STROBE Statement—checklist of items that should be included in reports of observational studies

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|  | Item No | Recommendation |
| **Title and abstract** | 1 | (*a*) Indicate the study’s design with a commonly used term in the title or the abstract: PROSPECTIVE |
| (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found  PAGES 1-3  **IN THE ABSTRACT THE FOLLOWING SECTIONS ARE FOUND**: **Design:** In 212 consecutive patients undergoing primary hip/knee arthroplasties for osteoarthritis, platelet counts and anti-PF4/heparin antibodies were evaluated pre- and post-operatively, while MTHFR, and factors II, V gene mutations were detected preoperatively. In 3 years minimum follow-up 196 patients were monitored for thrombosis development.  **Results:** From 196 patients 32 developed thrombocytopenia (insignificant correlation between anticoagulant and thrombocytopenia), 18 developed anti-PF4/heparin antibodies (12/173 for LMWH and 6/23 for fondaparinux – a significant correlation between the anticoagulant and antibodies formation was noted, p=0.005), 4 patients developed HIT (insignificant correlation between thrombocytopenia and antibodies), and 15 had factors II or V mutations. Five patients developed thrombosis: two had positive anti-PF4/heparin antibodies and two were heterozygotes for both factor II and V mutations. Thrombosis was not significantly correlated to platelet counts or HIT. The correlation of thrombosis to anti-PF4/heparin antibodies approached significance (p=0.076), and a significant correlation was found between thrombosis and factors II (p=0.043) and V (p=0.013). |
| Introduction | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported  **PAGE 4:** Total joint arthroplasties are associated with a percentage of proximal deep vein thrombosis of 1.5% and pulmonary embolism of 1% [1,3]. Apart of the factors related to surgery and the decreased mobility after major joint (hip and knee) arhtroplasties [2], mutations in genes that encode for blood coagulation factors V (FV Leiden) and II (Prothrombin 20210) [13], polymorphisms in genes involved in the metabolism of homocysteine (5,10 methylenetetrahydrofolate reductase [MTHFR] 677C/T), and immune-reactions related to anticoagulants (heparin-induced thrombocytopenia [HIT] and thrombosis) may also be responsible for potentially lethal thrombotic phenomena [13-16]. After orthopaedic surgery, the incidence of HIT appears particularly high [5]. The timely recognition of these factors may contribute to the decrease of thrombotic episodes after major joint arthroplasties. |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses  **PAGE 6:** The aim of the this prospective study is to evaluate the prevalence of factors, related to patients genetic profile and to the specific anticoagulants (LMWH or fondaparinux), in the development of a thrombotic episode after major joint arthroplasty for hip or knee osteoarthritis. The influence of different types of anticoagulation on platelet counts, anti-PF4/heparin antibodies and thrombotic episodes and the influence of MTHFR, FII, and FV mutations on thrombotic episodes are evaluated. |
| Methods | | |
| Study design | 4 | Present key elements of study design early in the paper **PAGE 6:** Two hundred twelve consecutive patients that underwent primary total hip or knee arthroplasty due to osteoarthritis during a period of nine months were enrolled prospectively in the study that was approved from the hospital’s ethical committee. Patients with previous thrombotic events were excluded from this study. |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection **PAGE 6-7:** Two hundred twelve consecutive patients that underwent primary total hip or knee arthroplasty due to osteoarthritis during a period of 12 months were enrolled prospectively in the study ….. Platelet counts were obtained from all patients preoperatively and daily for the first seven postoperative days, and at the 20th and 60th postoperative days, while the presence of anti-PF4/heparin antibodies was evaluated with an immunoassay during the preoperative check and at the 3rd, 7th, 20th and 60th postoperative days ……. Finally protein C, protein S, von Willebrand factor (vWF), Lupus Anticoagulant (LA), Antithrombin III (AT III), 677C/T mutation of MTHFR gene, factor V Leiden (G/A) and prothrombin gene G20210A mutations were detected. Genomic DNA was extracted from fresh blood using …....  All patients received anticoagulation for a total of 6 weeks, according to surgeon’s preference. ……….  All patients were followed for a minimum period of 3 years (36-44 months). Sixteen patients were lost to follow up and excluded from the study and thus 196 patients remained to the final follow-up…..Patients were monitored clinically for the development of arterial or venous thrombotic events, such as deep venous thrombosis (DVT), pulmonary embolism (PE), cerebrovascular accident, and myocardial infarction (MI). In suspicion of a thrombotic event the necessary examinations (triplex, pulmonary ventilation/perfusion scan) were performed, to rule out or to confirm the thrombotic incident. |
| Participants | 6 | (*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up **PAGE 6:** Two hundred twelve consecutive patients that underwent primary total hip or knee arthroplasty due to osteoarthritis during a period of 12 months were enrolled prospectively in the study that was approved from the hospital’s ethical committee. Patients with previous thrombotic events were excluded from this study. Patients with other causes of arthritis (rheumatoid arthritis, AVN, seronegative spondyloarthropathies, crystal deposition disease etc)as well as patients with previous thrombotic events or other major risk factors for thrombosis (including malignancy, diabetes, hypertension, BMI >35, age >80y, operative time >100min, congestive heart failure, arrhythmia, smoking, varicose veins)were excluded from this study. All patients were followed for a minimum period of 3 years (36-44 months). Sixteen patients were lost to follow up and excluded from the study and thus 196 patients remained to the final follow-up |
| (*b*)*Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed  **PAGES 7:** All patients were followed for a minimum period of 3 years (36-44 months). Sixteen patients were lost to follow up and excluded from the study and thus 196 patients (42 male and 154 female) remained to the final follow-up. The majority of these patients (173) received low-molecular-weight heparin (LMWH) for prevention of thrombosis, while 23 patients received fondaparinux. Patients were monitored clinically for the development of arterial or venous thrombotic events, such as deep venous thrombosis (DVT), pulmonary embolism (PE), cerebrovascular accident, and myocardial infarction (MI). In suspicion of a thrombotic event the necessary examinations (triplex, pulmonary ventilation/perfusion scan) were performed, to rule out or to confirm the thrombotic incident. |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable **PAGE 7:** Two hundred twelve consecutive patients that underwent primary total hip or knee arthroplasty due to osteoarthritis during a period of nine months were enrolled prospectively in the study ….. Platelet counts were obtained from all patients preoperatively and daily for the first seven postoperative days, and at the 20th and 60th postoperative days, while the presence of anti-PF4/heparin antibodies was evaluated with an immunoassay during the preoperative check and at the 3rd, 7th, 20th and 60th postoperative days ……. Finally protein C, protein S, von Willebrand factor (vWF), Lupus Anticoagulant (LA), Antithrombin III (AT III), 677C/T mutation of MTHFR gene, factor V Leiden (G/A) and prothrombin gene G20210A mutations were detected. Genomic DNA was extracted from fresh blood using …....  **PAGE 7-8:** Patients were monitored clinically for the development of arterial or venous thrombotic events, such as deep venous thrombosis (DVT), pulmonary embolism (PE), cerebrovascular accident, and myocardial infarction (MI). In suspicion of a thrombotic event the necessary examinations (triplex, pulmonary ventilation/perfusion scan) were performed, to rule out or to confirm the thrombotic incident  …..  Thrombocytopenia, defined as 50% decrease of platelets from the pre-operative (and pre-anticoagulant administration) platelet count  **PAGE 9:** HIT (defined as platelet counts <50% of preoperative values and presence of anti-PF4/heparin antibodies). |
| Data sources/ measurement | 8\* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group **PAGE 8:** All P-values were based on two-tailed tests and the level of statistical significance was set at P<0.05. The tests used to obtain p-values were the Pearson Chi-Square and Fisher’s Exact. Logistic regression analysis was used to study the significant correlations. Repeated measurements analysis was applied to test differences in platelets levels between the two anticoagulants. Analysis was conducted using SPSS 14 (SPSS, Inc., Chicago, IL). |
| Bias | 9 | Describe any efforts to address potential sources of bias  PAGE 6 : Patients with other causes of arthritis (rheumatoid arthritis, AVN, seronegative spondyloarthropathies, crystal deposition disease etc)as well as patients with previous thrombotic events or other major risk factors for thrombosis (including malignancy, diabetes, hypertension, BMI >35, age >80y, operative time >100min, congestive heart failure, arrhythmia, smoking, varicose veins)were excluded from this study. The mean age of the patients was 65.8 years (range, 43-80). None of the patients had any history of previous heparin exposure within the past 90 days. |
| Study size | 10 | Explain how the study size was arrived at  Due to extended number of variables and data acquired at different time points the authors decided to limit the sample of consecutive patients to 200. |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why  **PAGE 7:** Repeated measurements analysis was applied to test differences in platelets levels between the two anticoagulants. |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding  **PAGE 8:** All P-values were based on two-tailed tests and the level of statistical significance was set at P<0.05. The tests used to obtain p-values were the Pearson Chi-Square and Fisher’s Exact. Logistic regression analysis was used to study the significant correlations. Repeated measurements analysis was applied to test differences in platelets levels between the two anticoagulants. Analysis was conducted using SPSS 14 (SPSS, Inc., Chicago, IL). |
| (*b*) Describe any methods used to examine subgroups and interactions  **PAGE 7-8:** Logistic regression analysis was used to study the significant correlations. Repeated measurements analysis was applied to test differences in platelets levels between the two anticoagulants. |
| (*c*) Explain how missing data were addressed  **PAGES 7:** Patients that were lost to follow-up were excluded form the study |
| (*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed  **PAGES 8:** All patients were followed for a minimum period of 3 years (36-44 months). Sixteen patients were lost to follow up and excluded from the study and thus 196 patients (42 male and 154 female) remained to the final follow-up. The majority of these patients (173) received low-molecular-weight heparin (LMWH) for prevention of thrombosis, while 23 patients received fondaparinux. |
| (*e*) Describe any sensitivity analyses |

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| Results | | |
| Participants | 13\* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed  **PAGES 8:** All patients were followed for a minimum period of 3 years (36-44 months).  Sixteen patients were lost to follow up and excluded from the study and thus 196 patients  (42 male and 154 female) remained to the final follow-up. The majority of these patients  (173) received low-molecular-weight heparin (LMWH) for prevention of thrombosis,  while 23 patients received fondaparinux. |
| (b) Give reasons for non-participation at each stage  PLEASE REFER TO PREVIOUS COMMENT |
| (c) Consider use of a flow diagram |
| Descriptive data | 14\* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures  and potential confounders  **PAGES 7-8:** All patients were followed for a minimum period of 3 years (36-44 months).  Sixteen patients were lost to follow up and excluded from the study and thus 196 patients  (42 male and 154 female) remained to the final follow-up. The majority of these patients  (173) received low-molecular-weight heparin (LMWH) for prevention of thrombosis,  while 23 patients received fondaparinux |
| (b) Indicate number of participants with missing data for each variable of interest  PLEASE REFER TO PREVIOUS COMMENT |
| (c) *Cohort study*—Summarise follow-up time (eg, average and total amount)  PLEASE REFER TO PREVIOUS COMMENT |
| Outcome data | 15\* | *Cohort study*—Report numbers of outcome events or summary measures over time  **PAGES 7-10:** Thrombocytopenia, defined as 50% decrease of platelets from the  pre-operative (and pre-anticoagulant administration) platelet count [4,9,22-24] was  observed in 30 patients (15.3%) the first 4 postoperative days and in 2 additional patients  the postoperative days 5-7.  …….  Repeated measurements analysis for the comparison of the platelet counts  pre-operatively and the first post-operative days show a fluctuation in counts.  ……  Eighteen of 196 patients (9.2%) developed anti-PF4/heparin antibodies.  The incidence of these antibodies was 12/173 (6.9%) for LMWH and 6/23 (26.1 %) for  fondaparinux.  ……  There was a peak for the appearance of anti-PF4/heparin antibodies during the 7th  postoperative day (9/18 patients, 50.0%).  ……..  Four of 196 patients (2.04 %) developed HIT (defined as platelet counts <50% of  preoperative values and presence of anti-PF4/heparin antibodies). Two of these patients  developed the antibodies in the first postoperative week, while the other 2 patients  developed the antibodies later, between the second postoperative week and the third month.  ……..  The FV1691G/A mutation was detected in five patients (2.6%), while heterozygosity  and homozygosity for the G20210A mutation in the prothrombin gene was demonstrated  in 8 (4.1%) and 2 (1%) patients, respectively.  …….  Symptomatic thrombotic events were observed in five of the 196 patients (two incidents  of DVT, the 7th and 8th postoperative day; two incidents of PE, the 2nd and 14th  postoperative day; and one MI, the 5th postoperative week], while the suspicion for DVT  in 10 patients (between the 4th and 56th postoperative day) or PE in 2 patients (the 4th and 7th postoperative day) was not confirmed by the triplex or the pulmonary  ventilation/perfusion scan.  From the 5 patients that developed thrombotic complications, 2 had positive  anti-PF4/heparin antibodies and 2 patients (suffering from DVT and PE) were  heterozygote to both factor II and V mutations. …………….. |
| *Case-control study—*Report numbers in each exposure category, or summary measures of exposure (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included |
| (*b*) Report category boundaries when continuous variables were categorized |
| Main results | 16 | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period |
| Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses  PELASE REFER TO PAGES 4-7 |
| (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period |
| Other analyses | 17 | Summarise key results with reference to study objectives  PELASE REFER TO PAGES 4-7 |
| Discussion | | |  | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias |
| Key results | 18 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses,  results from similar studies, and other relevant evidence  **PAGE 12-16:** Although the number of patients of our series was relatively small, statistical  analysis indicated that symptomatic thrombotic events were not correlated to  thrombocytopenia or HIT. The correlation of thrombotic events to the formation of  anti-PF4/heparin antibodies approached significance whereas mutations of factors II and  V were significantly correlated to symptomatic thrombosis. Thus the evaluation of  mutations of factors II and V preoperatively and the monitoring for formation of  anti-PF4/heparin antibodies post-anticoagulants administration may reduce thrombotic  complications in patients undergoing major joint arthroplasties. In addition, both LMWH  and fondaparinux were found responsible for the formation of anti-PF4/heparin  antibodies-fondaparinux in larger percentages than LMWH. Consequently,  fondaparinux should not be administered as a substitute of LMWH in cases of HIT and  physicians should consider that in rare cases fondaparinux can also cause HIT. |
| Limitations | 19 | Discuss the generalisability (external validity) of the study results  **PAGES 12-16:** In the relevant literature, it is emphasized that all patients on heparin and  LMWH need close monitoring of platelet counts for early recognition of HIT and in  case of thrombocytopenia occurrence, heparin administration must be discontinued  and a test to detect anti-PF4/heparin antibodies should be made. If a diagnosis of HIT  is confirmed the treating physicians must be cautious to avoid all kinds of heparin.  However, in our series the simple measure of platelet monitoring is questioned, as it does  not necessarily uncover cases of formation of anti-PF4/heparin antibodies that may be  correlated to thrombotic events. A careful screening of the coagulation profile before a  major lower limb reconstruction procedure in combination to platelet monitoring  postoperatively and anti-PF4/heparin antibodies screening the 7th post-anticoagulants  administration day, is a safe way to minimize the risk of thrombosis. In confirmed HIT  syndrome heparin/LMWH must be replaced with other agents, however, the use of  fondaparinux as an alternative treatment of HIT is also questioned. |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses,  results from similar studies, and other relevant evidence  **PAGES 12-16:** Although the number of patients of our series was relatively small, statistical  analysis indicated that symptomatic thrombotic events were not correlated to  thrombocytopenia or HIT. The correlation of thrombotic events to the formation of  anti-PF4/heparin antibodies approached significance whereas mutations of factors II and  V were significantly correlated to symptomatic thrombosis. Thus the evaluation of  mutations of factors II and V preoperatively and the monitoring for formation of  anti-PF4/heparin antibodies post-anticoagulants administration may reduce thrombotic  complications in patients undergoing major joint arthroplasties. In addition, both LMWH  and fondaparinux were found responsible for the formation of anti-PF4/heparin  antibodies-fondaparinux in larger percentages than LMWH. Consequently,  fondaparinux should not be administered as a substitute of LMWH in cases of HIT and  physicians should consider that in rare cases fondaparinux can also cause HIT [8].  In the relevant literature, it is emphasized that all patients on heparin and  LMWH need close monitoring of platelet counts for early recognition of HIT and in  case of thrombocytopenia occurrence, heparin administration must be discontinued  and a test to detect anti-PF4/heparin antibodies should be made. If a diagnosis of HIT  is confirmed the treating physicians must be cautious to avoid all kinds of heparin.  However, in our series the simple measure of platelet monitoring is questioned, as it does  not necessarily uncover cases of formation of anti-PF4/heparin antibodies that may be  correlated to thrombotic events. A careful screening of the coagulation profile before a  major lower limb reconstruction procedure in combination to platelet monitoring  postoperatively and anti-PF4/heparin antibodies screening the 7th post-anticoagulants  administration day, is a safe way to minimize the risk of thrombosis. In confirmed HIT  syndrome heparin/LMWH must be replaced with other agents, however, the use of  fondaparinux as an alternative treatment of HIT is also questioned. |
| Generalisability | 21 | Give the source of funding and the role of the funders for the present study and, if applicable, for the  original study on which the present article is based  The study was supported by a grant form the Hellenic Association of Orthopaedic Surgery  and Traumatology.  The Hellenic Association of Orthopaedic Surgery and Traumatology, had no role in the  study design, collection, analysis and interpretation of data or in the writing of the  manuscript. |
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| Funding | 22 | The study was supported by a grant form the Hellenic Association of Orthopaedic Surgery  and Traumatology. |

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.