## The REMARK checklist

| Item to be reported | | Reported on Page Number/Line Number | Reported on Section/Paragraph |
|---|---|---|---|
| **INTRODUCTION** | | | |
| 1 | State the marker examined, the study objectives, and any pre-specified hypotheses. | Page 3-5 /Line 92-163 | Introduction/Paragraph |
| **MATERIALS AND METHODS** | | | |
| Patients | | | |
| 2 | Describe the characteristics (e.g., disease stage or co-morbidities) of the study patients, including their source and inclusion and exclusion criteria. | Page 6 /Line 173-177 | Methodology/Paragraph 11 |
| 3 | Describe treatments received and how chosen (e.g., randomized or rule-based). | - | - |
| Specimen characteristics | | | |
| 4 | Describe type of biological material used (including control samples) and methods of preservation and storage. | Page 6-7 /Line 191-239 | Methodology/Paragraph |
| Assay methods | | | |
| 5 | Specify the assay method used and provide (or reference) a detailed protocol, including specific reagents or kits used, quality control procedures, reproducibility assessments, quantitation methods, and scoring and reporting protocols. Specify whether and how assays were performed blinded to the study endpoint. | Page 5-11/Line 169-351 | Methodology/Paragraph 11-31 |
| Study design | | | |
| 6 | State the method of case selection, including whether prospective or retrospective and whether stratification or matching (e.g., by stage of disease or age) was used. Specify the time period from which cases were taken, the end of the follow-up period, and the median follow-up time. | Page 5/Line 169-177 | Methodology/Paragraph 11 |
| 7 | Precisely define all clinical endpoints examined. | Page 8/Line 243-251 | Methodology/Paragraph |
| 8 | List all candidate variables initially examined or considered for inclusion in models. | Page 10-11/Line331-351 | Methodology/Paragraph |
| 9 | Give rationale for sample size; if the study was designed to detect a specified effect size, give the target power and effect size. | - | - |
| Statistical analysis methods | | | |
| 10 | Specify all statistical methods, including details of any variable selection procedures and other model-building issues, how model assumptions were verified, and how missing data were handled. | Page 11/Line 344-351 | Methodology/Paragraph 31 |
| 11 | Clarify how marker values were handled in the analyses; if relevant, describe methods used for cutpoint determination. | Page 8/Line 243-247 | Methodology/Paragraph |

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| **RESULTS** | | | |
| Data | | | |
| 12 | Describe the flow of patients through the study, including the number of patients included in each stage of the analysis (a diagram may be helpful) and reasons for dropout. Specifically, both overall and for each subgroup extensively examined report the numbers of patients and the number of events. | Page 12/Line 372-375 | Results/Paragraph 32 |
| 13 | Report distributions of basic demographic characteristics (at least age and sex), standard (disease-specific) prognostic variables, and tumor marker, including numbers of missing values. | Page 12/Line 383-388 | Results/Paragraph 33 |
| Analysis and presentation | | | |
| 14 | Show the relation of the marker to standard prognostic variables. | Page 11-23/Line358-764 | Results/Paragraph 33-49 |
| 15 | Present univariable analyses showing the relation between the marker and outcome, with the estimated effect (e.g., hazard ratio and survival probability). Preferably provide similar analyses for all other variables being analyzed. For the effect of a tumor marker on a time-to-event outcome, a Kaplan-Meier plot is recommended. | Page 12-13/Line414-416 | Results/Paragragh 33-34 |
| 16 | For key multivariable analyses, report estimated effects (e.g., hazard ratio) with confidence intervals for the marker and, at least for the final model, all other variables in the model. | Page 13/Line 416-418 | Results/Paragraph 35 |
| 17 | Among reported results, provide estimated effects with confidence intervals from an analysis in which the marker and standard prognostic variables are included, regardless of their statistical significance. | Page 13/Line 413-425 | Results/Paragraph 35 |
| 18 | If done, report results of further investigations, such as checking assumptions, sensitivity analyses, and internal validation. | - | - |
| **DISCUSSION** | | | |
| 19 | Interpret the results in the context of the pre-specified hypotheses and other relevant studies; include a discussion of limitations of the study. | Page 23/Line 768-776 | Discussion/Paragraph 44 |
| 20 | Discuss implications for future research and clinical value. | Page 24/Line 776-779 | Conclusion/Paragraph 45 |

**From:** McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM: Reporting recommendations for tumor marker prognostic studies (REMARK). J Natl Cancer Inst 2005; 97: 1180-1184.

Article information: https://dx.doi.org/10.21037/jgo-23-984
*As the checklist was provided upon initial submission, the page number/line number reported may be changed due to copyediting and may not be referable in the published version. In this case, the section/paragraph may be used as an alternative reference.