PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Evaluation of the diagnostic accuracy of fractional exhaled nitric oxide (FeNO) in patients with suspected asthma: study protocol for a prospective diagnostic study
AUTHORS	Kellerer, Christina; Hapfelmeier, Alexander; Joerres, Rudolf; Schultz, Konrad; Brunn, Benjamin; Schneider, Antonius

VERSION 1 – REVIEW

REVIEWER	Bilun Gemicioglu Istanbul University-Cerrahpasa, Cerrahpasa Faculty of Medicine, Department of Pulmonary Diseases TURKEY
REVIEW RETURNED	17-Oct-2020

GENERAL COMMENTS	The study will respond really very interesting points in asthma diagnosis and follow-up. I have some minor comments; Initial asthma treatment may be different then just ICS (GINA Guideline Box 3-4A) with formoterol or with LABA or with low or medium dose of ICS. Which patient will be eligible for the study only step 2 patients who can be received low dose ICS? You must give information about that. What will be the rescue medication? The adherence to ICS therapy and inhaler technique are important points of asthma treatment. It's better checking all of this points. For rhinitis symptoms you didn't say if you will use any questionnaire or VAS. Are you giving any treatment for patients with perennial rhinitis like nasal steroids? How you will calculate the effect of rhinitis treatment? You didn't mention any funding for the measurement of pulmonary function and FeNO? Are they routine tests in Germany as well as for the research studies? Clarifying these points will be better for the audience.

REVIEWER	Jung Yeon Shim Sungkyunkwan University School of Medicine South Korea
REVIEW RETURNED	19-Oct-2020

GENERAL COMMENTS	1. Patients with suspected asthma symptoms will be included in this study. Asthma symptoms such as cough, wheezing, or chest tightness can be aggravated by respiratory viral infections (ex, rhinovirus, bocavirus, metapneumovirus….) However, patients with respiratory infection within 6 weeks before examination will be excluded in this study. How can researchers differentiate respiratory infections from asthma worsening? 2. It would be better to exclude patients with cardiac diseases, since they can present expiratory wheezing.

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	3. Authors should describe the diagnostic criteria of “AR”, since different cutoff of FeNO will be applied for patients with “AR” and “wheezing” 4. FENO can be affected by age, height and Th2 inflammation (ex, AR) as well as different devices. Evaluation of atopic status using skin prick test or immunoCAP will be necessary for checking Th2 inflammation. 5. I recommend to measure FeNO at 3 months follow-up (t2) of ICS treatment to evaluate effectiveness of FeNO in response to ICS therapy in asthma. 6. I also recommend to measure FeNO at 3 months follow-up of patients without asthma initially (t1) and persistent symptoms after 3 months, who will be re-evaluated with WBP and BP. 7. I also recommend to measure blood eosinophil count to evaluate FeNO in eosinophilic inflammation. 8. To evaluate ICS responsiveness after 3 months, patients’ compliance in daily ICS inhalation must be important. How can researchers check patients’ compliance during 3 months of ICS treatment? Which ICS will you provide patients? What is drop-off criteria from study? 9. It will be better to define diagnostic criteria of asthma using BP as provocative concentration of methacholine of 8 mg/mL rather than 960 ug. Also it would be better to describe obstructive airway as % predicted FEV1 or FEV1/FVC rather than lower limits of normal.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Reviewer Name: Bilun Gemicioglu

Institution and Country: Istanbul University-Cerrahpasa, Cerrahpasa Faculty of Medicine, Department of Pulmonary Diseases, TURKEY

Please state any competing interests or state ‘None declared’: None

The study will respond really very interesting points in asthma diagnosis and follow-up.
I have some minor comments;

1) Initial asthma treatment may be different then just ICS (GINA Guideline Box 3-4A) with formoterol or with LABA or with low or medium dose of ICS. Which patient will be eligible for the study only step 2 patients who can be received low dose ICS? You must give information about that. What will be the rescue medication?

Response: In the present study, each patient visiting one of the participating practices of pneumologists suspected to suffer from asthma will be included into the study regardless of the severity of symptoms and the corresponding medication for therapy (indicated population design). We clarified this aspect in the study protocol (page 5, lines 150-151). The evaluation of FeNO will have no impact on patient management.

2) The adherence to ICS therapy and inhaler technique are important points of asthma treatment. It’s better checking all of this points.

Response: This is an important issue but the study aims to mimic conditions of real practice, and we thus do not include special measures to check for the adherence to medication, except for the

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counting of regular prescriptions as documented in the patients' files. This aspect will be a limitation of the study and is now included in the discussion section (page 12, lines 405-407).

3) For rhinitis symptoms you didn't say if you will use any questionnaire or VAS. Are you giving any treatment for patients with perennial rhinitis like nasal steroids? How you will calculate the effect of rhinitis treatment?

Response: The history of allergic rhinitis will be reported by the patients and recorded by the treating physician. It will not be validated, for example, by nasal provocation. The study should reflect clinical reality and therefore we have to rely on the patient's clinical history without an objective validation of AR. We included this aspect as a limitation in the discussion section (page 12, lines 401-404).

4) You didn't mention any funding for the measurement of pulmonary function and FeNO? Are they routine tests in Germany as well as for the research studies? Clarifying these points will be better for the audience.

Response: The investigations with whole-bodyplethysmography and bronchial provocation tests are part of the clinical routine in practices of pneumologists in Germany. These measurements would also take place outside the study. Thus, no funding of these measurements is necessary. FeNO devices and measurements are provided by the Institute of General Practice and Health Services Research of the Technical University of Munich. We clarified these points in the manuscript (page 6, lines 198-206).

Reviewer: 2

Reviewer Name: Jung Yeon Shim
Institution and Country: Sungkyunkwan University School of Medicine, South Korea
Please state any competing interests or state 'None declared': none declared

1) Patients with suspected asthma symptoms will be included in this study. Asthma symptoms such as cough, wheezing, or chest tightness can be aggravated by respiratory viral infections (ex, rhinovirus, bocavirus, metapneumovirus….) However, patients with respiratory infection within 6 weeks before examination will be excluded in this study. How can researchers differentiate respiratory infections from asthma worsening?

Response: Only patients visiting one of the pneumological practices to clarify a potential asthma diagnosis for the first time will be included in the study. But indeed, we cannot completely exclude to enrole patients with postinfectious bronchial hyper-reactivity. Therefore, patients with positive bronchial provocation will be examined again after 3 months (follow-up investigation) to identify false positive findings.

2) It would be better to exclude patients with cardiac diseases, since they can present expiratory wheezing.

Response: In the present study, each patient visiting one of the participating practices of pneumologists suspected to suffer from asthma will be included into the study regardless of the severity of symptoms and the corresponding medication for therapy (indicated population design). The pneumologists are blinded against the FeNO results. Therefore, the evaluation of FeNO will have no impact on diagnostic decision making and patient management. Each patient visiting one of the participating practices of pneumologists suspected to suffer from asthma will be included into the study regardless of any comorbidities.

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3) Authors should describe the diagnostic criteria of “AR”, since different cutoff of FeNO will be applied for patients with “AR” and “wheezing”

Response: The information on the presence of allergic rhinitis is reported by the patient and known to the treating physician. This represents the typical procedure to include medical history in clinical practice. It is uncommon in German pneumological practices to verify the diagnosis, for example, by nasal provocation tests. We discuss this a limitation now (page 12, lines 401-404).

4 a) FENO can be affected by age, height and Th2 inflammation (ex, AR) as well as different devices.

Response: Thank you for this important comment. We will perform subgroup analyses taking into account anthropometric parameters and added this aspect in the statistical analysis section (page 10, lines 339-340). The device used in this study is generally accepted to yield valid readings. It is also, for example, used in the National German Cohort (NACO, subsample n=40.000), and it would be the topic of another study to compare devices.

4 b) Evaluation of atopic status using skin prick test or immunoCAP will be necessary for checking Th2 inflammation.

Response: There are no financial resources regarding the performance of skin prick tests or immunoCAP tests.

5) I recommend to measure FeNO at 3 months follow-up (t2) of ICS treatment to evaluate effectiveness of FeNO in response to ICS therapy in asthma.

Response: We will measure FENO at follow-up to evaluate ICS responsiveness exploratory (page 10, lines 336-337). However, this will have no impact on our analysis, because we cannot verify FeNO as index test with FeNO as reference standard. This would cause an incorporation bias.

6) I also recommend to measure FeNO at 3 months follow-up of patients without asthma initially (t1) and persistent symptoms after 3 months, who will be re-evaluated with WBP and BP.

Response: We will measure FENO in these patients, too. This is now given in the manuscript. However, the FENO changes will only be analysed in an exploratory manner.

7) I also recommend to measure blood eosinophil count to evaluate FeNO in eosinophilic inflammation.

Response: The measurement of blood eosinophils is not part of the diagnostic routine in the participating practices of pneumologists. Beyond that, there are no financial resources.

8) To evaluate ICS responsiveness after 3 months, patients’ compliance in daily ICS inhalation must be important. How can researchers check patients’ compliance during 3 months of ICS treatment? Which ICS will you provide patients? What is drop-off criteria from study?

Response: We will not apply objective controls for adherence beyond those used in clinical routine. This means that only the medication prescriptions and answers of patients will serve as an indicator of treatment. This aspect will be a limitation of the study and is now included in the discussion section (page 12, lines 405-407).

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9 a) It will be better to define diagnostic criteria of asthma using BP as provocative concentration of methacholine of 8 mg/mL rather than 960 ug.

Response: *As stated in `Reference Test´(line 211-214), BP is performed to determine bronchial hyperresponsiveness (BHR) to methacholine according to the 1-concentration-4-step dosimeter protocol. This protocol is validated (Merget R, Jörres RA, Heinze E, et al. Development of a 1-concentration-4-step dosimeter protocol for methacholine testing. Respiratory medicine. 2009;103(4):607-13) and widely used in the clinical routine of ambulatory care in Germany. This yields similar results as the ATS multi-concentration protocol but offers advantages in clinical practice (fast, safe, easier to use, and easier store). We will, however, perform subgroup analyses with different cut-off values of bronchial provocation and included this aspect in the study protocol (page 10, lines 338-339).*

9b) Also it would be better to describe obstructive airway as % predicted FEV1 or FEV1/FVC rather than lower limits of normal.

Response: *We prefer to use lower limits of normal to describe airway obstruction as these values are more reliable than %predicted and recommended by the guidelines. It could be shown that using FEV1/FVC in terms of LLN reduces the misclassification of airway obstruction (Swanney M, Ruppel G, Enright P et al. Using the lower limit of normal for the FEV1/FVC ratio reduces the misclassification of airway obstruction. Thorax 2008; 63: 1046-1051)*

VERSION 2 – REVIEW

REVIEWER	Jung Yeon Shim Sungkyunkwan University School of Medicine, South Korea
REVIEW RETURNED	19-Dec-2020

GENERAL COMMENTS	This is a study protocol to evaluate diagnostic accuracy of FeNO in asthma. In the diagnostic decision making (page 8), there were 2 criteria at t2 for evaluating ICS responsiveness. In the statistical analysis (page 12), one more criteria (change of ACQ) was added at t2 for ICS responsiveness. It makes confusion. In the statistical analysis (page 12), FeNO will be evaluated at t1 and at t2 for ICS responsiveness, but in study protocol (Fig.2), FeNO is checked only at t1. It makes confusion too. It will be better to note ICS dose for patients with asthma in study protocol to evaluate ICS responsiveness of FeNO. One of the criteria to see the ICS responsiveness was demonstrated as an increase of FEV1 from baseline by >12% and by 200 ml. Baseline means t1? If so, please add t1 in the text. If not, what dose baseline mean?

VERSION 2 – AUTHOR RESPONSE

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Reviewer: 2
Dr. Jung Yeon Shim, Sungkyunkwan University School of Medicine, Kangbuk Samsung Hospital

Comments to the Author:
This is a study protocol to evaluate diagnostic accuracy of FeNO in asthma.
(1) In the diagnostic decision making (page 8), there were 2 criteria at t2 for evaluating ICS responsiveness. In the statistical analysis (page 12), one more criteria (change of ACQ) was added at t2 for ICS responsiveness. It makes confusion.

Response: Thank you very much for your careful reading. We forgot to mention the change of ACQ in the diagnostic decision making part but now we included this criterion (page 8, line 260).

(2) In the statistical analysis (page 12), FeNO will be evaluated at t1 and at t2 for ICS responsiveness, but in study protocol (Fig.2), FeNO is checked only at t1. It makes confusion too.

Response: Thank you very much for this comment. We included the second FeNO measurement at t2 in figure 1 and figure 2.

(3) It will be better to note ICS dose for patients with asthma in study protocol to evaluate ICS responsiveness of FeNO.

Response: Thank you very much for this suggestion. We included a question on the type and daily dose of ICS into the protocol (page 6, line 188/189).

(4) One of the criteria to see the ICS responsiveness was demonstrated as an increase of FEV1 from baseline by >12% and by 200 ml. Baseline means t1? If so, please add t1 in the text. If not, what dose baseline mean?

Response: In this context baseline means t1. We clarified this aspect in the manuscript (page 8, line 258 and page 10, line 334).

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