Good morning and thank you to the organizing team for giving me this opportunity. I'll be discussing on a paper which was published in a general lung cancer, a non-invasive diagnosis of pulmonary nodules by circulating tumor DNA methylation. So, this was a prospective multicenter study conducted in Chinese hospitals. So, as we know about lung cancer screening,

most common cancer we already discussed and one of the most common cancers and lethal cancers. The NLST trial which used the low-dose CT scan, it showed that employing low-dose CT scan modality as a screening method for lung cancer in high-risk populations could reduce mortality by 20 percent. But the problem is that with the false positive, around I think 20 to 25 percent of patients had false positive rate and that led to invasive interventions. So, there are certain non-invasive models to predict

the risk of malignancy, there are two models which we are commonly using in the clinic. That is a Veterans Affairs model and other is a Mayo Clinic model. However, these models also have their limitations in terms of sensitivity and specificity. And then there are certain factors in the model which might not be very well relevant to Indian or the Asian settings like the one in upper lobe location which is in the Mayo Clinic model because there is a high risk, already high prevalence of tuberculosis in our setup.

So, what is liquid biopsy and how it can be used for pulmonary nodules? So, liquid biopsy uses the circulating tumor cells, nucleic acid and the proteins. And for lung cancer screening and for guiding whether it's a benign or a malignant pulmonary nodule, there are already certain commercial assays which have been proposed for identification of benign versus malignant. A few of them are early CDT lung, which assesses the presence of autoantibodies to a panel of seven lung cancer-associated

antigens and also there is a micro RNA signature assay which also has been proposed.

And now we are moving towards the CFDNA and CTDNA approaches. So we already know that CFDNA can be used to identify genetic mutations, abnormal DNA methylation and also fragmentation patterns. And if you look at the CTDNA-based methylation tests, then there are many studies which have reported that certain genes, the methylation on those genes can differentiate benign versus malignant pulmonary nodules.

So there have been certain papers which have proposed a test like ELSA sequence and PulmoSick which have utilized this methylation on these genes to identify and classify whether it is a benign or a malignant pulmonary nodule.

Further moving, PalmoSick+ model also incorporated methylation along with imaging and clinical parameters to improve the sensitivity and specificity of those models to identify benign versus malignant pulmonary nodules. So here in this paper, a lung track named model was used which was based on ctDNA methylation based assay.

And corresponding CT scan images were also used to certify and include patients who had 5 to 30 mm lung nodules. The study design was, it was a prospective multicenter diagnostic test in China and including patients of 40 to 80 years of age with a CT detected pulmonary nodules. And exclusion criteria was that if patients had any cancer, history of malignancy or if the pulmonary nodules were associated with corresponding mediastinal or hyaluronanopathy, those patients were excluded.

So here the FFP blocks were prepared and genomic DNA was extracted. Also the blood samples were collected and centrifuged for the method which we employed to extract the DNA. And identification of differential methylation regions. This was done to differentiate

ascertain which methylation patterns existed in the benign as well as in malignant tissues. So this was a study workflow. The stage one in the study was to identify the methylated markers distinguishing tissues of benign versus malignant. Stage two of the study was that targeted methylation sequencing assay was developed to identify these sequences and stage three the model which was

made out of identifying these methylation markers. This model was validated in a training set and the validation set and then also validated externally in another center's study. So this is the schema of the study in which the first methylation markers were identified

out of around 54 patients and then 467 patients were enrolled, out of which 60 were excluded and 401 patients were used for the study cohort. And then it was done in a training and the validation set and then again it was validated in a multicenter test.

So, first step was this identification of the differential methylation regions and then proportion of the discordant reads, that is the difference in the methylation of benign versus malignant was identified and then the model was made. So, what was the performance of this model of the lung track? So, in the training set, it had a AUC of

0.973 which was the highest because of training set. Sensitivity was 94% and specificity was 88%. Validation set had an AUC of 0.1 with a specificity of 73% and single center blinded cohort had an AUC of 0.81 with a specificity of 76.2%.

So this was the performance of the model. The topmost curve which is the training set as I already mentioned and followed by the validation and the single center. So AUC remained about 0.8 in all the three sets. Again this model was not biased and was not affected by any of the baseline clinical or radiological factors.

This set was again validated in a multicenter test which was different from the center where the study was conducted. And here the specificity was somewhere around 80% and AUC of 0.76.

How does it compare with the other models which I already mentioned, Mayo Clinic model and the Veteran Affairs model which we commonly use in our clinic? So, in the training cohort, again this model, the lung tract model was superior than the Mayo and the VA model which was with AOC of 0.68. In the validation cohort also, the lung tract model had a higher AOC that was 0.81 which was comparatively higher than the other two models.

Even in the single center model also lung track score was AOC was much higher than the Mayo and the VA. So what are the strengths of this study? So this model used 54 lung tissue samples to identify the methylation patterns and the proportion of differential methylation. And then it was

It was constructed on the basis of 401 plasma samples from the five centers. So it was a multicenter study. It was trained using both clinically and pathologically defined malignant nodules. So what I mean to say is when sometimes in clinic, we usually label a lung nodule as benign or pathological non-invasively also based on the rate of the growth of that nodule and evolution over time.

Specifically, it was developed to differentiate small size nodules, that is a stage 1A, 5 to 30 mm lung cancer from benign versus malignant. And compared to Mayo and the VA model, it had better

distribution of male-female patients and benign and malignant patients. And it had better performance as well. What are the limitations? So there was some imbalance in the benign-malignant ratio of cases, that is one third were benign.

sensitivity was decreased in the multicenter cohort as I have shown earlier the AUC decreased in the multicenter cohort because the training and the validation of this methylation based assay was done and was based on a single center so it lacked the diversity

Also, we know that DNA methylation patterns are very influenced by the environmental factors. It can vary from region to region. And some cases of clinically defined malignant or benign cases may need revision over time because of temporal evolution and that can also reduce the performance of the lung tract model. So what are the opportunities ahead? So the ctDNA-based methylation patterns can be

such studies can be collaborated globally to further validate this assay over the and to improve the sensitivity and specificity of such tests. Thank you.