquired infections that required hospital admission and hospital-acquired infections.

Statistical analyses

Statistical analyses were carried out using STATA version 14.0 (Stata corporation, College station, Texas, USA). Natural log (ln) transformation was used for CRP to achieve normal distributions. Only cases who had their CRP measured in both the hazard and a control period were included in the main analyses. Since CRP was measured upon request, this would yield the most conservative risk estimate. We used conditional logistic regression to obtain β coefficients with 95% confidence intervals (CI) for change in ln-CRP from control to hazard periods. If multiplied by 100, β coefficients from logistic regression on natural log transformed data can be interpreted as percentage difference, and thereby indicate the size of such a difference.[5] Further, as an estimate for VTE-risk, we calculated odds ratios (ORs) with 95% CI per one-unit change in ln-CRP. We additionally performed analyses comparing CRP in the hazard period with each individual control period, to investigate whether time to event influenced the association between inflammation, assessed by CRP, and risk of VTE.

Immobilization is a risk factor for VTE, and can coincide with inflammatory conditions such as cancer, surgery, trauma, infection and other acute medical conditions. We therefore included

immobilization as a covariate in the analyses. In a second model, we adjusted for infection, a common cause of acute inflammation and increase in CRP, to evaluate the impact of inflammation caused by conditions other than infection. For the same purpose, we also performed analyses stratified for infection. In these analyses, a VTE case with infection in the hazard period was compared to its control periods with infection, and a VTE case without infection in the hazard period was compared to its control periods without infection.

In the main analyses, we included only hazard and control periods in which CRP had been measured. The risk estimates from this conservative approach might be underestimations, as subjects with no hospital contact during a hazard or control period, or with a hospital contact without a CRP measurement most likely had a low CRP at that time. To address this concern, we performed sensitivity analyses where missing CRP values were set at the lower reported cut-off level of 5 mg/L. We also performed sensitivity analyses where we included only those CRP-measurements performed more than seven days before the date of VTE, to address potential bias due to reverse causation.

References

1. Jacobsen BK, Eggen AE, Mathiesen EB, Wilsgaard T, Njolstad I. Cohort profile: the Tromso Study. Int J Epidemiol 2012; **41**(4): 961-7.
2. Braekkan SK, Mathiesen EB, Njolstad I, Wilsgaard T, Stormer J, Hansen JB. Mean platelet volume is a risk factor for venous thromboembolism: the Tromso Study, Tromso, Norway. Journal of thrombosis and haemostasis : JTH 2010; **8**(1): 157-62.
3. Maclure M. The case-crossover design: a method for studying transient effects on the risk of acute events. Am J Epidemiol 1991; **133**(2): 144-53.
4. Kearon C, Ageno W, Cannegieter SC, Cosmi B, Geersing GJ, Kyrle PA. Categorization of patients as having provoked or unprovoked venous thromboembolism: guidance from the SSC of ISTH. Journal of thrombosis and haemostasis : JTH 2016; **14**(7): 1480-3.
5. Cole TJ. Sympercents: symmetric percentage differences on the 100 log(e) scale simplify the presentation of log transformed data. Statistics in medicine 2000; **19**(22): 3109-25.

Supplementary table 1.

Association of C-reactive protein[a] with risk of venous thromboembolism (VTE)

Sensitivity analysis, C-reactive protein measured more than seven days before VTE-diagnosis

	Hazard period compared to control periods		
		Adjusted for immobilization	Adjusted for infection
	β[b] (95% CI)	**β[b] (95% CI)**	**β[b] (95% CI)**
All cases	0.54 (0.35-0.74)	0.51 (0.31-0.70)	0.36 (0.15-0.58)
Cases with infection	0.37 (-0.03-0.77)	0.34(-0.09-0.77)	-
Cases without infection	0.55 (0.16-0.94)	0.54 (0.14-0.94)	-
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**
All cases	1.72 (1.42-2.09)	1.59 (1.30-1.95)	1.44 (1.16-1.78)
Cases with infection	1.45 (0.97-2.16)	1.40 (0.91-2.15)	-
Cases without infection	1.73 (1.18-2.55)	1.71 (1.15-2.55)	-

OR: odds ratio, CI: Confidence interval

[a] Natural log transformed C-reactive protein

[b] When multiplied by 100, β coefficients can be interpreted as percentage difference compared with the reference group

Supplementary table 2.

Association of C-reactive protein [a] with risk of venous thromboembolism

Sensitivity analysis, missing C-reactive protein values set to lower detection limit (CRP=5)

	Hazard period compared to control periods		
		Adjusted for immobilization	Adjusted for infection
	β[b] (95% CI)	**β[b] (95% CI)**	**β[b] (95% CI)**
All cases	0.86 (0.76-0.96)	0.69 (0.58-0.80)	0.49 (0.38-0.61)
Cases with infection	0.12 (-0.13-0.37)	0.12 (-0.14-0.38)	-
Cases without infection	0.97 (0.77-1.17)	0.83 (0.62-1.04)	-
	Odds ratio (95% CI)	**Odds ratio (95% CI)**	**Odds ratio (95% CI)**
All cases	2.36 (2.14-2.61)	2.00 (1.79-2.23)	1.64 (1.46-1.84)
Cases with infection	1.13 (0.88-1.45)	1.13 (0.87-1.47)	-
Cases without infection	2.64 (2.16-3.23)	2.29 (1.85-2.83)	-

CI: Confidence interval

[a] Natural log transformed C-reactive protein

[b] When multiplied by 100, β coefficients can be interpreted as percentage difference compared with the reference group