Novel data on needle technique at EBUS TBNA shows as few as 3 agitations of the needle is enough for adequate DNA on smears along with avoiding erythrocyte contamination of the smears.

D. Fielding*[a] (Dr), A. Dalley[b] (Dr), P. Simpson[b] (Dr), K. Nones[c] (Dr), V. Lakis[c] (Dr), S. Sharma[c] (Dr)

[a] RBWH, Brisbane; [b] University of Queensland Centre for Clinical Research, Brisbane; [c] Queensland Institute for Medical Research Berghofer, Brisbane, Australia.

Abstract

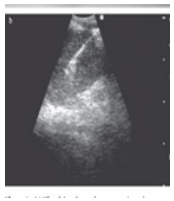
Background: Optimising the EBUS TBNA needling technique is important to maintain procedural simplicity and maximise sample quality. The number of agitations of the needle within the lymph node has not been studied for lung cancer, nor has which component of the content is placed on the smear- the first or the last drops out of the needle. Fewer agitations might mean less trauma to the node, but it would be important to confirm adequate DNA was available. **Methods**: We prospectively explored three versus 10 agitations of the needle in sequential passes into the lymph node using separate needles in EBUS TBNA for malignant nodes. Resulting Diff-Quik cytology smears were quantitatively assessed using microscopic (tumour cell cellularity, abundance scores, erythrocyte contamination) and DNA yields. **Results**: In 86 patients (45M, 41F), a mean of 5.3 smears were made per patient with a total of 459 smears scored by pathologists, and 168 paired smears extracted for DNA. There was significantly less contamination by erythrocytes from three agitations (X^2 p=0.008) as judged by microscopy. There was no significant difference between three versus 10 agitations for smear microscopy cellularity (p= 0.29) however there was significantly higher cellularity in smears made from the last drops out of the needle compared to the first drops (p= 0.01) . Overall there was no difference in DNA yield (469ng vs 488 ng, P=0.84) or DNA integrity (p=0.20). However there was significantly more DNA in the first pass into the node using three agitations than with other passes and with 10 agitations (Pass * Agitations interaction, p=0.031). **Conclusions:** Three agitations is non-inferior to 10 agitations for overall abundance of malignant cells and DNA content on smears. A smear with adequate DNA for panel sequencing could almost always be made with the first needle pass using three agitations.

Research Question

At EBUS TBNA is it possible to use as few as 3 agitations (versus 10) with each needle pass into a lymph node and still obtain good samples, as measured by microscopy cell percentage and DNA content on smears made from each pass using randomly assigned agitation number. Fewer passes may mean less trauma to the node and improved DNA recovery.

Methods

1.



Pass 1
- 3 (or 10) agitations
- First and last drops

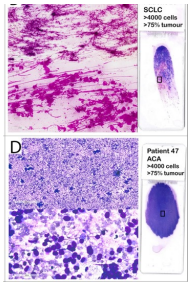
Pass 2
- 10 (or 3) agitations
- First and last drops

Etc up to 6 passes total

Smear for each pass using a separate needle for 3 or 10 agitations, labelled

No further smears when ROSE positive

2. Score Microscopy of smears

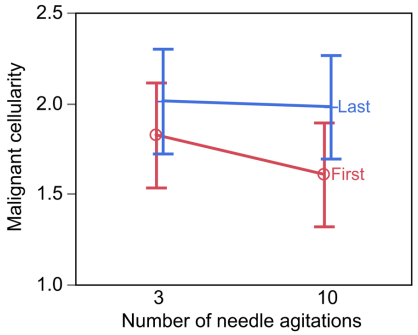


3. Extract DNA

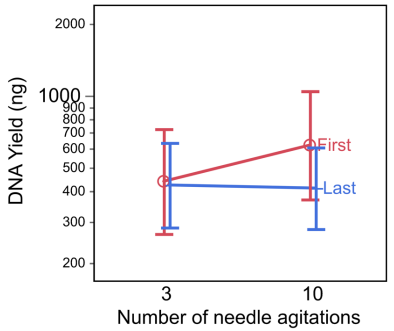
Results

A total of 459 smears were made from 86 patients with a final diagnosis of malignancy.

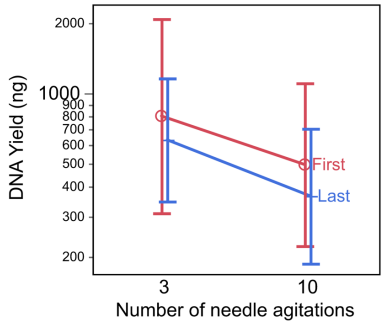
Microscopy overall showed no difference between 3 and 10 agitations for malignant cell percentage on smears. However there was a higher percentage n smears made from the last drops out of the needle (as opposed to the first drops) onto the smear- p-0.01.



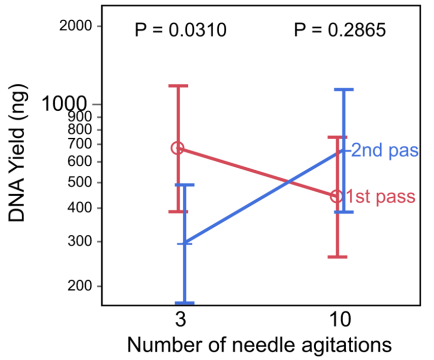
DNA extraction results from Diff-Quik slides. Comparing DNA yield from 3versus 10 agitations, and first drops (red) versus last drops (blue) of aspirate material deposited on the slide. Overall no significant differences.



DNA yield data from all passes into the node,



DNA yield data from only the first pass of needle into lymph node.



DNA yield data from first pass of the needle into the node (red) compared with second passes into the node (blue).

The best combination for DNA yield was the first pass of the needle using 3 agitations , which was better than 10 agitations and any other passes. (Pass * Agitations interaction, p=0.031

Discussion / Conclusions

DNA yield with 3 agitations is non- inferior to 10 agitations

The best combination of agitations and passes is the first pass with 3 agitations

Microscopy (but not DNA yield) is improved using the last drops out of the needle

Proceduralists could consider the importance of the first pass and 3 agitations
- By reserving that smear for DNA studies
- Alternatively where ROSE Diff Quik smears are not used proceduralists may elect to keep a saline or liquid medium pot of some or all of the first pass for molecular studies. (as opposed to only using additional passes at the end of the procedure, eg passes 4, 5 and 6, or later).

Aspirates with 3 agitations have fewer red cells (data not shown) and 3 agitations should simplify the procedure, with less node trauma.

The results support the notion that the sample in the needle is derived predominantly from capillary action into the needle, as opposed to mechanically shearing cells in by multiple passes

Acknowledgements

Cancer Australia
Cancer Council Queensland
Olympus Australia