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Key Words: DNA methylation, noninvasive test, non–small cell lung cancer, screening, sputum

Discussion

Presenter: Dr Harmik Soukiasian

Dr Usman Ahmad *(Cleveland, Ohio)*. That was fantastic work. Really exciting and well presented. The applications are endless. Now, you had approximately 40 patients with lung cancer in this cohort if I understand correctly. Did you see a correlation in the yield of the test and the stage of the tumor or central versus peripheral nature of the tumor?

Dr Harmik Soukiasian *(Los Angeles, Calif)*. We didn't do a subset analysis of that, and I am going to, but we were able to diagnose all of them. Well, 96%, but I did not stratify based on upper lobe, lower lobe, periphery versus central, and that is something that I will do in the article, now that you mention it.

Dr Ahmad. The other thought I had was another application in an area that we struggle with is diagnosing pleural effusions or malignant peritoneal ascites and so forth. Do you see this being applicable in those areas?

Dr Soukiasian. I see this as being applicable in areas like you said because routinely, 40% of pleural effusions that are malignant do not show up as being malignant. I think that has to do with the limitations of cytology. Same thing with the peritoneal analysis. So I think that if you applied this technique to it, it would probably help increase the yield, and these are studies that we can certainly easily do as a proof of concept as well.

Dr Ahmad. Particularly with that level of sensitivity. What your thoughts are about using this as a screening tool or surveillance after treatment of a lung cancer?

Dr Soukiasian. That is a good point, and I think one of the things is just a proof of concept, but if it proved to be useful and applicable on a broad spectrum, I think you could theoretically use it to screen patients who have lung nodules that you want to see what it is before you biopsy it or go to surgery. Certainly, you can use it also as a surveillance modality after resection. People are checking circulating DNA levels in blood. This could be an adjunct to that as well. It is

interesting technology that it's just from sputum, and you can just see the methylation, so it's right there.

Dr Dan Boffa *(New Haven, Conn)*. How important was it to link this to the pulmonary function tests (PFTs)? So all of these people had PFTs right before they had the sputum production?

Dr Soukiasian. This is induced sputum. Maybe I didn't say that, so thanks for clarifying that. The way we do the induced sputum in our hospital is at the PFT laboratory, and so we thought that it was just 2 birds with one stone kind of thing. But you could theoretically do it in your office if you wanted to, but we did it in the PFT lab because I don't really know how to induce sputum.

Dr Boffa. But they had undergone PFTs immediately before having the induction.

Dr Soukiasian. At the time of. Correct.

Dr Boffa. I would be interested to see if you have the same results. We used to look at circulating tumor cells, and you'd get a lot more right after PFTs compared with if they did not have PFTs, just the workup and you mentioned cell-free DNA. Why not just look at this using the same platforms as cell-free DNA? You would have so more sensitivity. Why look at this histologically? Why not just look at the fragments of DNA?

Dr Soukiasian. Well, because flow will usually do that, right? You can use flow cytometry. The problem with that is you're scouting for all kinds of DNA. So you don't know if its cytoplasmic DNA or nuclear DNA, and you're also going to include fragments of DNA from nonepithelial cells such as inflammatory cells and some other cells that are not contributory to cancer, so it could increase your false-positives.

Unidentified Speaker 1. What was the size of the tumors and how many of these were solid versus ground-glass opacity, the cancers?

Dr Soukiasian. The average size was about 2 cm. I just know the stages. I didn't quantify ground-glass opacity versus solid. We were just trying to see if we could determine cancers that we verified after surgery.

Unidentified Speaker 1. Were any 1 cm or less that were diagnosed?

Dr Soukiasian. Yes. For stage 1, some were 1 cm, and some were 0.9 cm.

Unidentified Speaker 1. All right.

Unidentified Speaker 2. From what I understand, a positive sample for malignancy is when you have at least 30% to 40% hypomethylated cells.

Dr Soukiasian. It's the angle underneath, with the colocalization of the [inaudible] and the 5MC. I mean, it's a flat regression line. In general, looking at a histogram, most of these patients who have lung cancer will have a hypomethylation state. In stage 3 and 4 lung cancers, only about 20% methylated, and in stage 1 and 2 cancers, about 30% to 50% methylated, so they are globally hypomethylated compared with normal or high-risk patients.