COVID Information Commons (CIC) Research Lightning Talk

Transcript of a Presentation by Ellen Foxman (Yale University), April 24, 2023

Title: Host response-based screening for unexpected or emerging respiratory viruses

Ellen Foxman CIC Database Profile

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Ellen Foxman:

All right, thank you so much. I hope - can I - am I good? Can everyone see the screen? Ok, great, all right. Well, thank you so much for having us all. It's fantastic to - I've been seeing these CIC emails and watching the other folks present, but it's really a pleasure to have a chance to present in the CICand be involved and reach out to this community. Today, I'd like to share some recent work from our our group on host response based screening for respiratory viruses

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I'll just start with the most important thing which is acknowledging everyone involved who made this project happen, which includes our funding from NIH and other sources. I also want to acknowledge Reddy Cheemarla and Amelia Hanron who did the screens I'm going to be talking about today as well as numerous colleagues at Yale School of Medicine and Yale School of Public Health who all work together to allow this project to happen.

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So the goal of this project was to really address the issue of surveillance for SARS-CoV-2 - well actually, surveillance for new emerging respiratory viruses. There's a new imperative and high priority to do this because of the COVID pandemic. As you all know, there's a lot of strategies already in place to look for emerging respiratory viruses and this is really important because if we can find a virus early we can develop diagnostic tests, we can develop antivirals, and

ultimately vaccines and hopefully reduce the impact of future viruses so it won't be as impactful as as this past few years of the pandemic has been.

This slide just highlights some of the current strategies. One is to look at zoonotic infections to see whether there's any viruses that have a good chance of jumping into humans, and that's very important work, as well as surveillance projects for outbreaks of pneumonia like the surveillance project that led to the discovery of SARS-CoV-2 in China. [This project] noted that there was a cluster of cases that of pneumonia with no virological diagnosis. So these strategies are very valuable but what I'm going to talk about today is an additional complementary strategy that leverages some untapped resources for emerging virus surveillance.

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So this is really based on the idea of using routine clinical specimens that we get in the course of patient care to do viral surveillance. This - a lot of tertiary care hospitals like our hospital at Yale New Haven Healthcare have a multiplex PCR panel that we use for patient diagnostics. What this is is a panel with PCR tests for many different respiratory viruses like the 10 or 15 most common respiratory viruses. Now this isn't what you're going to get if you go in the emergency room and you clearly probably have flu or COVID. You'll probably get a RAPID test or a fast PCR test for that one virus. This test is really deployed for more complicated cases where it's not clear what's going on. For example, somebody with fever, cough, shortness of breath, that has sort of non-specific symptoms. Often and you're not sure what the clinical picture is. Even at the peak of respiratory season when people like that get this test in our health care system only about a third of them turn out to be positive for viruses. About two-thirds of them aren't. And for the majority of those two-thirds they probably do not have a viral infection - they probably have something else causing their shortness of breath or their fever or so on. Hiding here, amongst the people where no virus was detected are probably a few cases of people that have a virus but they just have a virus that we can't test for on our PCR panel because the virus isn't on there. The reagents aren't on there to detect that virus. It would be great to be able to find these because some of these represent clearly viruses that we're really not thinking of as causing disease in in our patients or viruses that we don't even know about yet because they're emerging. To analyze all of these negative samples for undiagnosed viruses would be time consuming and costly and really prohibitive because we have hundreds of these per week so there's no way you could do deep virus discovery on each of these. It would just be, you know, not practical. So what we saw here is a way to screen these to enrich for samples that are most likely to contain an undiagnosed virus. You can sort of look for that needle in the haystack by just focusing in on the most important samples.

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The way that we chose to do this was to take advantage of the body's response to infection. When the airway tissue first becomes infected by a virus, it immediately initiates an antiviral response which involves secreting some antiviral proteins. In prior work from our group, we showed that some of these proteins, for example, I'll talk a lot about CXCL10 today also known as IP10 or interferon-induced protein 10. This protein is essentially one of the top secreted

proteins by nasal epithelial cells when they get exposed to a virus. We previously showed that levels of this protein - this protein elevated correlates highly with having a significant viral load in the nose of one of many different viruses as you see here. We sought to use this protein as a screen and say, okay, here we have all these people with no virus detected, but among those people who of those people are actually having an antiviral immune response in their respiratory tract. The way that we detect that is by using CSCL10 as a biomarker of antiviral defense in the upper respiratory tract. If we can screen for that we reason that these people with that biomarker are going to be the high yield samples because those are the people who are likely to have an undiagnosed infection.

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So we did two different screens. First, I'll tell you about the screen that Amelia did in January on samples from just a one single week of January 2017. This is a week where there's a lot of viruses circulating. That week we tested 359 samples on the respiratory panel and the majority of them, as I mentioned, are negative for all the viruses on the panel, which at that time was only 10 viruses now, we have more. Then we screened them for this biomarker CXCL10 and that resulted in only - out of these 251 only 60 samples had the antiviral response activated at the nose. We focused on those. But before doing virus discovery we thought, well, you know we only had 10 viruses on the panel we're missing some important ones like seasonal respiratory viruses. What if we test for those? So we did that and we found that actually half of these 60 samples were positive for seasonal coronaviruses, which sort of was a nice proof of concept of the strategy and also helped us focus in on the true unknowns which represented 32 samples. We were able to whittle down all those negative tests to only 32 samples that were of the greatest interest.

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Next, we chose to do metagenomic sequencing to look at all the nucleic acid in the samples and ask: are there any sequences from viruses there? Actually we only focused on the top 10 by biomarker level because we showed previously the biomarker level - if it's higher you're more likely to have a virus. So we did this RNA sequencing and we found that one of the samples -Sample A - had tens of thousands of reads in the RNA, in that sample, that were from influenza C virus. We felt like this is a pretty good proof of principle - proof of concept - because influenza C virus is a known viral pathogen but it's not so common and we don't usually test for it. It's not on our panel, but yet we were able to pick it up through this strategy. To prove that this virus was in the sample we inoculated primary human nasal epithelial cells with the - with the actual patient sample and we were able to observe cytopathic effect from the virus, syncytia formation, as well as that virus in these secretions of these nasal epithelial cells seven days later. So this, our first pass at this strategy worked pretty well in identifying a sample that had an undiagnosed clinically significant virus.

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The next screen I'll tell you about was - we were actually sort of wrapping up that project when COVID came along and we thought you know what I wonder if we could use this same strategy

to find undiagnosed cases of SARS-CoV-2 from the beginning of the pandemic. If you look at this graph here, the gray represents case counts in the United States in March of 2020 and then blue is New York state, which is pretty close to our hospital. Green is Connecticut and then the red is is our hospital. The bracket here shows this time between all these viruses entering the New York region and the time when our PCR test was up and running for SARS-CoV-2. In those two weeks we tested 375 samples on our respiratory panel that all tested negative. So actually there was - it was about a half or sixty percent of the samples from that period tested negative.

We thought well let's see if this biomarker would pick up any undiagnosed cases of SARS-CoV-2. We screened with the biomarker and in parallel we screened with the RQ-PCR, there's the typical PCR test for COVID.

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What we ultimately found was that there was four undiagnosed cases of COVID that we discovered by PCR as shown in these red dots here. Then this graph represents the level of the biomarker. What you can see is for the majority of samples for 92 percent of the samples the biomarker was below our cutoff threshold. For a small percentage, about, you know, eight percent of the samples, the biomarker was above that threshold. All of the COVID positive samples were in that group. So if we had just - if we had wanted to we could have done the biomarker screen first and only tested one tenth of the samples by PCR and still found all the undiagnosed cases. This was a really good way of getting a comprehensive view of how this strategy could work. In addition, the advantage of using these nasal samples for the screening is we were able to directly, with our colleagues from the Grubaugh Lab, get the sequence of those viruses and do molecular epidemiology which is their specialty in the Grubaugh Lab. What they found was that, interestingly, all these four isolates from that early time point in the pandemic were all genetically distinct. That represents that SARS-CoV-2 is coming into our health care system and into Connecticut by multiple different lines of transmission at that time. It wasn't all from one source. So that kind of gives you the bigger picture that the transmission was much more widespread than we realized by that time.

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Finally - I just don't have time to really discuss this today - but I just wanted to point you to the paper if you're interested and let you know that we did a lot more deep characterization of these unknown samples and compare them to known cases of virus positive and virus negative patients. We looked at the nasal host response to infection using RNA sequencing and cytokine profiling. You can see more subtle patterns. So mostly today I talked about elevation in one biomarker - CXCL10 - but there are actually numerous patterns that you can pick out that might be helpful in the future for developing more sophisticated host response screens and learning more about undiagnosed infections in the patient population.

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Just to summarize: the key findings of this study were that we used a single biomarker of the nasal mucosal response to viruses to enrich for samples containing undiagnosed viruses. We use

these patient samples testing virus negative and use the biomarker to highlight which samples we should further pursue. These screens diagnosed an udiagnosed case of influenza C virus as well as four undiagnosed cases of SARS-CoV-2 that were epidemiologically significant. What we're doing now is we would like to scale up this screening by making the workflows more automated and there's a bit about that in the paper. We're also interested in collaborating with people who might have samples from different geographic regions because obviously in different regions you may have different undiagnosed viruses circulating. We're also interested in using these nasal immunophenotyping to help further parse what undiagnosed infections are going around in in our patient populations.

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So with that, I'll conclude and I'd be happy to take any additional questions at the end of this - at the end of everyone's talk. So thank you very much for your attention.