Author's response to reviews

Title: Incident venous thrombotic events in the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER).

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Version: 2 Date: 17 December 2010

Author's response to reviews: see over
Response to Editors’ Comments

Associate Editor

1. Patients on warfarin treatment at baseline should have been excluded before the selection of cases and controls or alternatively all controls for the 4 cases should be excluded. You have excluded 4 cases and 5 controls. Does this mean that these 4 cases had only 5 controls? If not exclude the remaining three controls from the analysis.

The nested case control study was used for number of other analyses including genotyping analysis. This analysis was largely negative and underpowered and is not reported here. The coagulation markers analysis was also carried out on the full nested case control samples but when we were analysing the data we decided that we should not include those on warfarin as the drug treatment can influence the plasma analysis of hemostatic markers. Frequency matching was carried out by creating bins by age, smoking history and country, not by matching specific individual cases with specific controls. It is therefore not possible, nor necessary, to remove specific cases/controls for the warfarin subjects. To clarify this issue we have included Figure 1 – a flow diagram showing identification of cases, non-cases and control and we have clarified this in the methods section.

2. Consider sensitivity analyses where definite events are those events confirmed on the death certificate or from recorded evidence such as ultrasound venography ventilation perfusion lung scans or computed tomography pulmonary angiography. (i.e exclude those with at least 3 months continuous anti-coagulant treatment with warfarin or heparin). Repeat the analyses and report any difference in results.

We accept this point made by the associate editor and reviewer 1 about the definition of definite VTE. We have changed our definition of definite VTE to include those where death from VTE was confirmed on the death certificate or if there was recorded evidence from investigations such as ultrasound venography ventilation perfusion lung scans or computed tomography pulmonary angiography. This gives a new number of definite cases of n=48. The statistical analysis was repeated on this new dataset and the manuscript now presents all the analysis using the new, more rigorous definition. The results are not materially changed. We now refer to the analysis on the combined definite and probable VTE cases (n=72) in the text only.

3. The statistical analyses are confusing. Table 1 shows the baseline factors for cases and non-cases, while table 2 shows results for cases and controls. The results of the adjusted Cox model (your main analysis) are not reported in a table. Figure 1 has now been included to clarify the classification of non-cases and controls. Modelling adjusting for confounding covariates was only carried out for those variables that were univariately associated with VTE at the 5% significance level (i.e. MMSE, blood pressure, country and BMI). We have now quoted the full adjusted hazard ratios, confidence intervals and significance levels in the text for factors that remained significant on multivariate analysis.
4. You say "The time to VTE was quantified by hazard ratios calculated with Cox’s proportional hazard model?; this model is suitable for a cohort study. Since the study of the risk of VTE associated with statins (main analysis) is a cohort analysis, it is not clear to me why you also conducted a nested case-control analysis. Clarify this point and explain why data would be available only for the cases and controls. Was it because you had to retrieved them from the charts? Where data had already been collected on all participants in PROSPER (i.e. those listed in Table 1) all data was used and Cox’s proportional hazard model was used to calculate hazard ratios. This was data stored in the database and included information collected as part of the original study design, or as post hoc analyses for secondary studies which had been funded for the whole cohort (i.e. inflammatory markers). The current study undertook some new plasma analyses on coagulation factors. These assays are expensive and funding was only obtained to carry out a nested case control analysis. This is clarified at the end of the Blood Analyses section in the Methods “For blood analyses listed in Table 1 data was already available in the PROSPER database for all participants. For the coagulation analytes and IGF-1, the analyses were carried out specifically for the purposes of the current study and were performed in cases and matched controls only due to limited resource.”

5. The sentence "Other studies have analyzed effects of pre-existing statin medication on incident VTE [15-17] or have compared statin use in case control studies of VTE [18-20]? does not belong in the statistical section but in the Discussion section.

This sentence has been moved to the Background section (Paragraph 1) and has been expanded upon as requested by Reviewer 2 Point 29 below.

Editorial Comments

1. We recommend that you copyedit the paper to improve the style of written English. If this is not possible, you may need to use a professional copyediting service. Examples are those provided by the Manuscript Presentation Service (www.biomedes.co.uk), International Science Editing (http://www.internationalscienceediting.com/) and English Manager Science Editing (http://www.sciencemanager.com/). BioMed Central has no first-hand experience of these companies and can take no responsibility for the quality of their service.

We have made improvements to the written English and run a spell and grammar check on the manuscript.

2. Please include the name of the independent review board that approved your study in the Methods section of your manuscript.

The name of the independent review boards have been added to the Methods section.
Response to Reviewers’ comments

Reviewer 1.

Major comments

1. My major concern is the outcome assessment and classification. A definite VTE should be objectively confirmed by radiological procedures. A record of anticoagulant treatment for three months is not sufficient to confirm a diagnosis of VTE, and thereby classify an event as definite. Preferably, a more restrictive classification of definite VTE should be used.

We accept this point made by the associate editor and reviewer 1 about the definition of definite VTE. We have changed our definition of definite VTE to include those where death from VTE was confirmed on the death certificate or if there was recorded evidence from investigations such as ultrasound venography ventilation perfusion lung scans or computed tomography pulmonary angiography. This gives a new number of definite cases of n=48. The statistical analysis was repeated on this new dataset and the manuscript now presents all the analysis using the new, more rigorous definition. The results are not materially changed. We now refer to the analysis on the combined definite and probable VTE cases (n=72) in the text only.

2. In the present paper, almost 20% of the total cases were classified as probable cases (a number that would be even higher if definite cases were restricted to those objectively confirmed). The authors state in the discussion section, last paragraph, that analysis using definite VTE only, yielded similar results to those using the total VTE cases. Hazard ratios with 95% CI for definite VTE by pravastatin vs. placebo should also be provided in the results section.

We have now presented complete data on definite VTE only. Hazard ratios for combined definite and probable VTE are now included in the text.

3. The prospective nature of PROSPER do not allow adjustments for transient or precipitating risk factors due to lack of information on factors other than cancer in the whole study population. However, it would be intriguing to know whether cases were unprovoked or provoked, and whether there was any imbalance in the proportion of unprovoked/provoked VTEs between the treatment and placebo groups. Information on transient or precipitating risk factors among the cases, as well as information on the proportion of incident and recurrent VTE, would be preferable.

The only potential provoking factor that was pre-specified and recorded in a systematic manner in the PROSPER database was cancer. This was because it was listed as an adverse outcome in the original PROSPER study design. Unfortunately the data on other potential factors provided in the PROSPER database are insufficient for us to establish whether VTE were provoked or unprovoked. There was no systematic recording of immobilisation, major surgery or other potential factors that may provoke a VTE.

4. The dosage of pravastatin used in the trial should be provided in the text.

The dosage of pravastatin (40 mg/day) has been included in the methods.
Minor Comments

1. A table presenting the baseline characteristics of each treatment group would be helpful for the reader. A supplementary table of baseline characteristics in placebo (n=2865) and pravastatin treated (n= 2834) not on warfarin is included. This table can be included as a main table at the Editor’s discretion.

2. Results section, second paragraph, second line: The word “with” is missing Discussion section, second paragraph, sixth line: The word “greater” is superfluous

The word “with” has been added. The word “greater” has been removed.

Reviewer 2

Major compulsory revisions

1. The rationale for using a case-control analysis should be stated. It is not clear why such analysis was used since Cox regressions were also performed. If the case-control analysis was used because some variables were available for a portion of the population only (as suggested in the statistical analysis portion), there are questions that have to be answered: Why not use sensitivity analyses for missing variables in the whole cohort instead? If the variables were available only for a portion of the population, it is unclear why they were available for all cases, but not for the whole cohort. Could this be problematic? Also, it is not clear why patients on warfarin at baseline were not excluded before the matching process was performed (especially since they were excluded in the analyses involving the whole cohort). It is odd to exclude cases and controls afterwards. It means there are cases that are not matched to two controls and controls that are not matched to cases. More details should be given on how the matching process was performed (eg, could controls be chosen more than once?)

We accept the reviewer’s point that the selection of cases, non-cases and controls could be clarified. Figure 1 has now been included to clarify the classification of non-cases and control.

Where data had already been collected on all participants in PROSPER (i.e. those listed in Table 1) all data was used and Cox’s proportional hazard model was used to calculate hazard ratios. This was data stored in the database and included information collected as part of the original study design, or as post hoc analyses for secondary studies which had been funded for the whole cohort (i.e. inflammatory markers). The current study undertook some new plasma analyses on coagulation factors. These assays are expensive and funding was only obtained to carry out a nested case control analysis. This is clarified at the end of the Blood Analyses section in the Methods “For blood analyses listed in Table 1 data was already available in the PROSPER database for all participants. For the coagulation analytes and IGF-1, the analyses were carried out specifically for the purposes of the current study and were performed in cases and matched controls only due to limited resource.”

The nested case control study was designed to include other analyses including genotyping analysis. This analysis was largely negative and underpowered and is not
reported here. The coagulation markers analysis was also carried out on the full nested case control samples but when we were analysing the data we decided that we should not include those on warfarin as the drug treatment can influence the plasma analysis of hemostatic markers. The matching was carried out by creating bins by age, smoking history and country, not by matching specific individual cases with specific controls. It is therefore not possible, nor necessary, to remove specific cases/controls for the warfarin subjects.

Minor essential revisions

ABSTRACT

2. It would be informative for the reader to know that hazard ratios were used to evaluate the risk of VTE.

The evaluation of risk for VTE is presented as a hazard ratio in the Abstract “Pravastatin did not reduce VTE in PROSPER compared to placebo [unadjusted hazard ratio (95% confidence interval) 1.42 (0.80, 2.52) p=0.23].”

3. Since Cox regressions were used for the identification of the majority of factors associated with VTE, it should be clearly stated in the methods. The multivariate analysis should be announced in the methods (it appeared only at the end of the results section).

The type of analysis including the use of multivariate analysis is now stated in the Abstract “Risk for VTE was examined in non-warfarin treated pravastatin (n=2834) and placebo (n=2865) patients using a Cox’s proportional hazard model, and the impact of other risk factors assessed in a multivariate forward stepwise regression analysis.”

4. It is not clear why a case-control analysis was performed. In fact, from the abstract, the reader may think case-control analysis was used for identification of all factors. (Are the results of the case-control analysis essential for the abstract? Would it be easier for the reader if only the results from the cohort analysis are presented?)

As suggested by the reviewer we have removed the case-control analysis from the abstract.

BACKGROUND

5. At the end of the second paragraph, please give more details why “it was not certain that elderly people benefit”. Was it because there were too few of them included in the trials? Because of heterogeneity of the results?...

We have provided more details as to why it is unclear whether the elderly benefit “A meta-analysis suggested that statin treatment was likely to reduce the risk of VTE, however there was significant heterogeneity of study outcome [14], and as the majority of studies looked at middle-aged rather than elderly populations and there was no separate analysis by age, it was not certain that elderly people benefit.”

6. In the third paragraph, a sentence should be added after the first sentence to explain that the data from PROSPER was used for the present study. (The authors may refer to the way it is formulated in the abstract.)

The following sentence was inserted in the third paragraph “The present study is an analysis of incident VTE in this population of men and women aged 70-82 using data from the PROSPER database.”
METHODS

7. In general, the distinction between the analyses performed in the whole cohort and those performed within the case-control analysis should be made clearer. For example, on lines 16-17 of the paragraph under subject, it is stated, “For the nested case-control study non-cases were all individuals not identified as either a definite or probable case”. However, this also applies to the Cox regression. The term “cases” may lead to confusion, because it usually relates to case-control analyses, but is also used in the cohort. Also, instead of saying “where data were available only for the cases and controls…”, it may be more informative to state which plasma risk markers were available only for the case-control analysis (page 8, statistical analysis).

Figure 1 now shows more clearly the distinction between cases, non-cases and controls. In the methods the status of non-cases is clarified and more detail given on the two separate analyses. “Where data were available for the entire cohort (Fig 1, Table 1), the utility of a parameter in predicting risk of VTE was assessed using all cases (n=48 definite VTE, n=72 combined definite and probable VTE) and non-cases (n=5627) not on warfarin treatment. For hemostatic variables and IGF-1 (Table 2) a nested case control study was performed. We matched each probable and definite case (n=76) with 2 controls (n=152) selected at random from all non-cases on the basis of age (using two-year age categories), smoking status and country of origin (Fig 1). Patients on warfarin treatment at baseline (n=4 cases and n=5 controls) were excluded, leaving 48 definite VTE cases and 72 combined probable and definite cases and 147 controls in the nested case control study analyses (Fig 1).” The specific risk markers which were used in the nested case control analysis are now listed in the statistical analysis section.

8. At the end of the paragraph under subjects on page 6, it is not clear why VTE cases were separated into those who had and those who did not develop cancer during the study.

A brief rationale for the separation of cases into those who did and those who did not develop cancer during the study has been added to the methods “In order to assess the impact of cancer as a precipitating factor for VTE” and Results “There were more new cases of cancer in the pravastatin group than in the placebo group [15] (Supplementary Table 1) and since the etiology of VTE might differ between those with a diagnosis of cancer and those without we explored the possibility that baseline risk factor profile differed between cases who had cancer and cases who did not have cancer.”

9. When were blood analyses performed? At baseline? Please add the information on page 7.

Blood analyses were carried out on baseline blood samples. This information has been added to the blood analyses section in the methods, both in the subsection title and in the text.

10. In the statistical analysis, please give more information on multivariate analyses (eg, on page 9 in the results section, it is described that all variables significant at the 5% level on univariate analysis where allowed to enter the model. This information should be found in the method section instead).
This information has been moved to the methods section “In the multivariate analyses, forward stepwise regression was undertaken, where all variables significant at the 5% level on univariate analysis were allowed to enter the model, to determine the subsets of variables that were independently associated with VTE.”

11. Were only univariate analyses planned for the case-control analysis? Please add the information about multivariate analysis if appropriate.

Univariate analysis was carried out prior to multivariate analysis. Since there was only one significant univariate association for the risk factors assessed in the case control analysis (Table 2), we did not proceed to multivariate analysis.

RESULTS

12. The cancer diagnosis has not been well explained in the method section (it only appeared on page 6, where it was said that cases were separated according to cancer status.) To be adequate, the variable has obviously to be obtained for all cohort members. Please add the information.

Information on cancer frequency in the entire cohort is provided in Supplementary table 1 and hazard ratio for cancer as a risk factor for VTE is shown in Table 1. The following text is now included “Cancer is a recognised risk factor for VTE. In the current study cases were divided into cancer-associated (n=11) and non cancer-associated (n=37) VTE. The hazard ratio for cancer as a predictor of VTE was 4.58 (2.33, 9.01), P<0.001 when comparing cases with non-cases (Table 1).”

13. Please give the adjusted HR for BMI and country (page 9).

HR etc for BMI, blood pressure and country are now provided in the results.

14. The rationale for the risk factor profile (page 10) is not clear. Since cancer was a variable in the multivariate analysis, it is not clear what this analysis adds and where it stands relative to the study objectives. Was the risk factor profile also studied for the “non-cases”?

This analysis was carried out because we wanted to ask whether VTE cases secondary to cancer would have an etiology different to that of patients who had VTE in the absence of cancer. If this were the case then we might expect the risk factor profile to be different. One obvious potential difference is BMI due to the presence of cachexia in cancer. This has now been clarified in the text. “There were more new cases of cancer in the pravastatin group than in the placebo group [15] and since the etiology of VTE might differ between those with a diagnosis of cancer and those without we explored the possibility that baseline risk factor profile differed between cases who had cancer and cases who did not have cancer.” When this analysis was carried out using our new more robust definition of VTE there was no longer any difference in risk factor profile between those who had a VTE before and those who had a VTE after their cancer diagnosis. Thus this analysis could be excluded if the reviewer or Editor wishes. The risk factor profile of non-cases is provided in Table 1.

DISCUSSION

15. First sentence: the following could be deleted, “with a point estimate for risk above one”. The result is not statistically significant. It doesn’t make any difference whether it is above or below 1.

This text was deleted.
16. Second sentence, please specify again what was the population studied in JUPITER.
The JUPITER population was specified: “in middle-aged subjects with low LDL cholesterol and raised C-reactive protein levels,”

17. Third sentence. There is a need to explain in more details what the authors meant by comparing the confidence intervals and the point estimate. It could be hazardous to compare two different studies this way.
This text has been deleted.

18. Fourth sentence. How many person-years of follow-up were there in each study? Data on person-years of follow-up for PROSPER and JUPITER have been inserted into the text. “The PROSPER study had 48 VTE events (from 18,363 person-years of follow-up in those not on warfarin), slightly fewer than the 94 events seen in JUPITER (17,802 participants with median follow up of 1.9 years).”

19. The second paragraph of discussion would be more efficient if it followed the fourth sentence of the first paragraph.
The order of the paragraphs has been changed as requested.

20. First paragraph, last sentence: please specify that the similar lack of effect of statins was on VTE.
We have specified that the lack of effect was on VTE.

21. It may be informative to discuss the limits of having results from blood analyses only at baseline.
The use of baseline blood was to establish their ability to predict VTE outcome. Longitudinal blood analysis would not have enhanced this.

REFERENCES
22. Reference number 7 has been published (2010;31(10):1248-56)
Reference 7 has been updated (now reference 14)

TABLES
23. Table 1. It may be useful to use other terms than cases and non-cases to ensure there is no confusion with the case-control analysis.
The inclusion of Figure 1 now brings clarity to the definition of non-cases.

24. Table 2. Specify they are unadjusted Odds Ratios
We have specified in Table 2 that odds are unadjusted.

Discretionary revisions

BACKGROUND
25. The first sentence commences with “Venous thrombosis” whereas in the second, the term “Venous thromboembolism” is used. Do they refer to the same condition?
If so, please use only one term.
We have now only referred to venous thromboembolism throughout the manuscript.
26. In the first sentence, instead of defining the “old” in parentheses, the actual number could be used: (...) but is close to 1% per annum among those aged over 70 years.
We have changed “old” to “those aged over 70 years”.

27. In the fifth line of the first paragraph, please use the abbreviation VTE as it was already defined (see line #3). Please use it thereafter (eg, second paragraph, line 4).
We have used the abbreviation VTE throughout

METHODS
28. Editing: anticoagulant is written as “anti-coagulant” on line 11, and “anticoagulant” on line 14.
Anticoagulant is now used throughout

29. It would have been interesting to have more information on papers that are cited in the statistical analysis. Why were they not included as part of the introduction or discussed later in the article?
We have now moved this text to the introduction and expanded on it as requested by the reviewer.

DISCUSSION
30. In the first sentence, please add, “the study showed “with data from” a randomized trial …”.
We have inserted the text requested.

31. Second paragraph, third sentence. We find the same sentence in the introduction. It could be deleted in this paragraph.
The sentence has been deleted as requested.

32. Second paragraph, line 6-7. Please use one or the other (greater or #)
This has been corrected see Reviewer 1 comment 3 above.

Reviewer 3

The study has several limitations including the retrospective and the subgroup analysis of the original reported data. Furthermore there are few events and as a consequence the study may not detect a small benefit by pravastatin. Important risk factors such as immobilization, previous general surgery are not reported. As a consequence the conclusions should be tempered since this small study is not able to give an important contribution to the debate on the efficacy of statins in the prevention of VTE.
We have expanded the limitations section in the discussion. “It is a post hoc analysis on a subgroup from the PROSPER study.” “The number of VTE is small and we may not be able to detect a small benefit of pravastatin treatment.” “The PROSPER database did not have information on transient or precipitating risk factors, other than cancer, that may have preceded a VTE, such as immobilization or previous general surgery.”