The Scleroderma Education Project™

ANA and Antibody Series

Changes in ANA and Antibody Levels in Scleroderma

Background

This article was prompted by an excellent question that was recently sent to the Scleroderma Education Project:

> You state that antibody status does not change. Just to be clear, does that mean a negative result for any of the antibody tests for scleroderma is a non-indicator for scleroderma (in that it will never become positive?) Or can a negative result revert to positive at some point, and then once positive, never return to negative? I know the ANA will change levels and even change from positive to negative and back again - even though it isn't necessarily indicative of disease activity. I'm confused on the other antibodies however. Can you help clarify? Thanks!

Let's start this by looking at a very realistic scenario:

A 42-year-old female living in a small town visits her primary care clinician with the following complaints:

- Raynaud's symptoms that started about three years earlier and have gotten much worse over the past few months
- Severe fatigue
- Muscle aches and pains
- Heartburn

The clinician is concerned that something autoimmune might be going on, given the late-onset Raynaud's. She orders an ANA as an initial step. A few days later, the ANA result comes back. It is positive with a low titer of 1:80 so she refers the patient to a rheumatologist for a more detailed workup.

The rheumatologist sees the patient for an initial workup. He is unsure exactly what might be going on given the patient's cluster of symptoms and orders another ANA, with a comprehensive reflex autoimmune screening panel, should the ANA be positive. This time, however, the ANA comes up negative, and no further antibody testing occurs; he assumes that the first ANA result must have been a false-positive result. He reassures the patient that everything is basically OK, suggests over the counter medications for the heartburn and prescribes nifedipine for the Raynaud's. He says her fatigue and muscle aches and pains are probably stress-related and offers to give her a referral to a therapist who can probably help her deal with the stress in her life. He also advises her what to do if her Raynaud's and/or other symptoms worsen.

Unfortunately, the patient evolves into the early stages of rapidly progressing diffuse systemic scleroderma and probably will get a lot worse before she is eventually diagnosed correctly and started on systemic treatment. Anyone who listens to the stories of patients that are eventually diagnosed with scleroderma will tell you this is a very typical and realistic scenario.

In this article, we will look at what might be going on with the contradictory ANA results. Then we will address a more general question about whether or not ANA and/or antibody levels in scleroderma change over time and with what clinical significance, if any.

Why the Change in ANA From Positive to Negative?

The first possibility, raised by the rheumatologist, was that the initial positive ANA result was incorrect, probably due to a lab error or a different method of testing. So let's start by looking at the data on lab errors. Yes – there are occasional lab errors, but research suggests that this is actually quite rare. A recent study that closely looked at this question found that the overall rate of lab errors was about 1.4% [1]. Of these small numbers of errors, about 77% were actually the result of problems with specimen collection, labeling, and transport. Actual lab errors were only about 23% of the total errors (0.3% lab error rate) when the lab correctly received specimens in time. The pre-collection errors were primarily the result of problems like using the wrong type of collection tube, mixed up samples, or delayed transport when the sample was time-sensitive. So, while it is possible that the original positive ANA was the result of a lab error, that is not the most likely explanation for the difference between the first and second ANA result.

In fact, here is what probably happened. The patient was seen in a local clinic in a small town. In cases like this, the clinic's in-house lab is typically not set up to do the ANA testing so the specimen is collected and sent to a regional reference lab. In this particular example, we will assume that the ANA test was sent to RDL Reference Laboratory – a major national testing laboratory. While there are three different ways to perform an ANA test, this particular reference lab performs all of their ANA testing using a method called indirect immunofluorescence (IFA or IIF), considered by the American College of Rheumatology to be the "gold standard" in ANA testing for autoimmune diseases.

When the rheumatologist subsequently ordered the confirming ANA test, his clinic may have sent the specimen to a different national reference lab, in this hypothetical case we'll say LabCorp. The test he ordered was an ANA test where a positive result automatically triggers a second round of testing that looks for a number of different antibodies commonly seen in several different autoimmune diseases, including scleroderma and lupus. According to their website, LabCorp does their ANA testing using a method called Multiplex Flow Immunoassay - not the IFA "gold standard." It turns out that this particular (theoretical) patient actually has antibodies to RNA Polymerase III, which ,according to a 2011 study [2], is very likely to be missed when doing ANA testing using Multiplex instead of IFA, resulting in a falsely reported negative ANA result. The anti-RNA Polymerase III antibody is one of the two main antibodies associated with diffuse scleroderma (the other, anti-Scl-70), and will be missed if IFA methodology is not used.

This scenario was set up to illustrate a common problem that can arise when ANA testing methods are less than ideal. The referral rheumatologist was an experienced rheumatologist, in practice for many years. Back when he was in his rheumatology fellowship fifteen or twenty years ago, only two specific antibodies were known to be associated with scleroderma: anti-Scl-70 and anti-centromere antibody. The anti-RNA

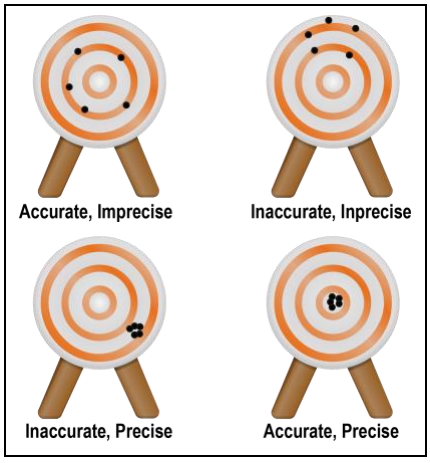
Polymerase III antibody is the third major scleroderma antibody and is about as common as Scl-70 or centromere antibodies. In addition, there are at least seven additional less common antibodies associated with the various types of scleroderma. Many rheumatologists may not be aware of the newer antibodies since they see relatively few scleroderma patients. However, the bigger problem is that many reference labs now do ANA testing using either Multiplex or another technique called ELISA, neither of which is routinely set up to detect any of the newer antibodies, thus missing important information needed for the diagnosis of scleroderma. In fact, some reference labs still include scleroderma-specific screening panels that *only* include anti-Scl-70 and anti-centromere antibodies, thus missing any of the newer scleroderma-related antibodies.

(For anyone wanting to better understand ANA and antibody testing for diagnosing systemic scleroderma, refer to a separate article in this series titled "How to Do Scleroderma ANA and Antibody Testing Correctly.")

Back to the Original Question – Do ANA and Antibody Levels Change Over Time?

In the original questions posed by the patient above, she was under the impression that ANA levels could change from positive to negative and back again. We have illustrated above a very common way that ANA may *appear* to change from positive to negative. There is one situation, discussed later, where ANA levels truly can drop dramatically and perhaps become negative, but let's first take a look at the more general question of how ANA and antibody levels can change over time and the clinical significance (if any) of these changes. However, before we can look at actual change, we need to discuss something called "precision" in clinical laboratory testing and how that differs from "accuracy." Basically, a measurement is considered "accurate" if it is very close to the actual value of something that is being tested. In contrast, a measurement is "precise" if repeated measurements give essentially the same results.

To illustrate this difference, let's look at a relatively simple laboratory test – hematocrit (abbreviated Hct) – which is a measure of the percentage of your whole blood that is made up of red blood cells. Normal range for hematocrit varies depending on age and sex, but for women, the typical normal range would be about 37% to 48%. Now, let's suppose that your Hct, if measured perfectly, is 42.1%. If we draw three separate blood samples and use a standard method of determining hematocrit and get the following values - 45.3%, 45.6%, and 45.4% - we could say that this was a very precise measurement since the three results are almost identical. On the other hand, it is not very accurate since the true value is 42.1%. In contrast, if we were to get the following three measurements - 40.0%, 43.9%, and 42.0% - we say that overall the measurement is pretty accurate since the average of the three numbers is very close to our target of 42.1%, but the measurement is not very precise.



So why is this important to our question as to how much ANA and antibody values change over time? Most laboratory measurements are like our example of hematocrit – a single value. In the case of ANA, for example, if you measure ANA levels using either ELISA or Multiplex testing methods, you do, in fact, get a single

number, just like hematocrit. However, if the ANA is done by IFA (as it should be), then instead of a single number, the result (if positive) looks something like this: 1:320; ANA is measured by how much a patient's blood sample can be diluted and still produce what is called a positive "staining pattern." The greater the dilution with a recognizable staining pattern, then the greater the ANA level, so a titer of 1:320 is higher than a titer of 1:80. If the lab technician sees a positive staining pattern, then the next step is to dilute the blood sample with 40 parts saline to one part blood, creating a dilution of 1:40 where the staining pattern is still visible. This process is repeated over and over again until the staining pattern finally is no longer visible, resulting in the final titer. This means that the possible ANA titers follow a precise sequence: 1:40, 1:80, 1:160, 1:320, 1:640, 1:1280, 1:2560, etc.

So what this means is that if you were to do an ANA by Multiplex and get values of 2.4, 3.6, and 2.9, you would intuitively understand that these values are pretty close together and probably do *not* reflect actual changes in ANA levels. However, if you are doing ANA tests by the preferred IFA method, the equivalence might be 1:320, 1:1280, and 1:640 and that 1:640 might in fact be the most "accurate" ANA titer. But if you didn't understand that 1:640 is next to and between 1:320 and 1:1280, you might think that your ANA level was fluctuating significantly, when it fact this is probably just normal testing precision variability. (Side note: While we have only been talking about ANA, it is worth noting that some individual antibody testing can also done by using IFA, in which case the results are also reported as a titer.)

Let's Look at the Research on Changes in ANA Levels

Now that we have the necessary background to understand when changes in ANA levels are actually significant instead of just being normal fluctuations in testing methodology, we can now look at the actual research on whether or not ANA levels change over time. To begin with, when someone is first coming down with an autoimmune disease that has a positive ANA, there is an initial period of time when his/her ANA levels may test very low, which can make early diagnosis challenging. For example, if you test the general population to see if they have a positive ANA, a significant percentage of the population (20 to 30%) over the age of 50 will have positive ANA titers at the 1:40 level, especially in relatives of patients with autoimmune diseases. So when a patient presents with clinical symptoms that are typical for an autoimmune disease such as lupus or scleroderma but his/her ANA level is 1:80 or below, many clinicians will appropriately be reluctant to make a formal diagnosis at that point, preferring to follow the patient closely and re-test ANA periodically. This can lead to a non-specific diagnosis of UCTD (undifferentiated connective tissue disease), basically meaning that the physician believes that patient may be developing an autoimmune disease, but it is too early to label the exact diagnosis.

Over time, the ANA level will typically rise if the patient does have an underlying autoimmune disease, and this can occur very slowly or very rapidly. Once the ANA titer reaches a level of 1:160 or 1:320 or higher, this higher titer is very unlikely to occur in a person without an underlying autoimmune disease. Then the focus becomes on identifying the underlying disease, starting with staining patterns or specific antibody profiles.

In systemic scleroderma patients, ANA often reaches a level and stabilizes. More importantly, according to a number of research studies, in scleroderma and most other autoimmune diseases the actual stabilized ANA titer does not specifically correlate with disease activity or severity. However, there are reports that in conjunction with certain immunosuppressant treatments, ANA and other individual antibody levels may decrease but rarely become negative.

A Few Scleroderma Patients Have Negative ANA

It is worth noting that about 5% of scleroderma patients repeatedly test negative for ANA using the IFA method. Although this is speculative, the most likely reason is that while ANA testing using the IFA method can test for up to 150 different potential antibodies, there may well be some rare antibodies associated with variants of scleroderma that are simply not detectable with current ANA testing methods.

A Known Exception to ANA Levels Remaining Stable Over Time

There is one apparent exception to the general rule that ANA levels remain relatively stable over time. While this has not yet been published in any research journals, in a recent private conversation with the head of one of the ongoing studies that are trying autologous hematopoietic stem cell transplantation (HSCT) to treat scleroderma, I was informed that ANA levels *do* drop significantly following HSCT. The ongoing HSCT research studies will be monitoring ANA and other scleroderma antibody levels over time to determine if there is a correlation between changes in ANA/antibody levels and clinical disease. The reduction in ANA / antibody levels makes theoretical sense since HSCT essentially destroys and restarts the patient's immune system, and it will be very interesting to see if ANA levels gradually increase over time or remain significantly reduced following this experimental treatment.

A Note About Staining Patterns with ANA by IFA Testing

When ANA testing is done using the IFA method, in addition to the titer, there is usually a distinctive staining pattern. A number of different staining patterns have been identified, and some of these are very specific to different autoimmune diseases. However, interpreting staining patterns can be a bit of an art form in some cases, and it is always recommended that confirming antibody testing be done rather than depending on the apparent pattern. For example, one of the patterns often seen in a subset of scleroderma patients is called "centromere." This pattern is highly correlated with the anti-centromere positive limited scleroderma (formerly called CREST syndrome). But since there can be human error in reading ANA staining patterns, and multiple staining patterns can occur in a given patient, specific follow-up antibody testing should always be done to verify the staining pattern.

What About Changes in Individual Antibody Levels?

When we switch the discussion from ANA to individual antibodies, the issue of a false negative result is much less likely. For example, while you can test for the presence of anti-centromere antibodies using any of the three ANA methods discussed (IFA, ELISA, Multiplex), it turns out that the newer testing methods (ELISA, Multiplex) generally do an excellent job of reliably detecting and measuring specific antibody levels. (An exception is that there is a significant occurrence of false positive anti-Scl-70 antibody results with ELISA and Multiplex testing. These will usually be low positive results. See the separate article in this series titled “False-positive Scl-70 (Topoisomerase) Antibody Testing: A Major Problem in Systemic Sclerosis Diagnosis” for more information about this important issue.)

Research on individual antibodies in scleroderma confirms that it is very rare for a patient to have more than one scleroderma-related antibody. Once you have a clearly identified antibody specific to a particular variant of scleroderma, this will generally not change over time. Like the overall ANA level, specific scleroderma antibody titers usually do not reflect clinical disease activity.

Summary

- ANA and other antibody levels often rise over time when the patient is developing an autoimmune condition. It is also known that antibodies may be detectable up to seven years prior to the clinical presentation of SLE (and possibly scleroderma). Eventually the ANA and antibody levels usually stabilize. In addition, since the absolute ANA or antibody level does not correlate with scleroderma severity or activity, ANA and antibody levels are rarely monitored once a laboratory diagnosis is firmly established.
- When ANA test results appear to switch from positive to negative or vice versa, the most likely reason is that different testing methods were used. The American College of Rheumatology strongly recommends using IFA for ANA testing with reflex specific antibody confirmation using ELISA [3].
- In patients receiving an experimental treatment called autologous hematopoietic stem cell transplantation (HSCT), ANA and antibody levels usually decrease. However, long-term studies are needed to see if ANA and antibody levels remain reduced or eventually return to pre-treatment levels after HSCT is stopped.

References

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