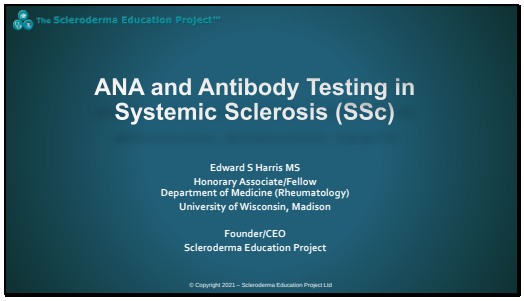
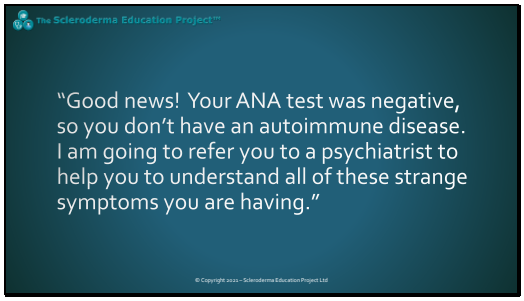
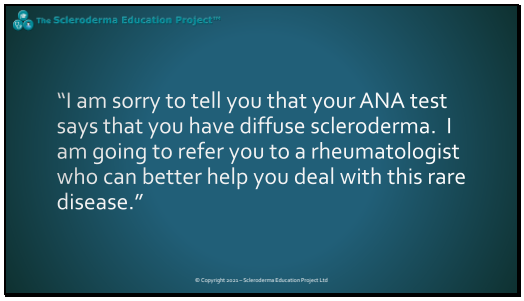
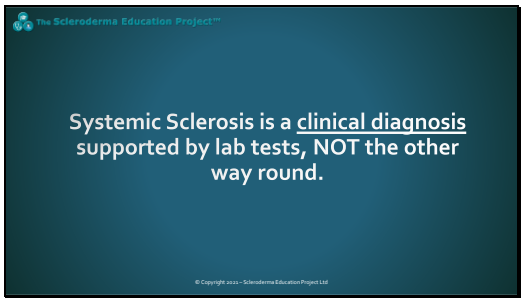
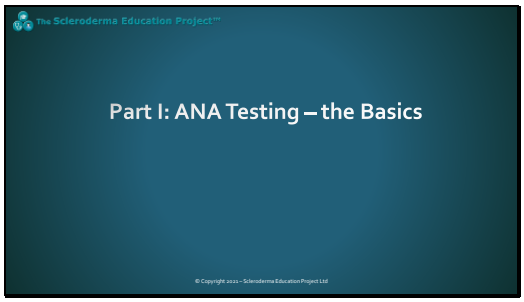
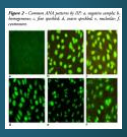
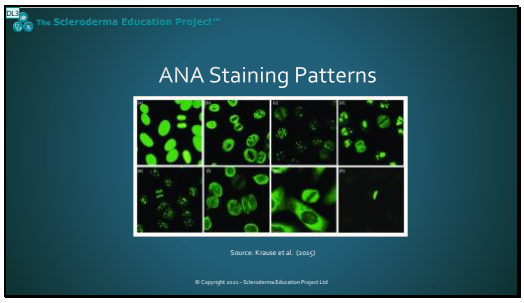
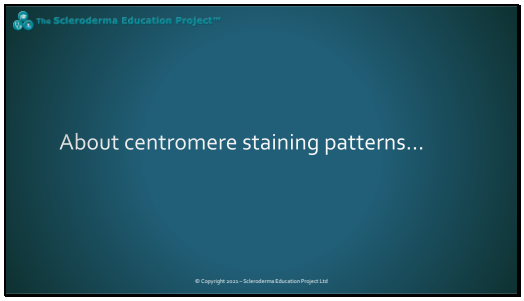
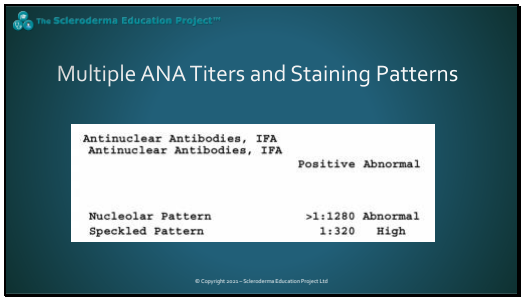
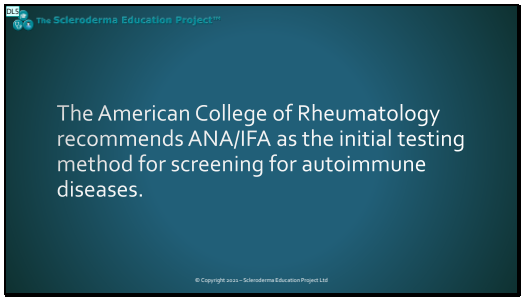
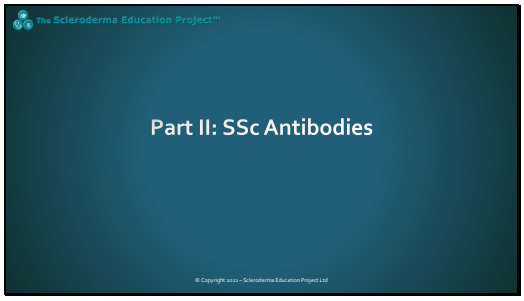
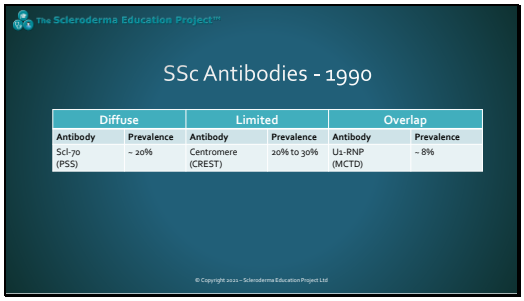
ANA and Antibody Testing in Systemic Sclerosis

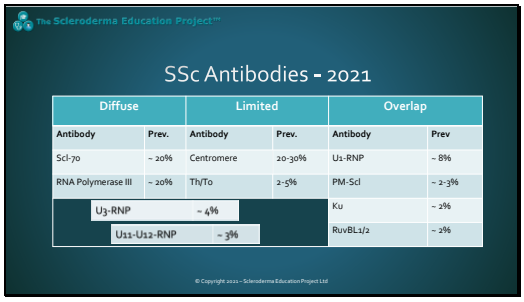
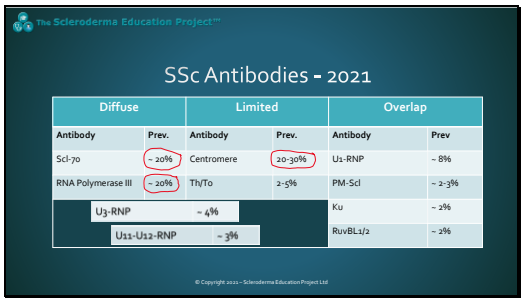
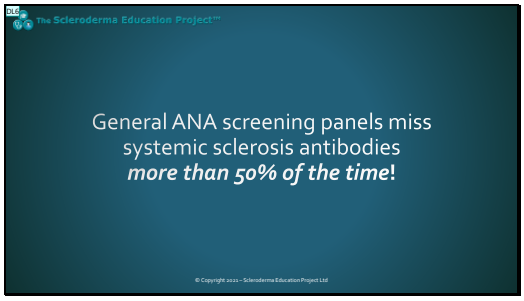
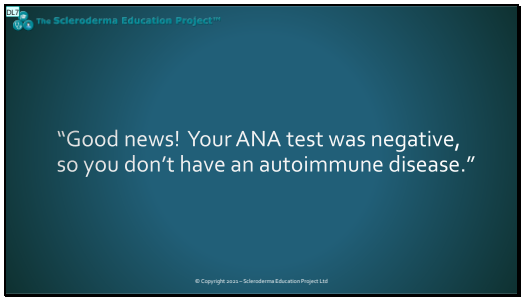
Edward S. Harris, MS

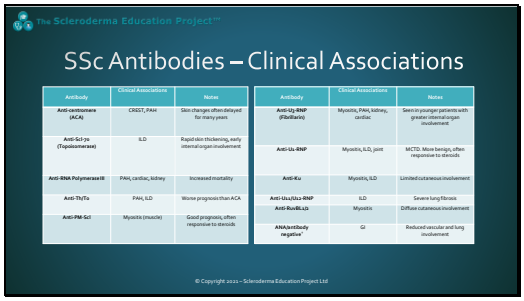
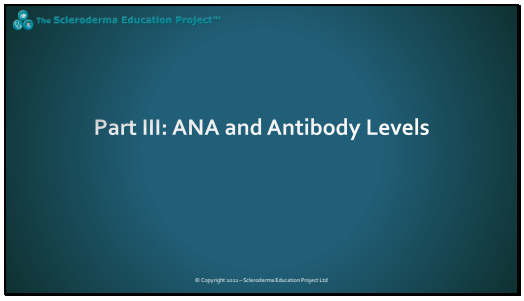
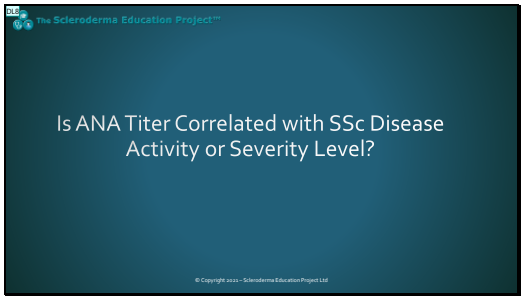
1		The topic of this talk is ANA and antibody testing in systemic sclerosis. In addition to covering the basics of ANA testing, we will also examine the role of ANA and antibody testing in systemic sclerosis diagnosis and treatment. When this presentation is posted on YouTube, there will be a link to a handout version of the presentation that will include detailed notes that you can refer to later. One important disclaimer. Some of the information presented here is US focused and may not be completely applicable in other countries where different testing methods are routinely used.
2		About seven years ago, I started writing a series of articles about ANA and antibody testing. What led to my doing this was frequently seeing two types of comments in patient support groups that greatly concerned me. This is the first type of comment. In many cases, when a clinician says something like this, correct diagnosis may be delayed for several years.
3		This type of comment is in some ways even more concerning. After the patient goes home in total shock, she will probably do a Google search for "diffuse scleroderma" where she will "learn" that she has a horrible, fatal disease and has only about five years to live. By the end of this talk, my goal is for you to understand exactly why both of these comments may be completely incorrect and should never be uttered by clinicians.
4		Before we start learning about ANA and antibody testing, I want to emphasize that systemic sclerosis is a clinical diagnosis supported by lab tests, NOT the other way around. This is the most important slide in this entire presentation.
5		Antinuclear antibodies are a type of antibody that attack the nucleus of a cell. These types of antibodies are usually, but not always, present in autoimmune disorders such as lupus, Sjoegren's, or systemic sclerosis.

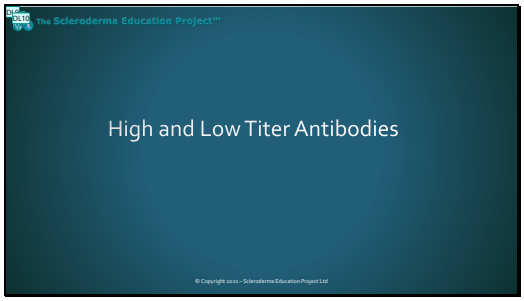
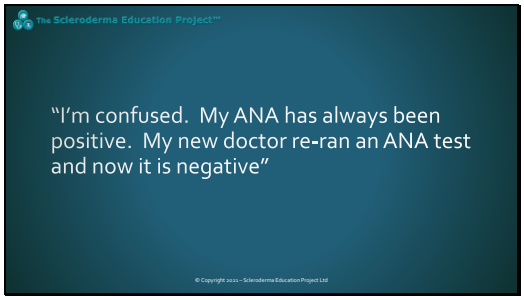
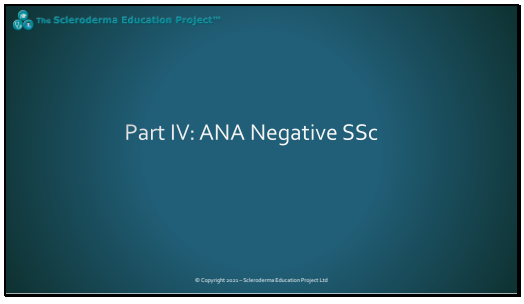
6	The Scleroderma Education Project™ Why do ANA / antibody testing for SSc? • Most of the time, it helps clinicians to make the correct diagnosis • Correct antibody identification helps to understand likely disease risks and complications © Copyright 2021 – Scleroderma Education Project Ltd	Correctly done ANA testing is very helpful in formally diagnosing systemic sclerosis. More than 90% of patients will have a positive ANA when ANA testing is done correctly. While sometimes very challenging to do, in most patients it is possible to identify the specific antibody that leads to a positive ANA result. As will be discussed shortly, correct antibody identification can be very helpful to the clinician by suggesting potential risks and complications, as well as having a role in developing the best possible treatment plan for the individual patient.
7	The Scleroderma Education Project™ ANA Testing by Indirect Immunofluouresence (IFA / IIF)   Source: Chauhan et al. (2019) © Copyright 2021 – Scleroderma Education Project Ltd	The "gold standard" method for doing ANA testing is called indirect immunofluorescence and is commonly abbreviated IFA or IIF. It is a time-consuming manual process. An ANA test done by IFA can detect the presence of up to 150 different antibodies but does not tell you which specific antibody or antibodies were detected. The main two results of an ANA/IFA test are called Pattern and Titer. Pattern is the way antibodies appear on the slide and Titer is a measure of the level of antibodies in the blood. The higher the titer, the higher the likelihood that the result is significant. This is in part because a significant number of people in the general population, especially older people, have low positive ANA titers that do not appear to be associated with any disease. The titer number indicates the degree to which the patient's blood sample can be diluted and still produce recognizable staining. In the US, initial testing is typically done with a dilution of 40 to 1 and is written as a two-part number such as 1:40. If no staining patterns are visible at this initial 40 to 1 dilution level, the ANA result is negative. However, if a staining pattern is seen, the dilution is doubled, and the technician again looks for a visible staining pattern. This means that possible ANA titers follow a pattern sequence, always starting at 1:40 and then doubling, so higher ANA tiers are 1:80, 1:160, 1:320, 1:640, etc.
8	The Scleroderma Education Project™ ANA Titers "My ANA doubled from 1:160 to 1:320! I am scared that my disease is getting much worse!" © Copyright 2021 – Scleroderma Education Project Ltd	Patients post comments like this all the time in support groups. It is important to understand that normal testing variance for ANA titers is plus or minus one titer level. This means that if your "real" ANA titer is 1:160, ANA/IFA testing of the same blood sample is likely to sometimes be either 1:80 or 1:320 in addition to the "expected" 1:160 result. In our example here, the 1:160 and 1:320 ANA titers are considered to be the same. If the ANA titer had changed from 1:80 to 1:640, that would be considered a significant change in titer level.

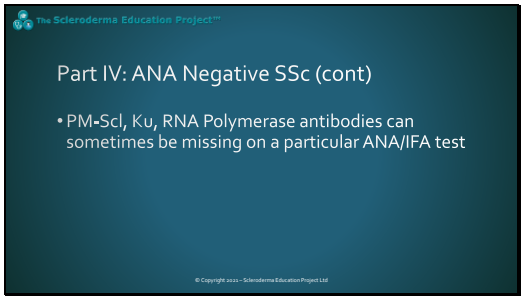
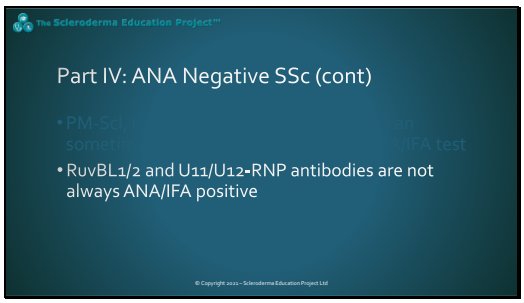
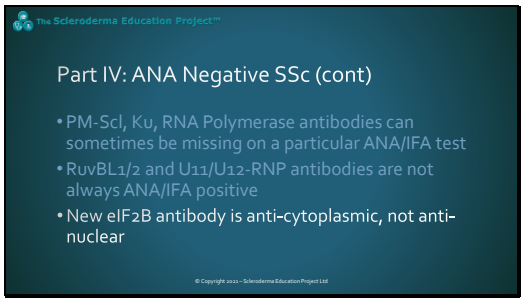
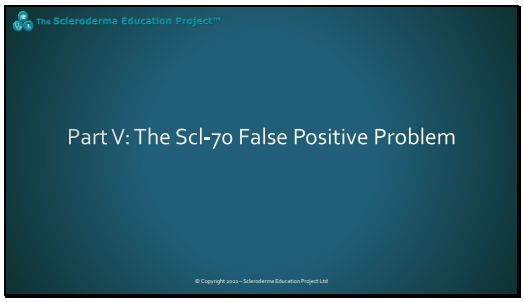
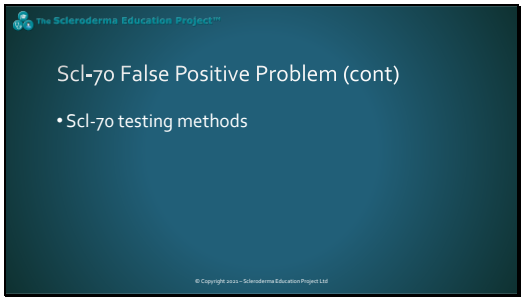
9		In addition to the ANA titer, a positive ANA/IFA also has a staining pattern. The four main types of staining patterns seen in systemic sclerosis patients include speckled, homogeneous, nucleolar, and centromere, and these are universally reported. While ANA staining patterns may suggest a possible type of autoimmune disease, in practice there is limited agreement among laboratories as to which additional ANA staining patterns should be identified and reported to clinicians. Therefore, it is recommended (e.g., International Consensus on Antinuclear Antibody (ANA) Patterns / ICAP), that a positive ANA/IFA test should always be followed up by detailed, specific antibody testing. The exact type of antibody testing depends on the patient's symptom profile, so if the clinician suspects lupus, they should order a different antibody panel than if they suspect systemic sclerosis.
10		One final point on ANA staining patterns. As noted earlier, ANA/IFA testing can detect the presence of up to 150 different antibodies. Of note, one staining pattern, centromere, is highly correlated with the presence of centromere antibodies. In fact, many research papers use a centromere staining pattern as sufficient criteria for indicating that the patient has centromere antibodies. However, some experts suggest that even with an ANA/IFA centromere staining pattern a clinical profile consistent with centromere antibodies, a follow-up centromere antibody test should be done to verify the staining pattern.
11		Just to complicate things, it is not uncommon to see ANA/IFA results showing two and occasionally three separate ANA titers and staining patterns, as in this example. What this means is that more than one autoantibody has been detected by the ANA/IFA test. Detailed antibody testing will often show which antibodies triggered this result.
12		In recent years, the standard method of doing ANA testing has started to change. Three alternative ways of doing ANA testing are now commonplace: solid phase immunoassays (ELISA or EIA), line immunoassays (LIA), or a related technique known as a Multiplex bead array. These new methods are faster, cheaper, and are generally very accurate. Unfortunately, they also introduce significant major problems – especially for patients with systemic sclerosis.

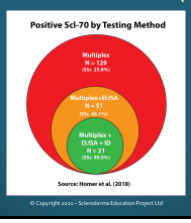
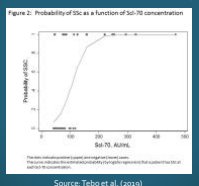
13		A few slides ago, I mentioned that ANA testing by IFA detects the presence of up to 150 different antibodies but doesn't tell you specifically which ones. In contrast, ELISA, LIA, and other Multiplex assays test for a limited number of specific antibodies, typically 12 or fewer. If you happen to have one of the antibodies that a particular ANA screening panel includes, the test will reliably detect the antibody. Most ANA screening panels are focused on more common autoimmune diseases than systemic sclerosis, primarily lupus and Sjogren's, and they do a very good job of detecting the most common antibodies in these diseases. However, when it comes to systemic sclerosis, it is a different story.
14		Here is the bottom line: because of the problem of ANA screening panels potentially missing antibodies for rare diseases, in 2011 the American College of Rheumatology issued a position statement recommending that initial ANA screening for the presence of autoimmune diseases should always be done by ANA/IFA testing, especially if the patient's symptom profile doesn't suggest a specific disease (van den Hoogen et al. 2013). However, if testing is being done for a specific autoimmune disease, screening by solid phase screening assays such as ELISA or Multiplex are often as accurate as ANA/IFA testing and, in some cases, can detect antibodies that can be missed by ANA/IFA testing, as will be discussed later.
15		If systemic sclerosis is the suspected diagnosis and ANA/IFA testing is positive, detailed antibody testing is the next step.
16		Let's go back 30 years for a moment. In 1990, only two antibodies, Scl-70 and centromere, were commonly screened for in cases of suspected systemic sclerosis, although researchers had identified other antibodies that were SSc specific, such as RNA Polymerase III and U3-RNP. One key difference between patients with Scl-70 and centromere antibodies was the degree of skin involvement. Scl-70 positive patients tended to have diffuse skin involvement potentially involving most of the body. In contrast, in patients with centromere antibodies who had skin involvement, the body areas were more limited, typically only including the face and lower limbs: hands up to the elbows and feet up to the knees, but not areas like the trunk or upper limbs. A third related disease, Mixed Connective Tissue Disease (MCTD), included many symptoms seen in systemic sclerosis but also symptoms commonly seen in lupus, rheumatoid arthritis, and myositis. MCTD is associated with U1-RNP antibodies, typically with a very high speckled ANA titer.

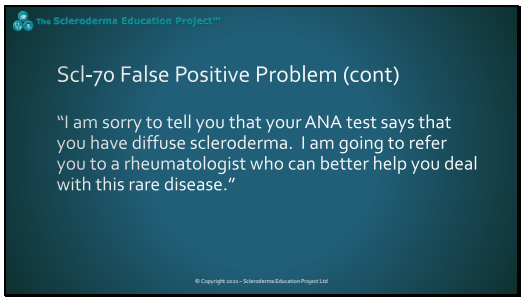
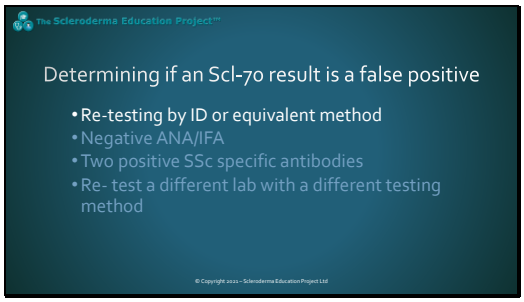
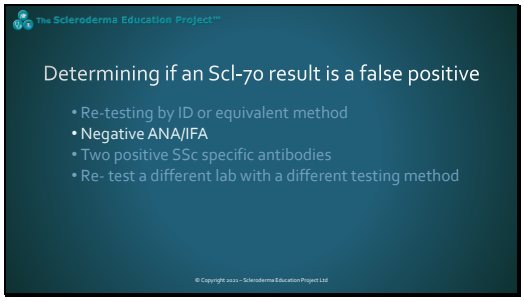
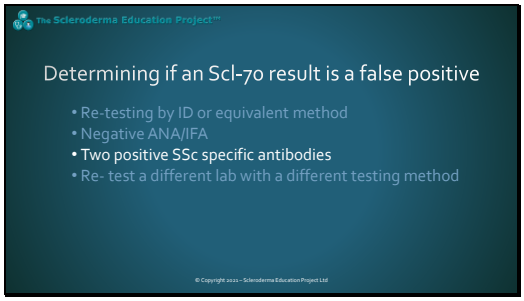
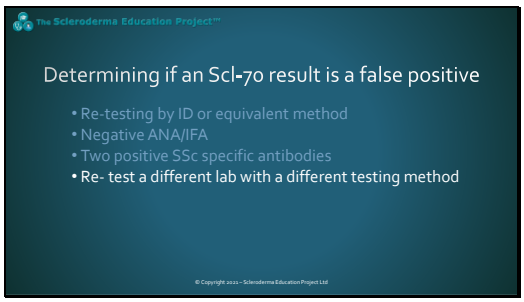
17		Fast forward to today. Now, most researchers include about ten antibodies in the systemic sclerosis family of diseases, and things are more complicated. Most of the antibodies tend to fit nicely into three general categories: diffuse, limited, or overlap syndrome. But, while patients with U3-RNP (fibrillarin) antibodies develop diffuse skin changes, several studies have shown that this is not universal (Tormey 2001, Sharif 2011). And, in the case of one of the newer discovered antibodies, U11/U12-RNP, about half of the patients have diffuse and half limited skin changes (T Medsger, personal communication, Dec 5, 2016).
18		Let's take a look at the antibody prevalence rates in this table. If you just look at the three most common antibodies in total, centromere, Scl-70, and RNA Polymerase III, this represents only 60 to 70% of the overall systemic sclerosis patient population.
19		All general ANA screening panels that I am aware of in the US include Scl-70. Some add centromere and occasionally U1-RNP, but none include RNA Polymerase III antibodies, which are about as common as Scl-70 antibodies. This means that depending on the particular ANA general screening panel, if a patient has systemic sclerosis, between 50% and 70% of the time, a general ANA panel will have a negative result. That leads to comments like this:
20		Initial ANA screening for a suspected autoimmune disease is often done by a primary care clinician. In many medical facilities, when the clinician orders an ANA test, what is generally done is an ANA screening panel using either ELISA or Multiplex testing rather than ANA/IFA testing. As you just learned, general ANA screening panels commonly used in the US miss systemic sclerosis antibodies up to 70% of the time. Since many primary care clinicians have no formal training in these complex ANA testing issues, it is not uncommon for an untrained clinician to incorrectly interpret a "negative" ANA panel result as indicating that the patient does not have any autoantibodies and therefore is very unlikely to have an autoimmune disease.

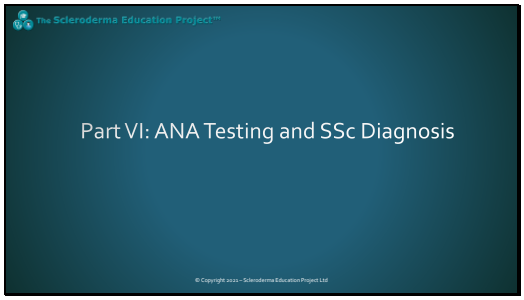
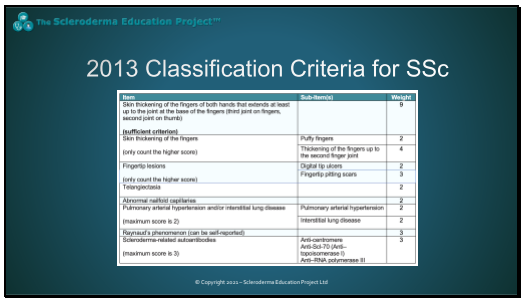
21		Over the years, we have learned that while patients vary widely on an individual basis, each antibody has its own unique clinical disease associations and specific risk profiles. This can be very important for managing patients. For example, patients with RNA polymerase III antibodies have a very high risk for developing scleroderma renal crisis (SRC), up to 35% in some studies. Because of this, these patients should monitor their blood pressure daily looking for a sudden spike in blood pressure that persists for several hours, as a sudden spike can be a leading indicator of developing SRC. In addition, patients with RNA Polymerase III antibodies should generally not be treated with other than very low doses of prednisone, as higher doses can trigger scleroderma renal crisis. In contrast, prednisone is often used to treat patients with some of the overlap syndromes such as Mixed Connective Tissue Disease since the risks of developing SRC are very low.
22		One question that systemic sclerosis patients are often confused by is whether there is any clinical significance to their ANA and antibody level, for example, does the fact that their ANA titer is 1:1280 mean that they have a more active disease than someone else who has an ANA titer of 1:160. In reality, in many cases, it is the exact opposite.
23		If you do a Google search for the question on this slide, you will quickly find that respected resources such as Medscape indicate that in patients with systemic sclerosis, ANA titers are not at all correlated with disease activity or severity, thus there is usually no clinical need to repeat ANA and specific antibody testing once the levels have been established. While this may be true in general, at least in the case of Scl-70 antibodies, there are a few papers that suggest the opposite, although the research has been mixed. A study published in 2003 (Hu et al. 2003) showed a positive correlation between total skin thickness scores and antibody levels in a group of 11 patients with Scl-70 antibodies. They also found that when antibody levels changed in 8 of the 11 patients, the changes correlated with changes in skin thickness scores. A more recent much larger study (Hasegawa et al. 2013) also found significantly positive correlations between antibody levels and total skin thickness scores. Several other studies have also shown this same correlation with skin scores. However, antibody levels did not correlate with other measures of disease severity such as lung involvement.

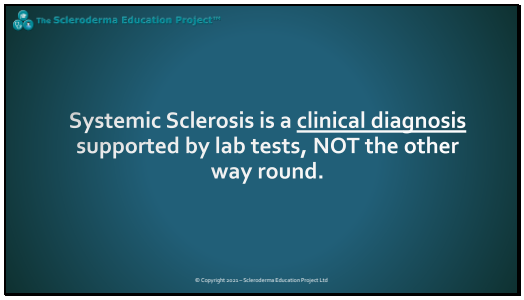
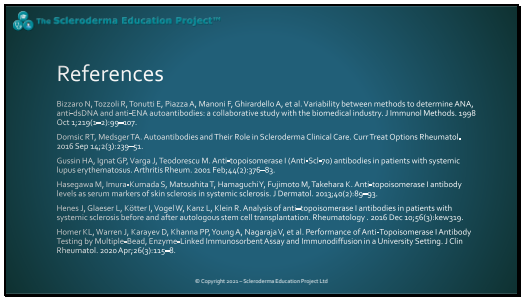
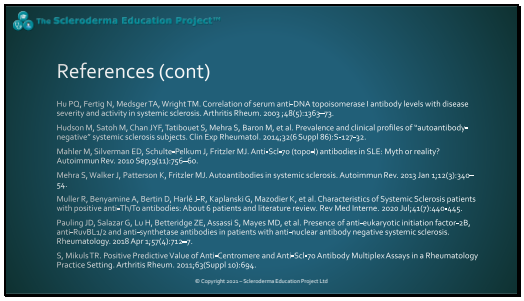
24		Earlier, I mentioned that patients are sometimes concerned that since they have very high ANA titers, for example, 1:1280 or above, this might be a bad sign since many other people seem to have much lower ANA titers, often in the 1:80 to 1:320 range. There is very little published data in the research literature on typical ANA titers for various SSc-specific antibodies. U1-RNP antibodies, which are associated with Mixed Connective Tissue Disease (MCTD) are known to typically have high ANA titers. All six patients in a recent study of six patients with rare Th/To antibodies (Muller et al. 2020) had ANA titers of 1:1280. SSc patients with Th/To antibodies are generally classified as limited SSc, although with a different overall disease profile than patients with centromere antibodies. A recent, informal self-report survey of 144 SSc patients conducted by this author showed that typical ANA titers for patients with centromere antibodies (n=86) were 1:1280 level or higher and patients with Scl-70 antibodies (n=29) had much lower ANA titers, mostly 1:320 or lower. We did not have a large enough number of respondents with RNA Polymerase III antibodies (n=14) to reach statistical significance, but average ANA titers for patients with this antibody were generally between the titer levels of patients with Scl-70 and centromere antibodies in this informal survey. The key takeaway here is that while it does appear to be the case that some SSc-specific antibodies tend to have higher or lower average ANA titers, the variability is very high for all antibodies and generally has little correlation with disease activity or severity.
25		This is also a common post. There are a couple of reasons why this can occur. In most cases, it is due to a change in testing method. Let's assume that the previous ANA test was done by IFA and you are positive for RNA polymerase III. If your new doctor re-runs an ANA test without specifying ANA/IFA, there is a very good chance that an ANA screening panel will be done by one of the solid phase assay testing methods that test for a limited number of antibodies. In that case, the ANA panel result will be negative since it is very unlikely that RNA polymerase III will be included in the ANA screening panel. A less frequent occurrence can occur with low positive ANA titers such as 1:80 or 1:40. Some labs use a 1:40 cutoff for a low positive and others a 1:80 cutoff. If your previous ANA titer was 1:40 and you retest at a lab with a 1:80 cutoff and ended with the same 1:40 titer, the new lab would report this as a "negative" result.
26		Several recent studies (Schneeberger 2013, Hudson 2014, Salazar 2015) have documented that about 5% of patients with formally diagnosed systemic sclerosis are ANA negative when testing is done by IFA. The question is why.

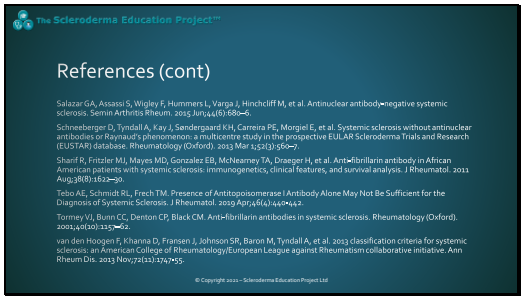
27		A 2013 paper (Mehra) indicates that in some cases, patients with Ku, PM-Scl, and even RNA Polyerase antibodies may be ANA/IFA negative. In some cases, this is dependent on the particular HEP-2 substrate tissue used, and testing at a different lab that uses a different substrate may yield a positive ANA/IFA result.
28		RuvBL1/2 antibodies are a newly identified SSc-specific antibody that are present in about 2% of SSc-patients. It is classified as an overlap antibody. A recent paper (Pauling 2018) notes that RuvBL1/2 antibodies can be ANA/IFA negative. Other rare antibodies such as U11/U12-RNP can also be ANA/IFA negative.
29		Another newly identified SSc-specific antibody, abbreviated eIF2B, is not an anti-nuclear antibody, but rather an anti-cytoplasmic antibody. Anti-cytoplasmic antibodies don't attack the nucleus of a cell and while detectable in an ANA/IFA test, some labs do not report cytoplasmic staining. It is important to note that currently not all researchers accept eIF2B as a systemic sclerosis specific antibody. Also, there is no published data as to what the overall prevalence rate is for eIF2B antibodies, although in a 2018 study (Pauling), 7 out of 128 ANA/IFA negative patients with a formal SSc diagnosis had eIF2B antibodies.
30		A false positive lab result means that the test result is positive when it should have been negative. While there are occasionally false positive (or false negative) testing issues with many lab tests, according to recent research, Scl-70 antibody testing appears to have a major problem with false positive results.
31		Historically, Scl-70 antibody testing was mostly done by a technique called double immunodiffusion, usually abbreviated ID. This is considered to be the most reliable Scl-70 antibody testing method (Domsic and Medsger 2016). However, ID testing is time-consuming and expensive. Because of this, almost all labs have switched to testing for Scl-70 antibodies using one of the solid phase assays such as ELISA, Multiplex, or LIA.

32	The Scleroderma Education Project™ Scl-70 False Positive Problem (cont) • Scl-70 testing methods • Scl-70 antibodies highly specific to SSc, but… © Copyright 2021 – Scleroderma Education Project Ltd	While Scl-70 antibodies are considered to be highly specific to systemic sclerosis (SSc), a number of studies (Meier and Mikuls 2011, Gussin 2001, Mahler 2010, Bizzaro 1998) have documented that patients without a clear diagnosis of SSc often test positive for Scl-70 antibodies when testing is done by either ELISA or Multiplex testing. This is sometimes seen in patients with a diagnosis of lupus. Notably, almost all of these positive Scl-70 results are low positives.
33	The Scleroderma Education Project™ Scl-70 False Positive Problem (cont) • Scl-70 Testing Methods • Scl-70 antibodies highly specific to SSc, but… • Repeat testing is not the answer © Copyright 2021 – Scleroderma Education Project Ltd	Some clinicians who are aware of the Scl-70 false positive problem order repeat testing at the same lab, thinking that this is just a testing precision issue. Unfortunately, this does not appear to be the case and repeat testing at the same lab is likely to continue to yield false positive results.
34	The Scleroderma Education Project™ Scl-70 False Positive Problem (cont)  Source: Homer et al. (2018) © Copyright 2021 – Scleroderma Education Project Ltd	Two recent papers have shed much more light on this important issue. A 2018 paper (Homer et al.) compared Scl-70 testing on a group of 129 patients by three different testing methods: Multiplex, ELISA, and immunodiffusion. All of the patients in this group were positive by Multiplex testing but only 26% had a formal diagnosis of SSc. If you also added ELISA testing, only 51 patients were positive by both methods and out of these 51, 45% of these patients had a formal SSc diagnosis. If you added ID testing, only 21 out of the original 129 patients were positive by all three methods, but more importantly, more than 90% of this group were formally diagnosed with SSc, suggesting that ID testing is significantly more specific clinically than either of the other two testing methods. One interesting finding of this study was that ELISA results that were five times higher than the normal range cutoff were highly correlated with a formal diagnosis of SSc. Unfortunately, this study did not look at Multiplex results to see if there was a similar pattern.
35	The Scleroderma Education Project™ Scl-70 False Positive Problem (cont)  Source: Tebo et al. (2019) © Copyright 2021 – Scleroderma Education Project Ltd	A second recent study (Tebo et al. 2019) looked at 46 patients who tested positive for Scl-70 antibodies by Multiplex testing and correlated the value of the test result against a diagnosis of SSc. Out of the 46 patients with a positive Multiplex Scl-70 antibody result, only 17 (37%) had a formal diagnosis of SSc. More importantly, only highly elevated results (200 AU/ml) were significantly correlated with this diagnosis. This is about five times the normal range cutoff, similar to the findings in the Homer study for ELISA testing. If you look at both studies and just consider Multiplex testing, between 63% and 75% of patients who were positive by Multiplex testing were negative by the “gold standard” ID testing method. This suggests that the false positive Scl-70 testing problem appears to be very common in routine clinical practice. However, it is also important to point out that since longitudinal follow up studies have not been done, there is no way to know if some of these patients will eventually test positive using ID and other similar testing methodologies.

36		Earlier, we saw what can happen when a clinician doesn't fully understand ANA testing and interprets a negative ANA screening panel as indicating that the patient does not have an autoimmune disease, let alone systemic sclerosis. This is the other side of the coin. Here the clinician suspects a possible underlying autoimmune disease, orders an ANA screening panel that is typically done by Multiplex or ELISA, and gets a positive Scl-70 result. As we just saw, in some cases this is a low-positive Scl-70 result that is a false positive. Unfortunately, many untrained clinicians believe that a positive antibody test means that the patient has systemic sclerosis, frequently leading to comments like this. We will learn about the role of ANA and antibody testing in diagnosing systemic sclerosis in the final part of this presentation.
37		There are a number of different ways to determine if an Scl-70 result is likely to be a false positive. The most reliable method is to re-test at a lab that offers ID testing or another method that does not have the false positive problem seen with Multiplex and ELISA testing. While no data has been formally published on this yet, I recently learned (anecdotally) that a new testing method called Chemilluminescence, done on the Bioflash platform, yields virtually identical results to ID testing but is less expensive and easier to do than ID testing. (I am pushing the researchers to publish the actual data, but no luck so far.)
38		If the results of an ANA/IFA are negative, this increases the likelihood that a positive Scl-70 result is a false positive. However, there is an important caveat here. If the lab uses a 1:80 cutoff for ANA/IFA testing, there is a possibility that an ANA/IFA result reported as negative has a titer of 1:40. In this case, a low positive Scl-70 result cannot be assumed to be a false positive.
39		In most cases, SSc patients test positive for only one SSc specific antibody. If the results of an ANA panel have a positive Scl-70 result and a positive second antibody, for example, centromere, this increases the chances that the Scl-70 is a false positive. Interestingly, a few years ago, one major testing lab included a note on results that reported more than one SSc specific antibody, stating that this was a rare occurrence and that the SCL-70 was likely a false positive.
40		While this is not a completely reliable method, re-testing Scl-70 at a different lab that uses a different testing method (ELISA, LIA, Multiplex) can sometimes be very helpful, as noted in the Homer paper discussed earlier. If one of these two tests is negative, this strongly suggests that the positive result was a false positive. However, it is entirely possible to have a second false positive, as we also saw in the Homer paper.

41		Formally diagnosing a patient with systemic sclerosis is often very challenging, even for an experienced scleroderma specialist. In the final part of this talk, I want to briefly discuss where ANA testing fits into clinical diagnosis.
42		In 2013, the American College of Rheumatology and the European League Against Rheumatism approved a new set of classification criteria for systemic sclerosis, replacing the older 1980 classification criteria. These classification criteria use a nine-point scale, with clear indications as to what signs and symptoms count towards the nine-point total. As you can see in this chart, the three most common scleroderma specific antibodies are included: centromere, Scl-70, and RNA polymerase III, but none of the rarer ones are. If you look closely, several common symptoms are missing from this table, for example GI symptoms such as GERD, difficulty swallowing, or small intestinal bacterial overgrowth. Also, these three antibodies only account for 60 to 70% of systemic sclerosis patients. So, why are these common symptoms missing from this chart? It turns out that the abstract for the paper that introduced this new point chart omitted a very important point, and as a result, this chart is often misused by many clinicians who are not SSc experts. If you read the actual paper (which in practice few clinicians do), you quickly discover that the intended purpose of this classification chart is for selection of patients for formal research studies, NOT for formal clinical diagnosis. While the point chart can be used as part of clinical diagnosis, the clinician is supposed to also factor in additional signs and symptoms, for example, typical GI symptoms, tendon friction rubs, and even very specific symptoms such as scleroderma renal crisis. To bring us back to the main topic of this talk, ANA and antibodies in systemic sclerosis, consider a patient who has Raynaud's, puffy fingers, abnormal nailfold capillaries, GERD, joint pain, and severe fatigue, but has a rare antibody such as U3-RNP instead of one of the three antibodies included in this chart. Despite having many systemic specific symptoms and an antibody that is disease specific, they only would have a total of seven points on this chart. A scleroderma specialist would almost certainly diagnose the patient with SSc given all these signs and symptoms. Unfortunately, in practice, many untrained clinicians rigidly look for a total of nine points and if not there, refuse to formally diagnose the patient with SSc, often giving the patient a tentative diagnosis such as undifferentiated connective tissue disease. This often leads to significant delays in correctly diagnosing the patient with systemic sclerosis, potentially delaying treatments that could slow down the course of the disease and failing to appropriately monitor for potential risk factors when following the patient.

43		To end this presentation, we return to our key slide. I hope that you now have a better understanding of why I still consider this to be the most important slide in today's presentation.
44		I would like to formally thank doctors Marvin Fritzler, Allan Metzger, Alan Bridges, and John Weiss for their invaluable contributions by reviewing and greatly improving these slides.
45		
46		
47		

48	 The Scleroderma Education Project™ References (cont) Salazar GA, Assassi S, Wigley F, Hummers L, Varga J, Hinchcliff M, et al. Antinuclear antibody-negative systemic sclerosis. Semin Arthritis Rheum. 2015 Jun;44(6):680–6. Schneeberger D, Tyndall A, Kay J, Søndergaard KH, Carreira PE, Morgiel E, et al. Systemic sclerosis without antinuclear antibodies or Raynaud's phenomenon: a multicentre study in the prospective EULAR Scleroderma Trials and Research (EUSTAR) database. Rheumatology (Oxford). 2013 Mar 1;52(3):560–7. Sharif R, Fritzler MJ, Mayes MD, Gonzalez EB, McNearney TA, Draeger H, et al. Anti-fibrillarin antibody in African American patients with systemic sclerosis: immunogenetics, clinical features, and survival analysis. J Rheumatol. 2011 Aug;38(8):1622–30. Tebo AE, Schmidt RL, Frech TM. Presence of Antitopoisomerase I Antibody Alone May Not Be Sufficient for the Diagnosis of Systemic Sclerosis. J Rheumatol. 2019 Apr;46(4):440–442. Tormey VJ, Bunn CC, Denton CP, Black CM. Anti-fibrillarin antibodies in systemic sclerosis. Rheumatology (Oxford). 2001;40(10):1155–62. van den Hoogen F, Khanna D, Fransen J, Johnson SR, Baron M, Tyndall A, et al. 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European League against Rheumatism collaborative initiative. Ann Rheum Dis. 2013 Nov;72(11):1747–55. © Copyright 2021 – Scleroderma Education Project Ltd	