PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Bronchoscopy with and without needle-based confocal laser endomicroscopy for peripheral lung nodule diagnosis: protocol for a multicenter randomized controlled trial (CLEVER trial)
AUTHORS	van Heumen, Saskia; Kramer, Tess; Korevaar, Daniël; Gompelmann, Daniela; Bal, Christina; Hetzel, Juergen; Jahn, Kathleen; Poletti, V; Ravaglia, Claudia; Sadoughi, Ali; Stratakos, Grigoris; Bakiri, Katerina; Koukaki, Evangelia; Anagnostopoulos, Nektarios; Votruba, Jiří; Šestáková, Zuzana; Heuvelmans, Marjolein; Daniels, Johannes; de Bruin, Daniel; Bonta, Peter; Annema, Jouke

VERSION 1 – REVIEW

REVIEWER	Guibert, Nicolas Centre Hospitalier Universitaire de Toulouse
REVIEW RETURNED	28-Nov-2023

GENERAL COMMENTS	Thank you and congratulations on the launch of this important trial I have no major comment on the very clear description of this RCT The evaluation of CLE in the context of lung peripheral nodules is highly needed My only comment on the design of the protocol rather than the manuscript is the lack of predefined number of GGO included in the trial and of a planned subgroup analysis, as they are the most challenging lesions (less real-time positioning tools with poor yield of rEBUS in particular)

REVIEWER	Manley, Christopher J. FCCC
REVIEW RETURNED	29-Nov-2023

GENERAL COMMENTS	A well designed study from a group with high level of expertise and experience in peripheral bronchoscopy and confocal microscopy. I have minor suggestions below. Fluoroscopy, EMN, VB, or ultrathin is optional; CBCT is not allowed; there is no mention of robotic bronchoscopy. I would recommend clarifying whether robotic bronchoscopy allowed or is prohibited from the study. Diagnostic yield for the control group (62%) is based on meta analysis of bronchoscopy with radial EBUS (citations 21 and 22) but control group allows for navigational bronchoscopy by institutional practice which may include EMN. If many providers will be using EMN, consider re-calculating sample size for yield in control arm closer to that of Navigate study (73%); this may require higher enrollment to demonstrate statistical significance.

REVIEWER	Bai, Chong Second Military Medical University First Hospital, Respiratory and Critical Care Medicine
REVIEW RETURNED	08-Dec-2023

GENERAL COMMENTS	Dear authors, The protocol by Heumen et al. aimed to investigate if nCLE-imaging integrated with conventional bronchoscopy results in a higher diagnostic yield compared to conventional bronchoscopy without nCLE. There is no doubt that this ongoing multicenter randomized controlled trial will provide interventional pulmonologist with important information about the added benefit of the nCLE technique for peripheral lung nodule diagnosis. Although this protocol is meticulous and rigorous, there are still some details that should be noted. 1. To evaluate the diagnostic yield and diagnostic sensitivity for malignancy of nCLE, nCLE diagnosis criteria should be predefined. Furtherfore, blinded raters should be unifiedly appointed in order to avoid the differences between participating centers. Also, as the imaging interpretation of nCLE has characteristics of subjectivity, the additions of the inter-observer agreement and intra-observer reliability of nCLE imaging is worthy of consideration. For the aforementioned points, this article below may be refered. “Bronchoscopic needle-based confocal laser endomicroscopy (nCLE) as a real-time detection tool for peripheral lung cancer” (doi: 10.1136/thoraxjnl-2021-216885) 2. This protocol focused on investigating if nCLE-imaging integrated with conventional bronchoscopy results in a higher diagnostic yield compared to conventional bronchoscopy without nCLE in diagnosing peripheral lung nodules, which should be highlighted in the title of this paper. For example, the title can be changed into “Design of a multicenter, randomized controlled trial on bronchoscopy with and without needle-based confocal laser endomicroscopy for peripheral lung nodule diagnosis (CLEVER trial)”. 3. Although multiple studies have demonstrated the excellent inter-observer agreement and intra-observer reliability, the fact that the imaging interpretation of nCLE has characteristics of subjectivity is unavoidable. As one of the exploratory endpoints, an algorithm for automated nCLE criteria recognition using machine- or deep-learning methods seems encouraging. As mentioned in the recent article entitled“The role of confocal laser endomicroscopy in pulmonary medicine”(doi: 10.1183/16000617.0185-2022), in the near future, it can be envisaged that a large atlas of CLE images for different pulmonary diseases will be established and can be identified automatically using artificial intelligence; as a result, the role of nCLE in diagnosing peripheral lung nodule will be further highlighted. In my opinion, a combination of nCLE and artificial intelligence should be involved in the section of Discussion in order to highlight the aforesaid exploratory endpoint. 4. For the description“rapid on-site evaluation (ROSE) is sometimes used for direct feedback on representativeness of the sample and forming a preliminary diagnosis. Nevertheless, diagnostic yield rarely exceeds 71%, (8) as it depends highly on factors such as nodule size, bronchus sign on pre-procedural CT, eccentric vs. concentric r-EBUS pattern and pretest probability of malignancy.”, sampling techniques should be added as the one of

	the influence factors (Mondoni M, Rinaldo RF, Carlucci P, Terraneo S, Saderi L, Centanni S, Sotgiu G. Bronchoscopic sampling techniques in the era of technological bronchoscopy. Pulmonology. 2022 Nov-Dec;28(6):461-471. doi: 10.1016/j.pulmoe.2020.06.007). Also noted: transbronchial lung cryobiopsy (TBLC) that can retrieve larger specimens with more preserved cellular architecture and fewer crush artifacts in comparison with conventional transbronchial forceps biopsy (TBFB), as an emerging technology for diagnosing PPLs, has been demonstrated to have the potential to resolve the clinical dilemma pertaining to currently available sampling devices (e.g., forceps, needle and brush) and become a diagnostic cornerstone for PPLs (Tang Y, Tian S, Chen H, et al. Transbronchial lung cryobiopsy for peripheral pulmonary lesions. A narrative review. Pulmonology. 2023 Oct 30:S2531-0437(23)00163-0. doi: 10.1016/j.pulmoe.2023.08.010); therefore, the addition of TBLC as another sampling technique besides TBNA and TBFB to acquire tissue for pathological evaluation may be worth considering.

REVIEWER	Wagh, Ajay The University of Chicago, Pulmonary and Critical Care
REVIEW RETURNED	11-Dec-2023

GENERAL COMMENTS	Thank you for the opportunity to review “Design of a multicenter, randomized controlled trial on needle-based confocal laser endomicroscopy guided bronchoscopy for peripheral lung nodule diagnosis (CLEVER trial).” This study is evaluating whether using confocal laser endomicroscopy can improve diagnostic yield during peripheral bronchoscopy. 1. Could the authors comment on whether industry representatives would be assisting during the procedure and/or with CLE image interpretation? (Page 12, lines 34-39). Are there predefined imaging standards? 2. Could the authors clarify on whether any additional imaging assessments may be used (including use of local registration, augmented fluoroscopy, etc) to update navigation and/or localization? Would such techniques be utilized pre-biopsy?

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Prof. Nicolas Guibert, Centre Hospitalier Universitaire de Toulouse Comments to the Author:

Thank you and congratulations on the launch of this important trial

I have no major comment on the very clear description of this RCT

The evaluation of CLE in the context of lung peripheral nodules is highly needed

My only comment on the design of the protocol rather than the manuscript is the lack of predefined number of GGO included in the trial and of a planned subgroup analysis, as they are the most challenging lesions (less real-time positioning tools with poor yield of rEBUS in particular)

Dear. Prof Guibert, thank you for your positive feedback on our study. You touch upon an important issue.

There indeed is no predefined number of GGO’s included in the study. One of the inclusion criteria of the study is that the nodule must have a solid component of at least 10 mm. As such, no GGO’s are included in this study but only solid and partially solid nodules are included. This is the reason there is

no predefined number of GGO mentioned in the manuscript and no subgroup analysis is described on this point.

Reviewer: 2
Dr. Christopher J. Manley, FCCC Comments to the Author:
A well designed study from a group with high level of expertise and experience in peripheral bronchoscopy and confocal microscopy.
I have minor suggestions below.
Dear dr. Manley, we appreciate the time and effort you invested in reviewing our work. Please see our answers below.

Fluoroscopy, EMN, VB, or ultrathin is optional; CBCT is not allowed; there is no mention of robotic bronchoscopy. I would recommend clarifying whether robotic bronchoscopy allowed or is prohibited from the study.
The primary aim of this study is to evaluate the added value of nCLE to conventional bronchoscopy including rEBUS and fluoroscopy. For the study part, augmented fluoroscopy and CBCT and robotic bronchoscopy is excluded.
In those centers were CBCT and/or robotic bronchoscopy is routinely used, these methods may be employed after all actions described in the study protocol have been done. The bronchoscopist can then decide that for optimal patient care it is best to also implement CBCT or robotic bronchoscopy.
The pathology samples will be analyzed separately. We have adapted this section manuscript to clarify these points (page 9).
Diagnostic yield for the control group (is based on meta analysis of bronchoscopy with radial EBUS (citations 21 and 22) but control group allows for navigational bronchoscopy by institutional practice which may include EMN. If many providers will be using EMN, consider re-calculating sample size for yield in control arm closer to that of Navigate study (73%); this may require higher enrollment to demonstrate statistical significance.
We acknowledge your concern and agree that in case of the use of EMN, the diagnostic yield would potentially be higher. However, only one of the participating centers users EMN sporadically meaning that the influence of the use of EMN will be minimal and therefore the diagnostic yield of the control group is not expected to be as high as reported in the Navigate study and closer to the reported yields from the meta analyses on rEBUS.

Reviewer: 3 Dr. Chong Bai, Second Military Medical University First Hospital Comments to the Author: Dear authors,
The protocol by Heumen et al. aimed to investigate if nCLE-imaging integrated with conventional bronchoscopy results in a higher diagnostic yield compared to conventional bronchoscopy without nCLE. There is no doubt that this ongoing multicenter randomized controlled trial will provide interventional pulmonologist with important information about the added benefit of the nCLE technique for peripheral lung nodule diagnosis. Although this protocol is meticulous and rigorous, there are still some details that should be noted.

Thank you, Dr. Chong Bai, for your detailed review of the manuscript and your feedback. Please see the answers to the comment below.

1. To evaluate the diagnostic yield and diagnostic sensitivity for malignancy of nCLE, nCLE diagnosis criteria should be predefined. Furtherfore, blinded raters should be unifiedly appointed in order to avoid the differences between participating centers. Also, as the imaging interpretation of nCLE has characteristics of subjectivity, the additions of the inter-observer agreement and intraobserver reliability of nCLE imaging is worthy of consideration. For the aforementioned points, this article below may be refered. “Bronchoscopic needle-based confocal laser endomicroscopy (nCLE) as a real-time detection tool for peripheral lung cancer” (doi: 10.1136/thoraxjnl-2021216885)

You touch upon a valid point regarding nCLE interpretation.
We indeed did not specify in the methods how the nCLE videos would be rated. We have clarified in the revised manuscript based on your comment which nCLE criteria will be used to interpret the images and clarified that blinded raters of the initiating center (Amsterdam UMC), with previous experience in interpreting nCLE images will be rating the videos. Please see page 10 for the added paragraph.
We understand your comment on the potential addition of reporting the inter-observer agreement and intra-observer reliability. Previous studies by our group have reported these parameters (DOI: 10.1183/13993003.01520-2018 and DOI: 10.1136/thoraxjnl-2021-216885 and DOI: 10.1111/resp.14542) and this is outside the main aim of this study.
However, the nCLE data matched with corresponding pathology will be further studies regarding IOA and IOR to further shed light on the interpretability of these images, possibly combined with AI analysis.

2. This protocol focused on investigating if nCLE-imaging integrated with conventional bronchoscopy results in a higher diagnostic yield compared to conventional bronchoscopy without nCLE in diagnosing peripheral lung nodules, which should be highlighted in the title of this paper. For example, the title can be changed into “Design of a multicenter, randomized controlled trial on bronchoscopy with and without needle-based confocal laser endomicroscopy for peripheral lung nodule diagnosis (CLEVER trial)”.

Thank you for this suggestion, the title has been changed to “Bronchoscopy with and without needle-based confocal laser endomicroscopy for peripheral lung nodule diagnosis: protocol for a multicenter randomized controlled trial (CLEVER trial)”.

3. Although multiple studies have demonstrated the excellent inter-observer agreement and intraobserver reliability, the fact that the imaging interpretation of nCLE has characteristics of subjectivity is unavoidable. As one of the exploratory endpoints, an algorithm for automated nCLE criteria recognition using machine- or deep-learning methods seems encouraging. As mentioned in the recent article entitled“The role of confocal laser endomicroscopy in pulmonary medicine”(doi: 10.1183/16000617.0185-2022), in the near future, it can be envisaged that a large atlas of CLE images for different pulmonary diseases will be established and can be identified automatically using artificial intelligence; as a result, the role of nCLE in diagnosing peripheral lung nodule will be further highlighted. In my opinion, a combination of nCLE and

artificial intelligence should be involved in the section of Discussion in order to highlight the aforesaid exploratory endpoint.
We absolutely agree with your comment of the future added benefit of implementing AI to interpret nCLE images for standardization purposes, easier implementation of the technique in centers and improving the inter-observer agreement and intra-observer reliability. We integrated this element in the discussion of the manuscript on page 14-15.
Additionally, based on the CLEVER data, we aim to report on the value of AI in image interpretation. This will be discussed in a separate manuscript.

4. For the description “rapid on-site evaluation (ROSE) is sometimes used for direct feedback on representativeness of the sample and forming a preliminary diagnosis. Nevertheless, diagnostic yield rarely exceeds 71%, (8) as it depends highly on factors such as nodule size, bronchus sign on pre-procedural CT, eccentric vs. concentric r-EBUS pattern and pretest probability of malignancy.”, sampling techniques should be added as the one of the influence factors (*Mondoni M, Rinaldo RF, Carlucci P, Terraneo S, Saderi L, Centanni S, Sotgiu G. Bronchoscopic sampling techniques in the era of technological bronchoscopy. Pulmonology. 2022 Nov-Dec;28(6):461-471. doi: 10.1016/j.pulmoe.2020.06.007*).

Thank you for this suggestion, this was indeed missing from the list of influencing factors. The revised sentence is as follows (page 3): “Nevertheless, diagnostic yield rarely exceeds 71%,(8) as it depends highly on factors such as nodule size, bronchus sign on pre-procedural CT, eccentric vs. concentric r-EBUS pattern, pre-test probability of malignancy and sampling tools used.”

5. Also noted: transbronchial lung cryobiopsy (TBLC) that can retrieve larger specimens with more preserved cellular architecture and fewer crush artifacts in comparison with conventional transbronchial forceps biopsy (TBFB), as an emerging technology for diagnosing PPLs, has been demonstrated to have the potential to resolve the clinical dilemma pertaining to currently available sampling devices (e.g., forceps, needle and brush) and become a diagnostic cornerstone for PPLs (*Tang Y, Tian S, Chen H, et al. Transbronchial lung cryobiopsy for peripheral pulmonary lesions. A narrative review. Pulmonology. 2023 Oct 30:S2531-0437(23)00163-0. doi: 10.1016/j.pulmoe.2023.08.010*); therefore, the addition of TBLC as another sampling technique besides TBNA and TBFB to acquire tissue for pathological evaluation may be worth considering. We agree that the implementation to TBLC is very interesting in the field of PPLs. Within this study we allow the use of TBLC during the procedures (if already regularly done at that center ensuring sufficient experience with the technique). It will be reported in how many cases TBLC was used.

Reviewer: 4

Dr. Ajay Wagh, The University of Chicago Comments to the Author:

Thank you for the opportunity to review “Design of a multicenter, randomized controlled trial on needle-based confocal laser endomicroscopy guided bronchoscopy for peripheral lung nodule diagnosis (CLEVER trial).” This study is evaluating whether using confocal laser endomicroscopy can improve diagnostic yield during peripheral bronchoscopy.

Dear Dr. Ajay Wagh, your feedback on our manuscript is much appreciated. Please find our answers to your questions below.

1. Could the authors comment on whether industry representatives would be assisting during the procedure and/or with CLE image interpretation? (Page 12, lines 34-39). Are there predefined imaging standards?

Industry representatives during the introduction of the nCLE technique and the first few clinical cases. There will be an emphasis on equipment handling. After a short introductory phase, industry representatives will only be present on indication.

2. Could the authors clarify on whether any additional imaging assessments may be used (including use of local registration, augmented fluoroscopy, etc) to update navigation and/or localization?

Would such techniques be utilized pre-biopsy?

For the present study, the use of rEBUS is a prerequisite and virtually all procedures will also include fluoroscopy. We also mention that the use of cone beam computed tomography often combined with augmented fluoroscopy is not allowed. Additionally, we have added based on a suggestion by another reviewer, that robotic bronchoscopy is also not allowed in this study and have clarified that the use of augmented fluoroscopy is also not used in this study in the methods section of the manuscript.
In those centers were CBCT and/or robotic bronchoscopy is routinely used, these methods may be employed after all actions described in the study protocol have been done. The bronchoscopist can then decide that for optimal patient care it is best to also implement CBCT or robotic bronchoscopy. The pathology samples will be analyzed separately. We have adapted this section manuscript to clarify these points (page 9).