PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Impact of anemia on clinical outcome in patients with atrial fibrillation undergoing percutaneous coronary intervention: insights from the AFCAS registry
AUTHORS	Puurunen, Marja; Kiviniemi, Tuomas; Nammas, Wail; Schlitt, Axel; Rubboli, Andrea; Nyman, Kai; Karjalainen, Pasi; Kirchhof, Paulus; Lip, Gregory; Airaksinen, Juhani

VERSION 1 - REVIEW

REVIEWER	Eugenia Nikolsky Rambam Medical Center Cardiology Department Haifa Israel
REVIEW RETURNED	28-Feb-2014

GENERAL COMMENTS	This paper by Puurunen et al. examined the impact baseline anemia on outcomes of 861 patients treated with percutaneous coronary intervention (PCI) in the prospective European AFCAS registry. The outcomes were stratified by presence/absence of anemia at baseline. According to results, baseline anemia was associated with worth clinical outcomes including higher rates of all-cause mortality, stent thrombosis and bleeding. By multivariable analysis, baseline anemia was an independent predictor of all-cause mortality at 12-month follow-up. The paper is on the important clinical topic and is well written. Comments: 1. In real life, PCI is not infrequently required in patients with serious comorbidities many of them resulting in anemia by different pathological pathways. Anemia is a syndrome combing a variety of clinical conditions including but not limited to chronic hemorrhage, inflammatory states, metabolic disorders, decreased red blood cell production, etc, and each of these conditions has different impact on prognosis. Identifying the reasons of anemia would have provided true insight on the reasons of worse outcomes post PCI in the studied population. Multiple clinical scenarios may be given with undoubtfully poor prognosis in patients with anemia as a symptom of serious underlying conditions. The patient with colon carcinoma who received chemotherapy and developed anemia or a patient with myelodysplatic syndrome or a patient with multiple myeloma has worse prognosis compared to the patient without this condition. The list of such examples may be easily extended. Given anemia is just a sign of serious comorbidities it is not surprising that patients with anemia have lower survival. In fact, the subgroup with baseline HGB < 10 g/dl has more than 30% mortality rate at follow-up, confirming

	that the authors deal with very sick population. 2. It is important to assess whether there was an impact of anemia on cardiac mortality? 3. What were the sources of bleeding in both groups? GI? Access site? Other? 4. What were the rates of blood product transfusions in 2 groups? 5. What was the timing of bleeding? In-hospital? At later F/U? 6. I recommend to analyze stent thrombosis rates in 2 groups in more details by the authors: was at acute, sub-acute or late ST? Occurrence of early ST has nothing to do with anemia and relates primarily to the technical issues. 7. More vessels were treated and total stent length was longer in patients with anemia. Did multivariable analysis account for these differences? 8. In the Discussion section, the authors try to explain the worse outcomes in the anemic patients by the compromised oxygen supply. This is barely correct explanation. The basic studies have shown than HGB levels need to be as low as 5-6 g/dl to impair oxygen delivery. 9. Figures depicting Kaplan-Meier curves need to be improved. The numbers at risk need to be designated underneath the horizontal axis at different time intervals. Please follow the recommendations for the use of figures in trial reports; 10. Pocock SJ, Travison TG, Wruck LM. Figures in clinical trial reports: current practice & scope for improvement. Trials. 2007;8:36.

REVIEWER	David Faxon Brigham and Women's Hospital Boston, MA USA
REVIEW RETURNED	01-Mar-2014

GENERAL COMMENTS	This is a report from a large prospective registry (AFCAS) evaluating patients with AF undergoing PCI. This sub-study looks at the impact of anemia on outcome. 861 patients were included and 30% had anemia defined as Hgb less than 13 for men and 12 for women. As expected outcome was less favorable for those with anemia than those without, with an increased MACE, mortality, MI, ST and bleeding. The type of antithrombotic therapy did not relate to outcome. The topic is of interest as an increasing percentage of patients undergoing PCI have AF and anticoagulant therapy is a concern in the setting of dual antiplatelet therapy. The study is well done and clear and consistent with the available literature on the impact of anemia in patients undergoing PCI. While this subgroup has not been previously specifically studied, it is not clear why the authors feel it would be different than prior studies of anemia following PCI. Another limitation of the study is that Hgb was determined only at one time before PCI. Studies have shown that improvement in anemia is related to an improved outcome while conversely a fall increases risk. As recognized by the authors the cause of the anemia is an important factor since these underlying conditions may be more important in contributing to the poor outcome than the anemia itself. Specific comments:

	P 1- Was this study registered? P 2- Please state the rationale for studying AF patients in the background. P 5- There is a considerable literature on triple therapy in AF undergoing PCI. In many of these anemia was evaluated but bleeding was a more potent predictor of outcome. P 6- Was Hgb measured at any other time point during this study? If so it should be reported. P 8- What variables were included in the Cox analysis? P 9- It seems likely that anemia due to GI bleeding or other significant bleeding risk would have impacted the use of Triple therapy as well as the use of BMS. Do the authors have any information on this? P12- Did any patient receive a transfusion during the hospitalization for the PCI. Any during follow-up? P 15- Since CKD is potent cause of anemia and also a significant risk factor for mortality in patients undergoing PCI, this variable should be carefully evaluated on its impact. It would be useful to look at not only those with a GFR <60 but also <45 and <30 (eg Stage 3a and 3b). P15- What was the relationship between ST, dual antiplatelet therapy use and bleeding? A common situation is that the patient of triple therapy develops bleeding and the antithrombotic therapy is discontinued and then ST occurs. Any information on the interrelationship of these factors? P 16- It would interesting to look at Hgb as a continuous variable.

VERSION 1 – AUTHOR RESPONSE

II Reviewer Dr. Nikolsky
We thank the Reviewer for constructive criticism and comments. Below please find our detailed answers to the comments.
1. In real life, PCI is not infrequently required in patients with serious comorbidities many of them resulting in anemia by different pathological pathways. Anemia is a syndrome combing a variety of clinical conditions including but not limited to chronic hemorrhage, inflammatory states, metabolic disorders, decreased red blood cell production, etc, and each of these conditions has different impact on prognosis. Identifying the reasons of anemia would have provided true insight on the reasons of worse outcomes post PCI in the studied population. Multiple clinical scenarios may be given with undoubtfully poor prognosis in patients with anemia as a symptom of serious underlying conditions. The patient with colon carcinoma who received chemotherapy and developed anemia or a patient with myelodysplatic syndrome or a patient with multiple myeloma has worse prognosis compared to the patient without this condition. The list of such examples may be easily extended. Given anemia is just a sign of serious comorbidities it is not surprising that patients with anemia have lower survival. In fact, the subgroup with baseline HGB < 10 g/dl has more than 30% mortality rate at follow-up,

confirming that the authors deal with very sick population.
Response: We completely agree with the comments. Unfortunately, it was not possible to get more detailed information on the background of anemia in this patient registry.
2. It is important to assess whether there was an impact of anemia on cardiac mortality?
Response. All-cause mortality was chosen as the primary endpoint in our study. Cardiac mortality was not a separate endpoint in the study setup and thus unfortunately cannot be added. However, MACCE and its derivates describe largely cardiac and cerebrovascular events in this cohort. Please see Table 4.
3. What were the sources of bleeding in both groups? GI? Access site? Other?
Response: We chose to use the Bleeding Academic Research Consortium (BARC) bleeding criteria, which largely depict the kind of bleeding event in question. Definition of BARC events is presented for the readers in Online Table 1. We have added in the revised manuscript the following information: the rate of access site complications, number of pseudoaneurysms, need for corrective surgery, red blood cell transfusions and the rate of prolonged hospitalization according the study groups. Please, see Table 4 for details.
4. What were the rates of blood product transfusions in 2 groups?
Response: Red cell transfusions were infused in 10 (3.9%) and 5 (0.9%) (p=0.002) of patients with anemia vs. those without during the in-hospital phase. This data has been added to Table 4 in the revised manuscript. However, comprehensive data after discharge is unfortunately not available.
5. What was the timing of bleeding? In-hospital? At later F/U?
Response: The majority of bleeding events occurred during the index hospital stay and within one month after the PCI. The mean survival time for bleeding events was 300 (95%CI 283 – 316) days vs. 312 (95%CI 302 – 322) days for those with anemia vs non-anemic patients (p=0.14). This information is shown in the revised Kaplan-Meier curve (Figure 1c).
6. I recommend to analyze stent thrombosis rates in 2 groups in more details by the authors: was at acute, sub-acute or late ST? Occurrence of early ST has nothing to do with anemia and relates primarily to the technical issues.
Response: Thank you for this important comment. Overall, nearly half (46.7%) of ST events occurred early (<30 days). Acute (<24h after index PCI); early (<30 days) and late ST (>30 and < 365 days) were detected in 1 (0.4%) and 1 (0.2%) (p=0.51); 4 (1.6%) vs. 2 (0.3%) (p=0.07); and 6 (2.3%) vs. 2 (0.3%) (p=0.01) of patients with anemia vs. those without anemia, respectively. This information has been added to the Results.
7. More vessels were treated and total stent length was longer in patients with anemia. Did multivariable analysis account for these differences?
Response: These factors were included in the Cox regression model in the original analysis. This has been clarified in the Results
8. In the Discussion section, the authors try to explain the worse outcomes in the anemic patients by the compromised oxygen supply. This is barely correct explanation. The basic studies have shown than HGB levels need to be as low as 5-6 g/dl to impair oxygen delivery.
Response: We agree with this comment. The underlying conditions in patients with anemia are most likely the more important cause for worse outcome. We have deleted this sentence from the discussion.
9. Figures depicting Kaplan-Meier curves need to be improved. The numbers at risk need to be designated underneath the horizontal axis at different time intervals. Please follow the recommendations for the use of figures in trial reports; Pocock SJ, Travison TG, Wruck LM. Figures in clinical trial reports: current practice & scope for improvement. Trials. 2007;8:36.

Response: The figures have been redrawn accordingly.
III Reviewer Dr. Faxon
We thank the Reviewer for constructive criticism and comments. Below please find our detailed answers to the comments.
1. This is a report from a large prospective registry (AFCAS) evaluating patients with AF undergoing

PCI. This sub-study looks at the impact of anemia on outcome. 861 patients were included and 30% had anemia defined as Hgb less than 13 for men and 12 for women. As expected outcome was less favorable for those with anemia than those without, with an increased MACE, mortality, MI, ST and bleeding. The type of antithrombotic therapy did not relate to outcome. The topic is of interest as an increasing percentage of patients undergoing PCI have AF and anticoagulant therapy is a concern in the setting of dual antiplatelet therapy.

The study is well done and clear and consistent with the available literature on the impact of anemia in patients undergoing PCI. While this subgroup has not been previously specifically studied, it is not clear why the authors feel it would be different than prior studies of anemia following PCI.
Response: Patients with AF undergoing PCI need intensive antithrombotic treatment after PCI. This population is most likely at high bleeding risk, which could be aggravated by the underlying anemia and its cause. The impact of pre-operative anemia on thrombotic and bleeding outcomes in this patient subset remains largely unknown. There are also no reports to our knowledge that would have assessed the effect of pre-procedural anemia on the choice of antithrombotic treatment at discharge.
2. Another limitation of the study is that Hgb was determined only at one time before PCI. Studies have shown that improvement in anemia is related to an improved outcome while conversely a fall increases risk. As recognized by the authors the cause of the anemia is an important factor since these underlying conditions may be more important in contributing to the poor outcome than the anemia itself.
Response: We are fully aware of the impact of possible correction or worsening of anemia on prognosis. Unfortunately only pre-Pci Hgb was recorded in the study and no information on the development of anemia is available.

3. Was this study registered?
Response: Yes, it was, the ClinicalTrials.gov number NCT00596570 has been stated in the Methods section.
4. Please state the rationale for studying AF patients in the background.

Response: The text in Introduction has been modified accordingly.
5. There is a considerable literature on triple therapy in AF undergoing PCI. In many of these anemia was evaluated but bleeding was a more potent predictor of outcome.
Response: We thank the Reviewer for this comment. To our understanding, the studies have not focused on the impact of anemia on outcome but it has been a secondary observation in most reports. The studies have also some methodological problems regarding e.g. study size, Hbg reporting etc. We believe that bleeding and anemia, even though closely related, reflect at least partially different causality in their effect on outcome.
6. Was Hgb measured at any other time point during this study? If so it should be reported.

Response: Hgb values were recorded only pre-PCI.
7. What variables were included in the Cox analysis?
Response: Baseline variables correlating at $p<0.10$ level with the dependent variable in univariate analyses were entered in the Cox regression model as covariates. Cox regression hazard model was used to identify the independent predictors of MACCE, and all-cause mortality at 12-month follow-up in the subgroup of anemic patients. This has been clarified on the Results.
In the revised manuscript, Results section stands as follows:
“In univariate analysis age above 75, diabetes, congestive heart failure, anemia, chronic renal impairment, ACS at presentation, and total stent length were strongly correlated with both MACCE and all-cause mortality at 12-month follow-up. In the Cox regression model including all the above variables, independent predictors of all-cause mortality were anemia (HR 1.62, 95% CI 1.05 – 2.51, $p=0.029$), ACS at presentation (HR 2.26, 95% CI 1.37 – 3.75, $p=0.001$), chronic renal impairment (HR 2.35, 95% CI 1.52 – 3.65, $p<0.001$), and diabetes (HR 1.76, 95% CI 1.15 – 2.70, $p=0.009$). In

contrast, anemia was not an independent predictor of MACCE at 12-months follow-up unlike age above 75 years (HR 1.7, 95%-CI 1.2-2.4, p=0.004), diabetes (HR 1.7, 95%-CI 1.2-2.3, p=0.002), ACS at presentation (HR 1.7, 95%-CI 1.2-2.3, p=0.003), and congestive heart failure (HR 1.5, 95%-CI 1.0-2.1, p=0.03)."

8. It seems likely that anemia due to GI bleeding or other significant bleeding risk would have impacted the use of Triple therapy as well as the use of BMS. Do the authors have any information on this?

Response: Patients with previous GI bleed were scarce. Anemic patients who had a previous GI-bleeding episode (N=9) had non-significantly less triple therapy at hospital discharge compared to those anemic patients without GI-bleeding episode (5/9 (55.6%) vs. 176/249 (70.7%), p=0.46). Dual antiplatelet therapy was used in 3/9 (33.3%) vs. 55/249 (22.1%) of patients (p=0.43), respectively. VKA+clopidogrel was used in 1/9 (11.1%) vs. 14/249 (5.6%) (p=0.42) of patients, respectively. Reflecting the more common DAPT use, median and mean use of clopidogrel was 12 [11] months vs. 3 [11] months (p=0.70) and 7.0 ± 5.9 months vs. 5.7 ± 5.1 months, for anemic patients with gi-bleeding episode vs. those without.

BMS was used in 62.5% vs. 73.3% of pre-procedurally anemic patients with previous GI bleed compared to those without (p=0.45).

9. Did any patient receive a transfusion during the hospitalization for the PCI. Any during follow-up?

Response: Red cell transfusions were infused in 10 (3.9%) and 5 (0.9%) (p=0.002) of patients with anemia vs. those without during the in-hospital phase. This data has been added to Table 4 in the revised manuscript. However, comprehensive data after discharge is unfortunately not available.

10. Since CKD is potent cause of anemia and also a significant risk factor for mortality in patients undergoing PCI, this variable should be carefully evaluated on its impact. It would be useful to look at not only those with a GFR <60 but also <45 and < 30 (eg Stage 3a and 3b).

Response: This is an important point. In multivariate analysis impaired renal function and anemia were independent predictors of worse outcome which allows us to conclude that the impact of anemia is not dependent on renal function. The number of patients with severe renal impairment is so low that statistical analysis in this subset of patients is not warranted.

11. What was the relationship between ST, dual antiplatelet therapy use and bleeding? A common situation is that the patient of triple therapy develops bleeding and the antithrombotic therapy is discontinued and then ST occurs. Any information on the interrelationship of these factors?

Response: We do unfortunately not have comprehensive data on this from every center, but this is the general practice which is most probably used. It is well known that discontinuation of triple therapy increases the risk of ST.

12. It would interesting to look at Hgb as a continuous variable

Response:The following has been added to Results.

" We performed the multivariate model also using Hemoglobin as a continuous variable. Independent predictors of all-cause mortality were pre-procedural hemoglobin (HR 0.82, 95% CI 0.72 – 0.93, p=0.002), ACS at presentation (HR 2.07, 95% CI 1.25 – 3.45, p=0.005), chronic renal impairment (HR 2.06, 95% CI 1.31 – 3.24, p=0.002), and diabetes (HR 1.75, 95% CI 1.14 – 2.70, p=0.01) in a Cox regression model including age over 75 years, total stent length and number of treated vessels as covariates.

On the contrary to what was found when assessing anemia as a categorical variable, hemoglobin as a continuous variable predicted also MACCE. Independent predictors of MACCE were pre-procedural hemoglobin (HR 0.89, 95% CI 0.81 – 0.98, p=0.016), ACS at presentation (HR 1.55, 95% CI 1.10 – 2.18, p=0.012), congestive heart failure (HR 1.45, 95% CI 1.03 – 2.04, p=0.035), age over 75 years (HR 1.77, 95%CI 1.27 – 2.45, p=0.001) and diabetes (HR 1.55, 95% CI 1.13 – 2.13, p=0.007) in a

Cox regression model including also total stent length, chronic renal impairment and number of treated vessels as covariates."

VERSION 2 – REVIEW

REVIEWER	Eugenia Nikolsky Rambam Medical Center and the Technion – Israel Institute of Technology, Haifa, Israel
REVIEW RETURNED	12-Apr-2014

GENERAL COMMENTS	Repeat review The paper by Puurunen et al. examined the impact baseline anemia on outcomes of 861 patients treated with percutaneous coronary intervention (PCI) in the prospective European AFCAS registry. The authors performed a comprehensive revision of the paper addressing in detail the raised questions. Several additional comments: 1. I would add triple vs. dual antiplatelet therapy at discharge into the MV model of bleeding and of mortality to assess the possible role of antithrombin/antiplatelet therapy in prognosis. 2. I don't think it makes sense to perform MV analysis of a composite endpoint (MACCE). Each of the individual endpoints of MACCE has dissimilar pathogenesis and therefore it is not recommended to look for predictors of different events combined in one endpoint. I recommend removing MV analysis of MACCE from the paper. 3. I recommend to include discussion on the possible reasons of higher incidence of late ST in patients with anemia (bleeding? Interruption of therapies? Other?). 4. Conclusion section: second sentence should be removed – this relates to patient's characteristics. 5. It will be nice for the readers' convenience to add values (%) on both K-M curves

VERSION 2 – AUTHOR RESPONSE

1. I would add triple vs. dual antiplatelet therapy at discharge into the MV model of bleeding and of mortality to assess the possible role of antithrombin/antiplatelet therapy in prognosis.

Response 1: In univariate analysis the use of triple therapy or dual antiplatelet therapy was not associated with bleeding and mortality. Nevertheless, we have now performed these Cox regression models with triple therapy or DAPT included. The results of the Cox regression model remained unchanged.

2. I don't think it makes sense to perform MV analysis of a composite endpoint (MACCE). Each of the individual endpoints of MACCE has dissimilar pathogenesis and therefore it is not recommended to look for predictors of different events combined in one endpoint. I recommend removing MV analysis of MACCE from the paper.

Response 2: We thank the reviewer for this comment. In the current PCI literature the use of combined endpoints, either MACE or MACCE is very common even though the pathogenesis of each individual component varies. The combined endpoint, however, describes the overall outcome of the patients with all clinically important events included. Therefore, we feel it would be of interest to the readers to include these results in our study.

3. I recommend to include discussion on the possible reasons of higher incidence of late ST in patients with anemia (bleeding? Interruption of therapies? Other?).

Response 3: This addition has been made in the discussion: We observed that the rate of definite or probable stent thrombosis was significantly higher in anemic versus non-anemic patients (p =0.002). ACS at presentation may have contributed to the higher rate of stent thrombosis in anemic patients, as patients with anemia more often presented with ACS versus those without anemia. In addition, in individual cases the presence of anemia may have influenced the choice of antithrombotic medication. Also, a bleeding event could have lead to interruption of combination antithrombotic therapy and thus to a higher risk of stent thrombosis. Consistent with our results, Pilgrim and co-workers, observed a higher rate of definite stent thrombosis at 4-year follow-up in anemic patients who underwent PCI with unrestricted use of drug-eluting stents, compared with non-anemic ones.[14] Interestingly, in a recent study, anemia was the only independent predictor of high residual platelet reactivity on clopidogrel in a series of patients undergoing PCI.[20] These observations warrant further studies to clarify the underlying mechanisms.

4. Conclusion section: second sentence should be removed – this relates to patient's characteristics.

Response 4: The second sentence has been removed, as suggested. The Conclusion is now as follows:
Anemia was a frequent finding in patients with AF referred for PCI. Anemia seems to be an independent risk factor for all-cause mortality during 12-month follow-up. Anemia is also associated with more MACCE, and a trend toward a higher rate of bleeding.

5. It will be nice for the readers' convenience to add values (%) on both K-M curves

Response 5: We have added the requested values in the revised Figures.