Reviewer's report

Title: Macrocytosis is associated with mortality in chronic hemodialysis patients

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Reviewer: Marlies Noordzij

Reviewer's report:

This manuscript describes the prevalence and distribution of macrocytosis in 150 hemodialysis patients. In addition, the authors study associations of macrocytosis at baseline with several clinical parameters and 9-month patient survival. In general, I think the topic is interesting and the manuscript is well written. However, I do have some concerns. Please find my comments below.

Comments to the authors:

Major Compulsory Revisions

My major concern refers to the statistical analysis of the data.

1) As the authors correctly state in their discussion section (page 10), their multivariate Cox regression models were probably over-fitted by incorporating many variables in their model with only 23 events. The authors should therefore decrease the number of variables in their model. A rule of thumb is that one can include 1 variable per 10 events. Another possibility is to perform a model in which they adjust for a propensity score. An advantage of this method is that one needs to include only 1 variable in the model which summarizes many covariates in one score so that over-fitting can be prevented.

2) I also have some concerns about the choice of the variables included in the multivariate models. If one wants to study the (causal) relationship between an exposure and an outcome, one wants to adjust for (potential) confounders which can obscure the association between the exposure and the outcome. To be a confounder, a variable needs to fulfil 3 criteria:

1) The variable needs to be related to the exposure (MCV /macrocytosis)

2) The variable needs to be related to the outcome (death)

3) The variable should not be an intermediate variable in the causal pathway between exposure and outcome.

In the current manuscript, the authors included a large amount of variables in their model which were found to be associated with mortality in previous studies in hemodialysis patients.

They do not take into consideration whether these variables are also related to the exposure (MCV/ macrocytosis), nor do they check whether the variables may be in the causal pathway. If the authors wish to make conclusions about causality, they should remove the variables that do not fulfil the criteria for confounding from their model. However, if the authors simply aim at assessing
whether macrocytosis adds prognostic power for mortality to standard death predictors in hemodialysis patients, then their choice of variables in their multivariate Cox model including is plausible. If this is the case, the sentence on causation should be removed from the discussion section (page 10). Therefore, I would recommend the authors to state the goal of their study more clearly: Do they aim to study whether macrocytosis is causally related to mortality, also after adjustment for potential confounders? Or do they aim to study whether macrocytosis can be used as a predictor for mortality in hemodialysis patients (prognostic approach)?)

3) Please also report at least one of the multivariate effect estimates in the abstract. This effect estimate is more important than the unadjusted effect estimate. In addition, the conclusion of the abstract is somewhat strong, while the final conclusion of the paper is stated more precisely.

Minor Essential Revisions

1) Table 1: Please explain in the footnotes that means ± standard deviations are reported for continuous variables (instead of in the Table itself).

2) Table 2: Please clarify in the footnote which variables were included in the multivariate logistic regression model.

3) Can the authors explain why they only included hemodialysis patients in their study? In their introduction they state that also the peritoneal dialysis patients in their centre had unexplained macrocytosis.

Discretionary Revisions

1) I would recommend spelling out the abbreviation CBC as well (page 5).

2) The results in table 3 could easily be included in Table 1.

3) Figure 1: Using the term “cross-sectional cohort” is somewhat confusing. The patients were selected for inclusion at one point in time (cross-section), so this means that prevalent patients are included. However, the patients were followed-up prospectively, so this study is in fact a prospective cohort study, and not a cross-sectional study.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests.