Reviewer A

The authors explored characterize precurved and steerable guiding sheaths (GS) in endobronchial targeting for lung biopsy using cone beam computed tomography (CBCT) based augmented fluoroscopy (AF) image guidance. To investigated the characteristics and feasibility of the new instruments, their study design consisted of three experimental phases, included phase 1: bench mode, phase 2: ex vivo, and phase 3: in vivo, and one case report. The authors showed that the bench to bedside feasibility of the use of AF guidance and endovascular steerable GS without a bronchoscope, together with intraprocedural CBCT, to successfully perform a biopsy if a lung lesion. Although more clinical research is needed for clinical application as an example of one case, it is a well-written paper with a detailed explanation. This research would provide some information for improving of diagnostic yield of peripheral lung lesions in the future.

Please consider the following minor suggestions.

1. The legends of Figures 3 and 4 do not match. Please confirm it.
   Reply: Thank you for pointing out this error that occurred in the uploading of figures 3 and 4, which has now been corrected in the current upload.
   No changes in the text. Figs 3 and 4, all figure legends and callouts in the text are now accurate with the implemented corrections.

2. There is no description for Table 2 in the manuscript. Please check it.
   What is table 1a-b? (page 13)
   Reply: Thank you again for pointing out our errors. Table 1a-b is incorrect and has been renamed table 1 and table 2, and a callout/reference for Table 2 was added to the text.
   Changes in the text: “Tables 1 and 2 summarize the baseline GS delivery angle and needle-induced angle loss for each GS/needle combination, as well as the delivery angle for the EM guide for the precurved GS. For precurved GS, needle-induced angle loss ranged from 2.5% for the Edge 45/21G combination to 74% for the Edge 180/TBAT combination (Table 1). Across all GS evaluated, the steerable DT was capable of the largest needle delivery angle of 114° with either the 21G or 19G biopsy needle in the working channel (Table 2)” (P 16, line 3-8).

3. Please check the reference 27 expression (lines 26-28, page 27)
   Reply: Thank you once again for pointing out our error. The reference refers to a book, and was incorrectly cited. We have corrected the reference citation according to journal guidelines for books.
Reviewer B

De Ruiter et al evaluated in their study “Endovascular Steerable and Endobronchial Precurved Guiding Sheaths for Transbronchial Needle Delivery Under Augmented Fluoroscopy and Cone Beam CT Image Guidance” the use of precurved and steerable guiding sheaths (GS) for lung biopsy with cone beam computed tomography (CBCT) based augmented fluoroscopy (AF) image guidance. The group did an exhaustive evaluation of the various catheters in benchtop, ex vivo, in vivo, and finally off-label use clinically in a patient. This is the first study that examines the question “Tool-in-Catheter deflection" problem in a systematic way. I congratulate the authors on their efforts.

Article is well written, descriptive, and provide clinically applicable information for the field. However, there are limitations.
Major comments:
1. Unclear why the study was done without bronchoscope support especially when the standard of care is done with bronchoscopy. Thus, the mechanical behavior of the GS inside the working channel of the bronchoscope is not characterized. Can authors explain why they did this study without the GS in the working channel of the bronchoscope?

Reply: With advances in imaging guidance technology, e.g., intraprocedural cone beam CT and augmented fluoroscopy, it is possible to accurately navigate catheters (of a caliber much smaller than a bronchoscope) within the airway and guide biopsy without the use of a bronchoscope. This represents an “out of the box” unconventional approach which is off label. The aim of this work was to characterize the inherent capabilities and limitations of existing guiding catheters and their performance without a bronchoscope. This is reflected in the stated hypothesis, “The hypothesis was that steerable GS with a deflectable tip could perform accurate transbronchial needle delivery without a bronchoscope and have improved steerability and direction compared to precurved GS, particularly in the setting of a steep delivery angle to a lung target in vivo.”

A conventional bronchoscopy approach was used in the clinical case, as well as, the unconventional off-label approach without a bronchoscope. No recommendation for such future use was intended or implied. It is possible that in specific scenarios, a bronchoscope might not have the flexibility or maneuverability of a “bare-back” guide catheter. Additionally, a guide catheter may have been manufactured with tapered edges, for example, intended not to cause endovascular injury, such as internal elastic laminal or emboli from endovascular plaques. However, these statements are highly speculative, are not stated or claimed in the paper, and are only presented here for the reviewer’s consideration.

Changes in the text: For clarification, the following text is inserted in the Introduction “The aim of this work was to characterize the inherent capabilities and limitations of existing guiding catheters and their performance alone without a bronchoscope.” (P 8, line 25-27)

2. 6.5 F Destino Twist is used alone for navigation purposes in the case study is interesting. However, with a 6.5 Fr 90cm DT catheter has an outer diameter of 2.8mm, this will fit in a 2.8 mm WC of the large therapeutic bronchoscope. If this does not, then one can use the 3.2 mm WC of the x-large therapeutic bronchoscope. One wonder about the clinical reasoning of why the catheter was used by itself. As the authors pointed out in their article: "Detection and management of pulmonary hemorrhage without the use of a bronchoscope remains a concern". Why did the authors design their study this way?

Reply: These are important clinical points. No clinical study was designed at all here. The case report was added because it happened to demonstrate an exemplary case of related use of endovascular catheters in the bronchial tree. In the case report, the biopsy was performed jointly by a pulmonologist and an interventional radiologist who was skilled in catheter-based, image-guided interventions and with experience in catheter-only pulmonary interventions, as well as augmented fluoroscopy in CBCT-based percutaneous and endobronchial interventions.

The case reported was not a study nor investigation, but rather the incidental use of off-label devices during a clinical need after a bronchoscopic approach alone approach failed to navigate
successfully. Rather, the decisions made were based on multi-disciplinary senior attending physicians’ judgements on what was in the best interests of the patient. As stated in the discussion, Tai et al. has shown that Pulmonary hemorrhage after TTLB is common, but rarely requires intervention. [https://pubs.rsna.org/doi/full/10.1148/radiol.2015150381].

Changes in the text: “The procedure was converted to the use of DT GS with CT and CBCT-based AF image guidance without the use of a bronchoscope, performed by an interventional radiologist with experience in catheter-based, image-guided procedures.” (P 19, line 15-17).

3. Using GS alone in peripheral nodule access with only AF supported by CBCT is somewhat difficult, as there are very limited adapters for endotracheal tube to maintain a complete seal. As such, the authors used a 9Fr sheath. This issue would have been solved with use of a bronchoscope. How did you address the CT-to-body divergence with lower lobe atelectasis that can interfere with your navigation (especially if you do not have a complete seal at entry to the airway)?

Reply: Great point. We agree that maintaining an airtight seal at the introduction site for the GS is essential. We used off-the-shelf devices with a 9 Fr sheath and maintained an adequate seal. New adapters could be designed if there were a demand in the future. Atelectasis was not present in this study. Since this approach allows real-time imaging (fluoroscopy, augmented fluoroscopy) and new intraprocedural CBCT acquisition, allowing the operator to update navigation, changes in the airway geometry could be recognized early on, when modifications might be made, such as length of the procedure. In fact, during CT-guided procedures of all kinds, it is quite common, if not almost "normal" to see significant atelectasis in the lower lobes during procedures. Presumably, atelectasis might be less with a tight seal, as was maintained here.

Changes in the text (Discussion): “In the case of intraprocedural atelectasis, a non-diagnostic biopsy, or airway deformation due to stiff scopes or needles, a CBCT may be acquired to assess the airway and fidelity to the original geometry and AF planning. Moreover, the issue of CT-to-body divergence can be addressed since the navigational planning may be updated based on new imaging data (33).” (P 21, line 17-20).

4. Not all systems have access to cone beam CT, blinded navigation prevents staging procedure to be done in the same setting. How do the authors envision this can be integrated into clinic practice – in the IR suite, hybrid OR, or Bronchoscopy suite?

Reply: Theoretically, such procedures could be performed in any room with fluoroscopy, CBCT capabilities, and an interventional operator with spatial skills and image guidance training. This may occur in all of the potential sites mentioned.

Changes in the text (Discussion): “The use of steerable GS may improve the diagnostic yield of transbronchial biopsy in settings with fluoroscopic and CBCT capability, whether in interventional radiology and bronchoscopy suites or hybrid operating rooms.” (P 23, line 11-15).

5. Tables: authors should consider a table to show comparative p-values between the steerable sheaths and the preformed sheaths in terms of angle deflection.
Reply: This is an excellent suggestion. However, this feasibility study was not designed or powered to report statistical differences between steerable GS and pre-curved GS. 

No changes in the text.

Reviewer C

This is an original study comparing the results of needle delivery of different guiding sheaths (endovascular steerable and endobronchial precurved) into lung targets using cone-beam CT based augmented fluoroscopy (AF). The authors investigated the guide sheaths and needle delivery in a bench model, ex vivo swine lung, in vivo swine lung, and also provided a case report utilizing the technology. The authors conclude that endobronchial needle delivery with augmented fluoroscopy guidance is feasible without a bronchoscope with the steerable endovascular guide sheaths outperforming the precurved endobronchial guide sheaths.

General Comments:
This is a very well-designed and performed study evaluating the accuracy as well as comparing different guide sheaths for endobronchial needle delivery for lung biopsy using advanced imaging techniques without the use of a bronchoscope.

Reply: Thank you for the kind words.

Questions/Comments:
1. Could the authors please comment if they observed any airway trauma or edema as a result of advancing the guide sheaths into the airway without endoscopic guidance?

Reply: We did not evaluate airway trauma, whether by bronchoscopy or explant pathology. A future device study addressing this could be an important part of the translation to clinical practice. We did perform CBCT after needle delivery to evaluate local needle delivery-related complications such as pneumothorax or pulmonary hemorrhage as represented by new consolidation or ground-glass opacity near the needle delivery site. These were not seen, but the study was not powered to make a statement on such, so it remains agnostic. Note that endovascular devices are specially designed with soft tips and tapered edges to minimize issues such as cholesterol thrombi (blue toe syndrome), which might be helpful here in the future.

No changes in the text.

2. Could the authors please comment on needle type/characteristics/flexibility used for the study? Could more flexible needles improve needle delivery?

Reply: In this study, we used two standard bronchoscopy needles and the stiffer TBNA tool. The study demonstrated that stiffer needles (e.g., TBAT) have a higher loss of flexion, thus reducing the maximum needle delivery angle. The devices and their characteristics are described in Methods (p9, line 21-24). We agree that newer, more flexible needles might have better performance. Newer, less stiff needles such as the Olympus needle were not evaluated here but may be delivered at a steeper angle with less loss of flexion.

Changes in the text:
Methods: Endobronchial working channel instruments that were evaluated with each GS included the EM locatable guide with a blunt tip (Edge™ Locatable Guide); 21G*8 mm aspiration needle; 19G*8 mm aspiration needle (superDimension, Medtronic, Minneapolis, MN, USA); and an EM trackable Transbronchial access tool (TBAT) (superDimension, Medtronic, Minneapolis, MN, USA). “ (P 9, line 24-25).

Discussion: “These characteristics may allow steerable GS to reach higher-generation airways, navigate at larger GS delivery angles, or to deliver biopsy tools capable of obtaining larger tissue samples. These results may be extrapolated to the use of next-generation more flexible needles with reduced stiffness, resulting in reduced GS loss of flexion, thus increasing the maximum needle delivery angle. Although speculative, this may contribute to higher diagnostic yield rates in both adult and pediatric patients (6,28).” (P 20, line 12-17).

3. Could the authors please comment on whether they encountered any fatigue of the precurved endobronchial guide sheaths with loss of curve over time of use (beyond the mentioned needle-induced angle loss)? Could this contribute to reduced needle delivery accuracy as well? Did the endovascular steerable sheaths fatigue at all – or were the steering angles able to be maintained during all portions of the procedure?

Reply: Re-use of the precurved catheters for multiple bench and ex vivo deliveries may contribute to fatigue. Although fatigue was not quantified and cannot be ruled out, a new pre-curved GS was substituted before a significant change in the resting curve was subjectively noted. Moreover, exposure to body temperature may potentially impact performance but we did not specifically study the impact of temperature on precurved or steerable GS performance. In the in vivo study, a new steerable GS was used for each animal and assessed prior to each target delivery for acceptable performance. Although fatigue was not quantified, any potential change in performance could be compensated for by increasing the GS curvature to maintain the planned needle angle in steerable sheaths, with some limitations in memory, depending upon the specific alloy. Use of un-kinkable materials or nitinol might alter this issue.

Changes in the text:

Methods: “A new pre-curved GS was substituted when a significant change in the resting curve was noted on the bench. A new steerable GS was used for each animal and assessed prior to each target delivery for acceptable performance.” (P 13, line 25 -28).

Discussion: “The effect of fatigue and exposure to body temperature on device shape and performance were not evaluated in this study”. (P 23, line 6-7).

4. Could the authors comment on how mediastinal/hilar staging could be performed if required in a clinical context? Would this be performed with EBUS bronchoscopy? Or could it be performed using CBCT/AF with a steerable sheath? In the latter case – what would be the radiation exposure?

Reply: Mediastinal staging was not within the scope of this paper. An EBUS scope may also be used in a procedure that includes a steerable GS.
5. Could the authors comment on radiation exposure utilizing CBCT/AF as in this study compared with conventional TTNA utilizing CT?

Reply: The study of radiation exposure, including dose protocols, was beyond the scope of the paper. We agree this is an important question which we partially addressed in a previous preclinical study (de Ruiter et al.) demonstrating that the use of optimized lung protocols will significantly reduce radiation dose. Figure 6 illustrates the use of proper radiation protection.

Changes in the text (Discussion): “Although CBCT-guided procedures have higher radiation exposure in comparison to EM-guided bronchoscopy procedures (20), radiation doses may be decreased by using optimized radiation dose settings for fluoroscopy and CBCT in CBCT-guided bronchoscopy procedures and adequate shielding (22).” (P 21, line 19-22).

Reviewer D

This study evaluated the flexibility of 4 precurved and 2 steerable GSs during AF and CBCT procedures. It demonstrated that the angle loss of steerable GSs was less than the precurved ones when biopsy instruments were inserted and suggested using the steerable GS rather than the precurved GS is preferred during AF/CBCT guided biopsy or ENB. This manuscript may provide some useful information for readers. My comments are as follows.

Major:
1. The loss of angle of GSs depends on the flexibility and the length of inserted needles and the flexibility of the GS. Now, more flexible needles are available (e.g. Periview flex, Olympus), and the bending angle of the GS may be less affected by more flexible needles. Which needle did you use (model, manufacturer)? Are they the most common type of needles during AF/CBCT and ENB? The standard biopsy instruments are biopsy forceps. Please give some comments for other promising biopsy instruments.

Reply: The use of super dimension needles (21Gx8mm, and 19Gx8mm) is described in Methods and represents the most common needle type used by our collaborating IP with ENB. More flexible needles may contribute to improved navigation and target accuracy. Please see our response to reviewer C question 2 above. The change in text (Discussion) is repeated below.

Changes in the text (Discussion): “These characteristics may allow steerable GS to reach higher-generation airways, navigate at larger GD delivery angles, or to deliver biopsy tools capable of obtaining larger tissue samples. These results may be extrapolated to the use of next-generation more flexible needles with reduced stiffness, resulting in reduced GD loss of flexion, thus increasing the maximum needle delivery angle. Although speculative, this may contribute to higher diagnostic yield rates in both adult and pediatric patients (6,28).” (P 20, line 12-17).
2. Please reduce the length of the manuscript (by one-third).
   Reply: We have reduced the discussion but added additional text based on reviewer's comments. Some methods information was moved to a supplementary index.
   **No changes in the text.**

**Minor:**

1. The results of the DT GS were better than the Morph GS. In your opinion, do you recommend the use of the DT GS? Or, is the use of either DT GS or Morph GS an acceptable option?
   Reply: We hope the results of this study will help guide physicians in device selection, but we cannot make recommendations on clinical practice.
   **No changes in the text.**

2. What is the difference between commercially available AF software and prototype AF software? Did you compare them in this study? What was the role of prototype one in this study?
   Reply: We provided additional details on the differences between both versions in Methods. We compared the feasibility of both in the ex vivo study. Tumor segmentation is available with both generations; however the prototype software also includes airway segmentation, navigation, and delivery pathways, CBCT-CBCT lung registration, and optimized endobronchial workflow for endobronchial procedures.
   **Changes in the text (Methods):** “While both provide for tumor segmentation, the prototype software has features that enhance AF workflow including CBCT airway segmentation, navigation pathway trajectory analysis, and CT-CBCT registration and needle planning.” (P 11, line 27-29).

3. Page 11 line 25 to page 12 line 11. The paragraph "Clinical case report," should be transferred to the Result Section.
   Reply: We have moved the paragraph "Clinical case report" to the Result section as suggested.
   **Changes in the text as noted above.**

4. Page 16, Page 23. The sentences on "ROSE" seem to be unnecessary.
   Reply: We agree and have removed this section from the discussion.
   **Changes in the text as noted above.**

5. Figure 3 and 4. It looks like the legends and the images may be reversed.
   Reply: Thank you for pointing out this error that occurred in the uploading of figures 3 and 4, which has now been corrected in the current upload. Please see our same response to reviewer A question 1 above.
   **No changes in the text.** Figs 3 and 4, all figure legends and callouts in the text are now accurate with the implemented corrections.