

# Endotheliopathy and Platelet Dysfunction as Hallmarks of Fatal Lassa Fever

*[Announcer] This program is presented by the Centers for Disease Control and Prevention.*

[Sarah Gregory] Hello, I'm Sarah Gregory, and today I'm talking with Dr. Brian Sullivan, a researcher and instructor at La Jolla Institute for Immunology. We'll be discussing endotheliopathy and platelet dysfunction in Lassa fever.

Welcome, Dr. Sullivan.

[Brian Sullivan] Hi Sarah. It's great to be here.

[Sarah Gregory] Endotheliopathy and platelet dysfunction are outcomes of Lassa fever. Start with telling us what Lassa fever is.

[Brian Sullivan] Lassa fever is a disease that's caused by the arenavirus, Lassa virus.

[Sarah Gregory] How many people get it annually?

[Brian Sullivan] Well, it's been estimated that 300–500,000 people get it annually. And it's interesting because it really has been an understudied disease, so we...we don't really have a good grasp of how many people actually have it. But it covers an area of West Africa where about 300 million people live, and we know that the seropositivity in many places is quite high. So we think it's pretty widespread.

[Sarah Gregory] Explain to us what endotheliopathy and platelet dysfunction is.

[Brian Sullivan] Sure, I'd love to. So, the endothelium is the barrier that lines our blood vessels and has very important functions. And when these functions are disrupted, you get a disease of the endothelium, and that's what we call endotheliopathy. And so during these viral infections (such as Lassa fever), you disturb that endothelial barrier and it becomes porous for various reasons. Platelets are also very important in maintaining this barrier function. As you know, I think the most famous part about platelet function is the ability of your blood to clot. So everybody knows that they get a scratch, that when they start bleeding their blood clots and forms a barrier to the outside. Well, platelets have that function as well as many other functions to maintain that blood vessel system. And they also have very important functions in immunology that I think we're just really beginning to understand.

[Sarah Gregory] What are the symptoms Lassa fever and is it fatal?

[Brian Sullivan] So, the symptoms can vary quite a bit. And I think that's one of the problems with Lassa fever in West Africa, is that the disease mimics many of the diseases that are endemic in the area. So, many people would think of these symptoms as flu-like symptoms (malaise, fever), but once the disease progresses you can have very specific symptoms like bleeding from the mucosal areas, edema (which is swelling) which is when you have liquid going into various tissues, you would have a swelling of the neck area, and sometimes in the very severe cases you'd have neurological symptoms like confusion and disorientation. And it certainly is fatal. Many people are likely infected with Lassa virus and only a small subset of those people show these very severe symptoms. But among those people, it's very fatal. Anywhere between 30 and 70% of people that show these more severe symptoms and go to the hospital in West Africa end up dying from them...from the disease.

[Sarah Gregory] That's actually a huge number. Dr. Sullivan, how is it transmitted?

[Brian Sullivan] Well, most times the disease is transmitted from a rodent to human. So, rodents will invade a person's house and the rodents would, you know, make a mess, urinate or...or defecate on...on various items in their house and that would end up causing the person to inhale some of this, what we call "excreta." And so, it can also be passed from human to human, but most of the time that happens within a household with somebody who's very ill or within a hospital setting (what we call nosocomial infection). But most of the time it's...it's from a rodent to human transmission. Which actually makes it very hard to eliminate because those rodent hosts are pretty widespread throughout West Africa.

[Sarah Gregory] And widespread in people's houses.

[Brian Sullivan] Right, yes.

[Sarah Gregory] Is there a treatment or a vaccination?

[Brian Sullivan] So there's no treatment or vaccination to Lassa fever. Currently, there's different vaccines being tried out and developed in the coordination with the Center for Innovation Preparedness. But there's no currently licensed vaccines available. As for treatments, there's no accepted or licensed treatments available either, although some people have used Ribavirin, which is an antiviral. But it's an off-label use, and really its efficacy hasn't been thoroughly tested. We...we think that at the time that most people arrive at the hospital sick with Lassa fever, that Ribavirin really isn't effective because it has to be given pretty early on in the infection for it to be effective.

[Sarah Gregory] Which is true of all antivirals, they usually need to be within at least 78 hours, correct?

[Brian Sullivan] Right, yes. That's true.

[Sarah Gregory] Why is Lassa fever classed by CDC as a bioterrorism agent and...and what does that actually mean?

[Brian Sullivan] Well, the CDC classifies bioterror agents under certain criteria. So, certainly diseases that can be easily disseminated or transmitted from person to person (which certainly Lassa is, under certain conditions). When diseases result in high mortality rates, you know, that would have a potential for public impact. Certainly diseases like Ebola would certainly fit into that...into that realm. But Lassa is a very similar virus, although it doesn't spread from person to person as easily. And...and diseases that could cause public panic or some...some kind of social disruption and would require a special action for public health. It's also important to note that as we had mentioned, there's no treatment or vaccine for this disease as well, which is really makes it a strong contender for a...for a bioterrorism agent and to really cause panic among population if it was to disseminate more widely.

[Sarah Gregory] So, it's not like it can be gathered like anthrax and put in people's mailboxes.

[Brian Sullivan] Right. It certainly can't be disseminated like that. I mean, the virus itself is not...it's not a very robust virus physically. So, you know, it will only last on surfaces for...for several hours most likely. But certainly the disease can be spread from person to person and it causes pretty substantial symptoms that...you know, like...it's very similar to Ebola, which does certainly cause a lot of panic in people (as it...as it should because of its high mortality rate).

[Sarah Gregory] Right, yes. What time period did your study cover?

[Brian Sullivan] Well, we used samples from the viral repository at the Kenema Government Hospital in Sierra Leone. And that viral repository holds samples from...since the end of the civil war there, 2003. But we limited our samples to more recent samples (from around 2016–2018) just to get...make sure that our samples were the best samples available and the freshest we could use.

[Sarah Gregory] What geographic locations is it endemic in? Besides...I mean, you said West Africa, but that's a pretty large area.

[Brian Sullivan] Sure. So, when I...when I say West Africa, I mean the entire area covering from Nigeria all the way west to Sierra Leone and Guinea. The most widely studied regions for Lassa fever are Nigeria and Sierra Leone and Liberia. However, it's been more widely studied now (the area between Sierra Leone and...and Nigeria). Those gaps of countries also have Lassa fever endemic in those populations. As I said before, there's about 300 million people in that...in that general area. Lassa fever is really mostly endemic in the rural parts of West Africa. So we're talking about smaller towns and villages, not necessarily in cities. Which actually makes it much harder to study when many of these places do not have the healthcare infrastructure that you need to study Lassa fever.

[Sarah Gregory] Is this the same area where we see Ebola?

[Brian Sullivan] So this is the same region where the West African Ebola outbreak took place. Many of the other Ebola outbreaks (which take place in Central Africa and Uganda) are in different regions where Lassa isn't endemic. But there are also other similar viruses endemic in that area that I think are understudied as well.

[Sarah Gregory] That must be very challenging to separate out initially...which bloodletting fever you have.

[Brian Sullivan] Right. Well, the...the problem is those symptoms are so common to malaria (which is very common in the same area) and typhoid fever (which is also common), that everything else gets tested before Lassa fever does. And there's not a very good recognition of the impact of...of Lassa fever in West Africa because there just isn't enough testing done.

[Sarah Gregory] Help us to understand what this means for public health.

[Brian Sullivan] Sure. So, our colleagues have shown that through sequencing of the...of the virus in different parts of West Africa that actually in the last few hundred years, Lassa fever has been on the move. It seems to have originated in Nigeria thousands of years ago, but actually only more recently moved west towards Sierra Leone and Liberia and Guinea in about the last several hundred years. So we can imagine impacts of...of people movement impact, of further spread of human population, impacts of climate change...might lead to this rodent host spreading further in different areas of the world. So, certainly understanding Lassa fever and its rodent host would be important if Lassa fever...especially if Lassa fever is spreading throughout other places in Africa, or ended up spreading to other areas around the world as well.

[Sarah Gregory] Is there an economic impact of this disease? I would imagine there is.

[Brian Sullivan] So, certainly I think there is. The economic impact is unclear because the...it just hasn't...Lassa fever just hasn't been studied very thoroughly. Much more epidemiology has to be done with this virus. And some is really starting to be done with recent surveys in Nigeria and Sierra Leone. The Nigerian CDC has really done an excellent job in...in promoting and making people aware of Lassa fever, and now many more surveys (or serosurveys) are being done in different areas in Nigeria to understand really how widespread Lassa fever is. Certainly this is also happening in Sierra Leone, and we have learned that some towns have much higher seropositive rates than other towns. So, so far the economic impact is unclear. But, you know, this disease also affects people of all ages. So once somebody gets Lassa fever, they could be young, they could be middle-aged, they could be older, and certainly that would affect their ability to...to produce economically with both a high fatality rate, but

also that people that even survive with Lassa fever also have a post-viral syndrome that causes them to have much more health difficulties in their life following...following survival. So this is very similar to what people have been seeing in...in coronavirus (COVID) survivors (who...who they call "long haulers") who have problems even much later after they have cleared the virus and have been released from the hospital. There's...there's definitely an economic impact, I think, from...those individuals as well.

[Sarah Gregory] Yes. The longterm economic impact of people (as you said "long haulers") from COVID is...is truly worrisome. Why did you do this study?

[Brian Sullivan] Well, we had been interested in Lassa fever for a while. And...and when a very talented medical fellow—an infectious disease medical fellow—in our lab (Lucy Horton) came...came to study with us, she was interested in why people haven't really studied...this is termed as a viral hemorrhagic fever, but people haven't really studied what's causing this hemorrhage or...or endothelial disruption. And so she had noticed that really maybe the term hemorrhagic fever isn't very accurate, because many times you don't see people having overt hemorrhages. What you see are signs of endothelial dysfunction. So, people will have coughs and that's because fluid is filling up in their lungs, people will have swollen areas like I had mentioned before (edema), but...and some bleeding maybe from mucosal surfaces but not a very overt bleeding in many...many cases. So, she was...wanted to understand what are the changes that occur in people that survive Lassa fever and people that succumb to Lassa fever. And maybe by studying those two populations, we could understand a little bit more of the mechanisms of...of what is occurring in these most severe cases where you see such high impact on people's endotheliums.

[Sarah Gregory] How did you go about doing the study? I mean, what kind of data did you use?

[Brian Sullivan] Sure. So we used, like I mentioned, we used plasma samples from patients that were stored in the viral repository that had been admitted to the Lassa fever ward in Sierra Leone. And it was important that we had used time-of-admission samples, because then we can distinguish...we...we could ask questions of whether we could distinguish between fatal and non-fatal cases, and we could ask what is going on in severe cases, and how would we begin to think about treating those individuals that have the most severe cases so we can start lowering the case-fatality rate. And so, what we did was looked at various proteins that were present in the plasma of people that had been admitted to the ward—proteins that were important in coagulation, proteins that were important in endothelial barrier disruption, and proteins that were important in figuring out what's occurring with...with platelets that, of course, are essential for maintaining the endothelial barrier.

[Sarah Gregory] Do you want to take a moment to tell us briefly about your study?

[Brian Sullivan] Sure. So, we...we analyzed plasma samples, like I said, to identify differences in these circulating proteins that are different in Lassa fever, specifically different in people that have died and people that survived from Lassa fever. We compared these to healthy controls and people with...with other similar diseases that weren't Lassa fever so we could understand what are the specific differences that occur in Lassa fever, and then what are the specific differences in people that survive and people that die from Lassa fever.

Again, these proteins that we looked at are involved in coagulation (which is the ability of blood clots to come together and block any disruption in the endothelium), proteins that are important in fibrinolysis (which is the resolving of those clots, the breaking up of those clots after they've done their job), and in endothelial cell activation. Again, that endothelium is both a barrier on your blood vessels but it also has

many other functions—letting different immune cells into tissues, signaling to immune cells that there's been an infection or disruption in the barrier. So, the endothelial cells are very important. And we thought that we'd see differences in people with very severe cases as compared to people with milder cases of Lassa fever.

And then, we were certainly interested in looking at platelet function. We knew that platelet numbers go down in people that have Lassa fever, but they don't go down so dramatically that you're still not able to, let's say, clot your blood if...if you get a cut. So, we call this overt hemorrhaging. So we didn't see platelet numbers so low that...that would have happened. So, we thought that maybe there was a deficiency in the platelets themselves.

So, this is all tied together in a very complex system—the coagulation, the fibrinolysis, the endothelial cell activation, and the platelet function. And really I think we're just scratching the surface of what's going on. But it's important to really start answering these important questions so when we can further develop hypotheses in mouse models and nonhuman primate models and in humans to really understand what's going on on the molecular level.

[Sarah Gregory] Were there any other findings you want to mention?

[Brian Sullivan] Sure. We found that a specific pathway called the protein C pathway wasn't able to function to protect the endothelial integrity. And this is a very interesting pathway that has several different functions—it has an anticoagulation function and it has a cytoprotective function. And these functions are mediated depending on the state of the receptors of endothelial cells. So, we observed that certain receptors on endothelial cells have been cleaned off, and these receptors are important in the cytoprotective pathway that is protecting these endothelial cells that maintain the vascular barrier.

There was a study done a few years ago where people had given an activated protein C to nonhuman primates that had Ebola infection and showed an increase in survival. We think that might not be able to happen here, because those receptors that are so important in that signaling pathway that protects the endothelium are likely no longer on these endothelial cells in the most severe cases. So, that gives us one window of understanding what might be lacking in these...in these individuals that have very severe cases of Lassa fever.

[Sarah Gregory] Is there a way to stop cases from increasing, like public outreach or public education programs? Is there anything that can be done?

[Brian Sullivan] Sure. And I think education is...is key here and I want to give special recognition to the outreach team in Sierra Leone that have been doing this for many, many years in the Lassa fever program. They have been going to different towns and villages, and talking to people about Lassa fever and what can be done to avoid rodents in their houses (so to clean up areas or protect areas that might have food that might attract rodents). And...and this is really essential to both educate people that Lassa fever is out there so that if they get sick they can seek the proper treatment, and also to mitigate them getting infected in the first place.

Again, I think much more epidemiology has to be done on Lassa fever to really understand how most people are getting infected. It seems that some studies have shown that because of the thatched roofs in many people's houses, that the rodents can run across the ceiling and the roof and perhaps drop...drop their droppings down into the living areas, and maybe that's how people are getting infected. But we really don't understand how most people are getting infected, other than they're getting infected from these rodents. Certain studies have shown that the level of infection of Lassa fever in any particular town

is directly correlated with the number of rodents that have Lassa fever. So, there's a very direct correlation with the presence of these rodents and the rodents that have Lassa fever and the...and the infections that people are getting. So if we can figure out a way to stop these rodents from...from encroaching into people's homes, I think that would be the most effective to try to avoid getting Lassa fever.

[Sarah Gregory] Were there any challenges to this research? I imagine there were.

[Brian Sullivan] Certainly when you're working in environments that don't have as many resources as developed countries, there's always challenges. But rather than focus on the...on the challenges, I just want to say what a pleasure it was to work with our African partners and the expertise that they had in both treating Lassa fever and identifying patients with Lassa fever and doing community outreach and education, certainly the people that we worked with in Sierra Leone, experts in identifying the various rodents that could carry Lassa fever. And they have actually shared this knowledge with other individuals in Nigeria and trained people across West Africa. So while there's certainly challenges—even getting to these areas are sometimes very difficult in the rainy season and the conditions of the infrastructure, challenges in...in regular access to electricity is always challenging—but we were able to overcome these challenges mainly because of the expertise of our African collaborators.

[Sarah Gregory] Are there any actions or further studies that you would like to see? I know you said that it needs lots more epidemiology and studies along that line. Can you elaborate a little bit?

[Brian Sullivan] Sure. So, certainly there's...there's a lot to learn when it comes to Lassa fever. In terms of furthering our study, we'd certainly like to see studies in nonhuman primates exploring some of the avenues that we've identified as potential mediators of this endothelial barrier dysfunction. And again, as you mentioned, certainly many more epidemiological studies need to be done. The highest risk is among pregnant women and their...and their unborn babies where nearly all of pregnant women that are infected lose their...lose their pregnancy, and it's a very high maternal mortality rate if pregnant women are infected in their last trimester. So the impact of Lassa fever, I think in this population, has specifically been understudied. These are areas (where Lassa fever is endemic) where you have the highest maternal mortality rate. And certainly I think part of the reason might be the poor healthcare infrastructure, but the impact of Lassa fever in that case hasn't really been studied. And I think if a population such as that were identified, it would actually be a lot better for...it would be a good target population for...for vaccination, and certainly that has a very big healthcare impact.

Also women who have Lassa fever when they give birth, they pose a specific hazard to healthcare workers as well. Because of all the fluids that are generated during pregnancy, it could be easily passed to...to healthcare workers. And there's certainly been many cases that have occurred in Kenema of pregnant...of infected pregnant women having stillbirths in...in the maternal wards there and infecting some of the healthcare workers.

[Sarah Gregory] Do you have any additional recommendations to help people not get Lassa fever other than looking into ways to keep rodents out of houses?

[Brian Sullivan] Right. Certainly I think keeping rodents out of houses is the most important part, but washing your hands, cleaning areas...other people in South America where they have similar arenavirus infections have used people that are immune because they had been previously infected by these arenaviruses to collect rodents on the outskirts of town to keep the numbers of rodents in the town minimally...minimally present. So certainly, I think programs like that could help rodent trapping programs, surveillance programs from the various public health units that are present in these areas to

understand what is the impact or the potential impact of infection on each of these smaller towns and villages.

[Sarah Gregory] So if you have it, you're immune then. You don't get it again, like...like West Nile or something where you can just keep getting it.

[Brian Sullivan] Right. So, if you're...if you've had it before, it doesn't mean you can't be infected again. But you won't have the severe disease again. The immunity that you get from Lassa is not what you call a "sterilizing immunity." So, you can get infected again and the virus will replicate a little bit in your body, but you won't get the severe disease that you did get the first time.

[Sarah Gregory] I see. Ok, so tell us about your work and what you enjoy most about it.

[Brian Sullivan] Well certainly, like all scientific work I think the thrill of discovery of learning something new that nobody else has learned is really...I think the biggest part of it. But also, with the studies (this and various other studies) we've done in West Africa, just working with the people in these regions, learning from the experts there that have been working with Lassa virus for...just dozens of years has really been very rewarding. And hopefully, we're going to start seeing an impact of this work on both patient treatment and perhaps with...with vaccines hopefully being developed, that Lassa fever won't have such a great impact in the future than it does today.

[Sarah Gregory] You live in a fabulously beautiful part of the country—southern...southern California (La Jolla, San Diego). What do you do...what do you enjoy doing in your personal time there?

[Brian Sullivan] Well, you know, in the pandemic we've spent a lot of time in our houses and I think that we're lucky down here as we have a lot of natural beauty. So, my family and I have certainly gone hiking several times which is a, you know, good...good social distance activity outdoors to enjoy the beauty of both the mountains and...and the beach and ocean areas here. We definitely enjoy a lot of outdoor hiking and camping activities.

[Sarah Gregory] Well thank you for taking the time to talk with me today, Dr. Sullivan.

[Brian Sullivan] Thank you so much. It's been a real pleasure.

[Sarah Gregory] And thanks for joining me out there. You can read the November 2020 article, Endotheliopathy and Platelet Dysfunction as Hallmarks of Fatal Lassa Fever, online at [cdc.gov/eid](https://www.cdc.gov/eid).

I'm Sarah Gregory for *Emerging Infectious Diseases*.

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