Thrombophilia ad dies vitae

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Chapter 7

Venous thrombosis is associated with hyperglycaemia at diagnosis: a case-control study

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Abstract

Background

Patients with (undiagnosed) diabetes mellitus, impaired glucose tolerance or stress-induced hyperglycaemia may be at greater risk for venous thrombosis and present with relative hyperglycaemia during the thrombotic event.

Objectives

To assess whether venous thrombosis is associated with hyperglycaemia at diagnosis.

Methods

We performed a case-control study, derived from a cohort of consecutive patients referred for suspected deep venous thrombosis. Cases were patients with confirmed symptomatic venous thrombosis of the lower extremity. Controls were randomly selected in a 1:2 ratio from individuals in whom this diagnosis was excluded. We measured plasma glucose levels upon presentation to the hospital.

Results

A total of 188 patients with thrombosis and 370 controls were studied. The glucose cut-off level for the 1st quartile was < 5.3 mmol/l, the 2nd 5.3-5.7 mmol/l, the 3rd 5.7-6.6 mmol/l and the 4th quartile ≥ 6.6 mmol/l. When adjusted for body mass index, a known history of diabetes mellitus, age, sex, ethnicity and whether known risk factors for deep venous thrombosis were present, the OR for deep venous thrombosis in the 2nd, 3rd and 4th quartile of glucose levels compared to the 1st quartile was 1.59 (95% CI 0.89-2.85), 2.04 (95 % CI 1.15-3.62) and 2.21 (95% CI 1.20-4.05), respectively, *P* for trend =0.001.

Conclusions

Increased glucose levels measured at presentation were associated with venous thrombosis. Experimental evidence supports a potential causal role for hyperglycaemia in this process. As this is the first report about the association between (stress) hyperglycaemia and venous thrombosis, confirmation in other studies is required.

Introduction

Venous thromboembolism (VTE) is a common disease with an annual incidence of 2-3 per 1,000 inhabitants.[1] Both acquired and inherited risk factors are known to play a role in the development of thrombosis.[2] Nevertheless, in approximately 25% of patients with VTE neither an acquired nor an inherited risk factor can be demonstrated.[3,4] Evidence is growing that classic risk factors for arterial disease are also involved in the development of VTE.[5,6] Hyperglycaemia is associated with arterial thrombosis[7-9] and, indeed, patients with diabetes mellitus or the metabolic syndrome[10]) also have an increased risk of VTE.[11,12] This increased risk of VTE can in part be explained by the platelet activation and hypercoagulability present in diabetes mellitus.[13] Activation of the coagulation system has also been observed in acute experimentally induced hyperglycaemia in healthy male volunteers.[14] During acute illness such as myocardial infarction, a phenomenon called stress hyperglycaemia may occur independently of the presence of known diabetes.

Since hyperglycaemia stimulates coagulation, we hypothesized that higher glucose levels - independent of known diabetes mellitus- would be more common in patients presenting with acute VTE.

Methods

We performed a case-control study. Cases and controls were selected from the Amsterdam Case-Control Study on Thrombosis (ACT) which was initiated in 1999 to identify new risk factors for DVT.

All consecutive outpatients older than 18 years referred to the Academic Medical Centre in Amsterdam between September 1999 and August 2006 with clinically suspected deep venous thrombosis (DVT) of the lower extremity were eligible for this study. The study protocol was approved by the Medical Ethics Review Committee and all participants provided written informed consent. At presentation, the patient's medical history was obtained through a standardized questionnaire including specific questions about symptom duration, presence of known risk factors (concomitant malignancy, pregnancy, use of Hormonal Replacement Therapy, Oral Contraceptives or selective oestrogen receptor modulators, recent trauma (within last 60 days), bedridden > 3 days, uncommon travel (> 6hrs) within the last 3 months, paralysis of the symptomatic leg, or surgery within the last 4 weeks), concomitant diseases and medication use. In addition, body mass index (BMI) was calculated. Cases were patients with thrombosis of the lower extremity confirmed by compression ultrasonography, including proximal DVT (i.e. proximal thrombosis of the

iliac or superficial femoral vein, calf vein thrombosis, involving at least the upper third part of the deep calf veins), symptomatic calf vein thrombosis, and superficial thrombophlebitis. The diagnosis was confirmed following a diagnostic management strategy, based on the Wells' criteria[15] and a Tinaquant D-dimer assay (Roche diagnostics, Basel, Switzerland), followed by compression ultrasound if indicated as validated and described before.[16] Controls were selected in a 2:1 ratio from those individuals in whom thrombosis was ruled out using the above mentioned strategy. Selection was performed randomly, only taking into account the male/female ratio of the cases.

Sample storage and laboratory analysis

On admission and prior to diagnostic testing, blood samples were drawn and collected in tubes containing 0.109 M trisodium citrate. Within one hour after collection, platelet poor plasma was obtained by centrifugation twice for 20 min at 1600 g and 4°C. The plasma was stored in 2 ml cryovials containing 0.5 ml plasma at -80° C.

Glucose was measured using the HK/G-6PD method (Roche/Hitachi, Basel, Switzerland) and corrected for the 10% dilution with sodium citrate. To assess whether elevated glucose levels were related to an acute phase response induced by the thrombotic event itself, we analyzed C-reactive protein (CRP) levels from 20 cases and 20 controls, randomly selected from each quartile, hence in a total of 160 patients.

Statistical analysis

Results are presented as mean ± standard deviation (SD) or median with interquartile range (IQR), depending on the observed distribution. The primary objective of this study was to assess the relationship between glucose levels at presentation and VTE, which was expressed in Odds Ratios (OR), with 95% confidence intervals. Glucose levels of the controls were divided into quartiles, as distribution of glucose levels is typically non-normally distributed. Subsequently, the cases were assigned to these quartiles according to their admission glucose values. We used binary logistic regression. The regression model was created based on clinical relevant potential confounders (BMI, concomitant known diabetes mellitus, sex, ethnicity, age at diagnosis, and whether known risk factors for VTE were present). Furthermore, ORs were calculated for cases in which the diagnosis of thrombosis was restricted to DVT only, excluding calf vein thrombosis and superficial thrombophlebitis. To assess the correlation between glucose levels and CRP levels we

performed a scatter plot and calculated the correlation coefficient (expressed in r). All statistical analyses were performed in SPSS version 15.0.

Results

In total 188 patients with confirmed thrombosis and 370 controls were included in this study, as blood samples were not available for 2 cases and 10 controls. The baseline characteristics of the two study groups are displayed in Table 1. Mean age and gender distribution were comparable. The control group consisted of a smaller proportion of white patients and a greater proportion of black patients as compared to the cases. The mean BMI was higher in the control group, as tended to be the number of patients with known diabetes mellitus. Median glucose levels were 5.9 mmol/l (IQR 5.3-6.6) in patients with thrombosis, and 5.6 mmol/l (IQR 5.2-6.6) in the controls.

Table 1. Baseline characteristics of the two study groups

	Cases (n=188)	Controls (n=370)
Age, years (mean ±SD)	57±17	56±16
Female (%)	57.4	58.1
Ethnicity (%)		
White	78.7	69.2
Black	10.1	18.1
Asian/Pacific islander	2.7	4.2
Other	8.5	8.5
BMI kg/m² (median, IQR)	26.6 (23.9-29.1)	27.2 (24.2-31.3)
Diabetes type 1 or 2 (%)	3.7	10.0
Glucose mmol/l (median, IQR)	5.6 (5.2-6.6)	5.9 (5.3-6.6)

Known risk factors: concomitant malignancy, pregnancy, use of Hormonal Replacement Therapy, Oral Contraceptives or selective oestrogen receptor modulators, recent trauma (within last 60 days), bedridden > 3 days, uncommon travel (> 6hrs) within the last 3 months, paralysis of the symptomatic leg, or surgery within the last 4 weeks

In thrombosis patients, 38% had one or more acquired risk factors and the distribution of thrombosis was: 82% DVT, 6% calf vein thrombosis, and the remaining 12% had superficial thrombophlebitis.

The cut-off glucose levels for the 1st quartile was < 5.3 mmol/l (n=141), the 2nd 5.3-5.7 mmol/l (n=134), the 3rd 5.7-6.6 mmol/l (n=139) and the 4th quartile ≥ 6.6 mmol/l (n=144) (Table 2a).

After adjustment for BMI, concomitant known diabetes mellitus, sex, ethnicity, age at diagnosis, and whether known risk factors for VTE were present, the OR for thrombosis in the 2nd, 3rd and 4th quartile of glucose levels compared to the 1st quartile were 1.40 (95%

confidence interval (CI) 0.82-2.38), 1.69 (95 % CI 1.00-2.87) and 1.94 (95% CI 1.12-3.39), respectively, *P* for trend = 0.01. The same trend of an increasing OR could be observed when adjusting only for BMI, age, sex and known diabetes mellitus.

Table 2a. Odds Ratio (OR) for all thrombotic events (including calf vein thrombosis and superficial thrombophlebitis)

Quartile (glucose mmol/l)	Cases	Controls	Crude OR (95% CI)	Adjusted OR* (95% CI)
1st : < 5.3	39	102	*reference*	*reference*
2nd : 5.3-5.7	46	88	1.37 (0.82-2.28)	1.40 (0.82-2.38)
3rd : 5.7-6.6	52	87	1.56 (0.94-2.59)	1.69 (1.00-2.87)
4th : ≥ 6.6	51	93	1.43 (0.87-2.37)	1.94 (1.12-3.39)
			P for trend = 0.14	*P* for trend = 0.01

CI=confidence interval, * adjusted for BMI, known diabetes mellitus, sex, age, ethnicity and known risk factors

As most risk factors predominantly relate to DVT a separate analysis was planned for DVT only, thus excluding patients with superficial thrombophlebitis or calf vein thrombosis, leaving 154 cases and 370 controls (Table 2b). Also here, the OR increases with glucose levels: 1.59 (0.89-2.85), 2.04 (1.15-3.62) and 2.21 (1.20-4.05) for the 2nd, 3rd and 4th quartile of glucose levels respectively (see Table 2b, *P* for trend = 0.001). Finally, the Spearman's rank correlation coefficient for CRP and plasma glucose was 0.09, with a *P*-value of 0.27.

Table 2b. Odds Ratio for deep venous thrombosis only

Quartile (glucose mmol/l)	Cases	Controls	Crude OR (95% CI)	Adjusted OR* (95% CI)
1st : < 5.3	28	102	*reference*	*reference*
2nd : 5.3-5.7	37	88	1.53 (0.87-2.70)	1.59 (0.89-2.85)
3rd : 5.7-6.6	46	87	1.93 (1.11-3.34)	2.04 (1.15-3.62)
4th : ≥ 6.6	43	93	1.68 (0.97-2.93)	2.21 (1.20-4.05)
			P for trend = 0.05	*P* for trend = 0.001

CI=confidence interval, * adjusted for BMI, known diabetes mellitus, sex, age, ethnicity and known risk factors

Discussion

In this case-control study, increased glucose levels measured at the time of presentation were associated with venous thrombosis. This could be a relevant clinical concept as the general population is becoming increasingly glucose intolerant. The relation between glucose and DVT was not readily explained by an acute phase reaction due to the thrombotic event itself.

Whereas our results indicate that increased glucose levels and VTE coincide, it is impossible with the current design to demonstrate a causal relationship. However, a causal relationship seems plausible. To assess this one can apply the diagnostic criteria for causation.[17]

A causal relationship is supported by the available biological evidence from experiments in humans. Stegenga and co-workers showed that experimentally induced acute hyperglycaemia activates the coagulation system in healthy volunteers.[14] From a pathophysiological point of view, hyperglycaemia is known to induce coagulation activation through glycocalyx damage[18], up regulation of tissue factor[19,20], non-enzymatic glycation and the development of increased oxidative stress.[21] Long term exposure to hyperglycaemia such as in diabetes mellitus, is a known risk factor for VTE.[22] In addition, the effect of hyperglycaemia on coagulation seems modifiable in diabetes patients, as treating hyperglycaemia among these patients lead to down regulation of coagulation activation in several randomized controlled trials.[23,24] Furthermore, our results are in line with the findings by Mraovic and colleagues who demonstrated that hyperglycaemia increases the risk of pulmonary embolism after major orthopaedic surgery.[25] Thus, direct and indirect evidence supports a possible association for acute and chronic hyperglycaemia in the development of VTE. Furthermore, the association is consistent from this study to other studies, which is in line with the criterion for repetitive demonstration of causality.

In this study, an adjusted OR of 2.21 (95 % CI 1.20-4.05) for DVT was observed in the highest quartile which suggests a strong relationship. In comparison, the OR for the well established risk factor for VTE, the prothrombin 20210A mutation is 2.8 (95% CI 1.4-5.6).[26] The OR for venous thrombosis is increasing with increasing glucose levels, from 1.40 (0.82-2.38) in the 2nd quartile to 1.69 (1.00-2.87) in the 3rd quartile and 1.94 (95 % CI 1.12-3.39) in the 4th quartile. We tested for differences in ORs amongst the quartiles of glucose levels and found a significant linear trend (P=0.01). This is in concordance with a dose-response gradient, another criterion for causality.

The question arises whether elevated glucose levels during a VTE result from the inflammatory and counter regulatory hormone action initiated by the VTE event itself, or whether hyperglycaemia preceded the VTE event. Although a significant proportion of the patients with hyperglycaemia during an episode of VTE will have an undisturbed glucose tolerance at follow-up,[27] undiagnosed impaired glucose tolerance is likely to have been present in a proportion of patients before the VTE event itself, which may therefore have contributed to the development of thrombosis. We therefore suspect a temporal

relationship. In addition, no correlation was found between the acute phase reaction, measured by CRP, and glucose levels. The presence of stress hyperglycaemia during a thrombotic event, independent of its cause, could be relevant: it has been shown to have evident clinical consequences in patients with myocardial infarction and patients admitted to the Intensive Care Unit (especially without known diabetes mellitus)[28,29], although results from recent intervention trials have been disappointing.[30,31]
Interestingly, we found a greater proportion of patients with diabetes in the control group compared to the case group. In fact, this higher rate can be caused by referral bias. Patients with diabetes are usually under chronic medical care and are prone to leg- and foot problems which can resemble DVT, such as erysipelas. Thus, they are more easily referred for suspicion of DVT. We had no information on the use of anti-diabetic drugs. Differences in distribution of diabetes treatment in both cases and controls could be a source of bias. However, we believe this effect to be limited. The model was adjusted for diabetes mellitus as a potential confounder.

Our study has several limitations. Firstly, the control group consisted of patients with complaints of their legs instead of healthy controls. Consequently, it may be possible that glucose levels were increased in the controls due to an underlying disease such as infection. However, this would have led to an underestimation of the association between glucose and DVT. Secondly, the glucose levels were measured on admission, and it was unknown whether these were fasting or non-fasting samples. However, due to the study design there were no differences between cases and controls with respect to the time of presentation and therefore larger dispersion of glucose levels would have affected cases as much as controls.
Thirdly, citrated plasma is not the plasma of choice for determining glucose and CRP levels, because of the dilution with sodium citrate. Also, sodium citrate does not inhibit ex-vivo glycolysis. However, we corrected glucose levels for the dilution and the obtained blood samples were centrifuged and stored within one hour thereby minimizing glycolysis. Again, as blood samples of both cases and controls were obtained and processed in the same manner, the results of glucose levels were affected equally. Since this is the first report about the association between stress-hyperglycaemia and VTE, confirmation in other studies is required.

In conclusion, our findings suggest that higher glucose levels are a risk factor for the development of venous thrombosis. It will therefore be of importance to analyse whether disturbed glucose homeostasis persists after the acute phase of venous thrombosis.

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References

1. Cushman M, Tsai AW, White RH, Heckbert SR, Rosamond WD, Enright P, Folsom AR. Deep vein thrombosis and pulmonary embolism in two cohorts: the longitudinal investigation of thromboembolism etiology. The American Journal of Medicine 2004; 117:19-25.
2. Rosendaal FR. Venous thrombosis: a multicausal disease. The Lancet 1999; 353:1167-73.
3. Lensing AW, Prandoni P, Prins MH, Buller HR. Deep-vein thrombosis. The Lancet 1999; 353:479-85.
4. Spencer FA, Emery C, Lessard D, Anderson F, Emani S, Aragam J, Becker RC, Goldberg RJ. The Worcester Venous Thromboembolism study: a population-based study of the clinical epidemiology of venous thromboembolism. J Gen Intern Med 2006; 21:722-7.
5. Eliasson A, Bergqvist D, Bjorck M, Acosta S, Sternby NH, Ogren M. Incidence and risk of venous thromboembolism in patients with verified arterial thrombosis: a population study based on 23 796 consecutive autopsies. Journal of Thrombosis and Haemostasis 2006; 4:1897-902.
6. Sorensen HT, Horvath-Puho E, Pedersen L, Baron JA, Prandoni P. Venous thromboembolism and subsequent hospitalisation due to acute arterial cardiovascular events: a 20-year cohort study. Lancet 2007; 370:1773-9.
7. Bruno A, Levine SR, Frankel MR, Brott TG, Lin Y, Tilley BC, Lyden PD, Broderick JP, Kwiatkowski TG, Fineberg SE. Admission glucose level and clinical outcomes in the NINDS rt-PA Stroke Trial. Neurology 2002; 59:669-74.
8. Malmberg K, Ryden L, Hamsten A, Herlitz J, Waldenstrom A, Wedel H. Effects of insulin treatment on cause-specific one-year mortality and morbidity in diabetic patients with acute myocardial infarction. DIGAMI Study Group. Diabetes Insulin-Glucose in Acute Myocardial Infarction. Eur Heart J 1996; 17:1337-44.
9. Laakso M. Hyperglycemia and cardiovascular disease in type 2 diabetes. Diabetes 1999; 48:937-42.
10. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC, Jr., Spertus JA, Costa F. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 2005; 112:2735-52.
11. Ay C, Tengler T, Vormittag R, Simanek R, Dorda W, Vukovich T, Pabinger I. Venous thromboembolism a manifestation of the metabolic syndrome. Haematologica 2007; 92:374-80.
12. Petrauskiene V, Falk M, Waernbaum I, Norberg M, Eriksson JW. The risk of venous thromboembolism is markedly elevated in patients with diabetes. Diabetologia 2005; 48:1017-21.
13. Grant PJ. Diabetes mellitus as a prothrombotic condition. J Intern Med 2007; 262:157-72.
14. Stegenga ME, van der Crabben SN, Levi M, de Vos AF, Tanck MW, Sauerwein HP, van der Poll T. Hyperglycemia Stimulates Coagulation, Whereas Hyperinsulinemia Impairs Fibrinolysis in Healthy Humans. Diabetes 2006; 55:1807-12.
15. Wells PS, Anderson DR, Bormanis J, Guy F, Mitchell M, Gray L, Clement C, Robinson KS, Lewandowski B. Value of assessment of pretest probability of deep-vein thrombosis in clinical management. The Lancet 350:1795-8.

16. Kraaijenhagen RA, Piovella F, Bernardi E, Verlato F, Beckers EA, Koopman MM, Barone M, Camporese G, Potter Van Loon BJ, Prins MH, Prandoni P, Buller HR. Simplification of the diagnostic management of suspected deep vein thrombosis. Arch Intern Med 2002; 162:907-11.
17. How to read clinical journals: IV. To determine etiology or causation. Can Med Assoc J 1981; 124:985-90.
18. Nieuwdorp M, van Haeften TW, Gouverneur MC, Mooij HL, van Lieshout MH, Levi M, Meijers JC, Holleman F, Hoekstra JB, Vink H, Kastelein JJ, Stroes ES. Loss of endothelial glycocalyx during acute hyperglycemia coincides with endothelial dysfunction and coagulation activation in vivo. Diabetes 2006; 55:480-6.
19. Khechai F, Ollivier V, Bridey F, Amar M, Hakim J, de Prost D. Effect of advanced glycation end product-modified albumin on tissue factor expression by monocytes. Role of oxidant stress and protein tyrosine kinase activation. Arterioscler Thromb Vasc Biol 1997; 17:2885-90.
20. Min C, Kang E, Yu SH, Shinn SH, Kim YS. Advanced glycation end products induce apoptosis and procoagulant activity in cultured human umbilical vein endothelial cells. Diabetes Res Clin Pract 1999; 46:197-202.
21. Ceriello A. Coagulation activation in diabetes mellitus: the role of hyperglycaemia and therapeutic prospects. Diabetologia 1993; 36:1119-25.
22. Ageno W, Becattini C, Brighton T, Selby R, Kamphuisen PW. Cardiovascular Risk Factors and Venous Thromboembolism: A Meta-Analysis. Circulation 2008; 117:93-102.
23. Caballero AE, Delgado A, Guilar-Salinas CA, Herrera AN, Castillo JL, Cabrera T, Gomez-Perez FJ, Rull JA. The Differential Effects of Metformin on Markers of Endothelial Activation and Inflammation in Subjects with Impaired Glucose Tolerance: A Placebo-Controlled, Randomized Clinical Trial. J Clin Endocrinol Metab 2004; 89:3943-8.
24. De Jager J, Kooy A, Lehert P, Bets D, Wulffele MG, Teerlink T, Scheffer PG, Schalkwijk CG, Donker AJ, Stehouwer CD. Effects of short-term treatment with metformin on markers of endothelial function and inflammatory activity in type 2 diabetes mellitus: a randomized, placebo-controlled trial. J Intern Med 2005; 257:100-9.
25. Mraovic B, Hipszer BR, Epstein RH, Pequignot EC, Parvizi J, Joseph JI. Preadmission Hyperglycemia is an Independent Risk Factor for In-Hospital Symptomatic Pulmonary Embolism After Major Orthopedic Surgery. J Arthroplasty 2008.
26. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3′-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 1996; 88:3698-703.
27. Ishihara M, Inoue I, Kawagoe T, Shimatani Y, Kurisu S, Hata T, Nakama Y, Kijima Y, Kagawa E. Is admission hyperglycaemia in non-diabetic patients with acute myocardial infarction a surrogate for previously undiagnosed abnormal glucose tolerance? Eur Heart J 2006; 27:2413-9.
28. Ishihara M, Kojima S, Sakamoto T, Asada Y, Tei C, Kimura K, Miyazaki S, Sonoda M, Tsuchihashi K, Yamagishi M, Ikeda Y, Shirai M, Hiraoka H, Inoue T, Saito F, Ogawa H. Acute hyperglycemia is associated with adverse outcome after acute myocardial infarction in the coronary intervention era. Am Heart J 2005; 150:814-20.
29. Whitcomb BW, Pradhan EK, Pittas AG, Roghmann MC, Perencevich EN. Impact of admission hyperglycemia on hospital mortality in various intensive care unit populations. Crit Care Med 2005; 33:2772-7.
30. Mehta SR, Yusuf S, Diaz R, Zhu J, Pais P, Xavier D, Paolasso E, Ahmed R, Xie C, Kazmi K, Tai J, Orlandini A, Pogue J, Liu L. Effect of glucose-insulin-potassium infusion on mortality in patients with acute ST-segment elevation myocardial infarction: the CREATE-ECLA randomized controlled trial. JAMA 2005; 293:437-46.
31. NICE-SUGAR Study Investigators, Finfer S, Chittock DR, Su SY, Blair D, Foster D, Dhingra V, Bellomo R, Cook D, Dodek P, Henderson WR, Hebert PC, Heritier S, Heyland DK, McArthur C, McDonald E, Mitchell I, Myburgh JA, Norton R, Potter J, Robinson BG et al. Intensive versus conventional glucose control in critically ill patients. N Engl J Med 2009; 360:1283-97