



## Technical Article Series

### **Scleroderma Blood Hyperviscosity: Implications for Research and Treatment**

In 1979, a research paper authored by Kahaleh, Sherer, and LeRoy titled “Endothelial Injury in Scleroderma” included the following passage:

*Many theories exist regarding the etiology and pathogenesis of scleroderma: endocrine dysfunction, nervous disorder, infection, physical trauma of various types, and immune factors. Many, if not all, of the manifestations of scleroderma can be explained on the basis of functional and structural vascular compromise after repeated vascular insults, subsequent healing of vascular walls with proliferative vascular response, and luminal narrowing. The coagulation cascade may be triggered by the intimal lesion, leading to fibrin deposition, reduced blood flow, and local ischemia.*

Basically, what this is saying is that while we don't know exactly what triggers Scleroderma, all symptoms that ultimately arise may be the result of a chain of events that starts as a result of repeated damage to the cells that line the smallest blood vessels (endothelial layer on the inside of microcapillaries). While this disease / endothelial damage model is now commonly accepted by Scleroderma researchers, many theories have been floated about the specific mechanisms of that damage, usually centered around various biological processes and cellular level interactions.

Based on this theory, we propose that a simplified model for Scleroderma symptom development and progression can be divided into five stages, as shown below:

#### **Scleroderma Disease Stages and Treatment Approaches**

Stage	Description	Treatment Approaches
1	Something triggers changes to the immune system that leads to the development of Scleroderma-related autoantibodies in genetically susceptible individuals. Possible triggers include environmental toxins such as silica dust or organic solvents, or infectious processes triggered by mycoplasma or other bacterial/viral organisms.	For the small subset of scleroderma patients that have ongoing infections that continue to trigger changes in the immune system, antibiotic treatment using Minocin and other antibiotics would be considered a Stage 1 intervention. For patients with no ongoing underlying infectious processes, antibiotics would be considered a Stage 2 immune system regulating treatment.
2	The altered immune system generates destructive autoantibodies on an ongoing basis.	Immunosuppressive and immune regulation drugs such as Cellcept, Cytoxan, Plaquenil, Methotrexate,

		Intravenous immunoglobulin (IVIG).
3	These autoantibodies, either directly or indirectly, cause repeated damage to the endothelial layers of the microcapillaries. A possible mechanism of action is discussed below.	Plasmapheresis; drugs or other treatments that target red blood cell clumping (see below).
4	The repeated damage to the endothelium triggers a cascade of cellular level events that starts the fibrosis process.	Drugs that target the fibrosis process directly. These drugs are currently in early research stages.
5	The fibrosis eventually affects multiple organ systems, including the skin as well as internal organs.	Symptom specific treatments, e.g., calcium channel blockers or Phosphodiesterase Type 5 (PDE5) inhibitors for Raynaud's; proton pump inhibitors (PPIs) for reflux; Angiotensin converting enzyme (ACE) inhibitors for scleroderma renal crisis.

Current systemic treatments for Scleroderma are mostly focused on Stage 2 of the disease process. The logic is simple – suppressing the production of the destructive autoantibodies using various types of immunosuppressive drugs should help to control the damage by reducing the number of circulating autoantibodies. While research indicates that immunosuppressive treatments may result in modest systemic improvement, any benefit stops when treatment stops and symptoms then resume “normally”. And, since most immunosuppressive treatments are potentially very toxic, the stronger immunosuppressive drugs can only be used for a relatively short time period. Until a fundamental treatment is found that fixes the immune system so that it again functions normally (probably a long way off), any treatment for Scleroderma is likely to be needed on a life-long basis. This means that standard immunosuppressive therapies that can only be used for a limited amount of time are unlikely to be an effective long-term treatment for Scleroderma. Standard immunosuppressant and disease modifying drugs, including side effects, are listed in Table 3 in the *Scleroderma FAQ*.

A number of researchers are now focusing on Stage 4 of the disease process - reducing or preventing fibrosis and subsequent organ damage. An example of this is early stage research that is being done at Michigan State University (Haak et al. 2014). Treatments that can reduce or prevent fibrosis can potentially be very helpful, but at this point it is too early to know whether these treatments will be safe enough to be used on a long-term basis without causing other systemic problems. Since this type of intervention is relatively late in the disease process, any treatment that can prevent or reduce fibrosis will need to be continued on a permanent basis. Because of this, safety will be a major consideration in whether or not drugs developed to deal with the fibrosis stage of Scleroderma will be useful over the long run.

Once symptoms are clearly established, treatment focus usually switches to individual symptom management (Stage 5) even as general immunosuppressant treatments may be continued. There are a number of treatments for specific symptoms that are reasonably effective, at least initially. Standard treatments for various symptoms that occur frequently in Scleroderma patients are covered in detail in the *Scleroderma FAQ*.

It is worth noting that antibiotic protocol (AP) therapy targeted at the small subset of patients that have ongoing infections may be considered a Stage 1 intervention. The logic is that if there is an ongoing underlying infectious process that is triggering the generation of Scleroderma-related autoantibodies, then treating the underlying infection might reduce or even stop the future production of the harmful autoantibodies. However, once the infection has altered the immune system in a manner that leads to the development of autoantibodies, it may be too late to completely restore the immune system to its pre-disease state. Note that in patients that do not have an underlying ongoing infectious process, drugs such as minocycline are also considered DMARDs (disease-modifying antirheumatic drugs) and may have additional immune regulating benefit. This would be considered a Stage 2 intervention for the majority of Scleroderma patients that do not appear to have an underlying infection as the active trigger for their disease.

This paper focuses on the Stage 3 of the disease process – damage to the endothelium – and proposes a simpler explanation for the cellular damage stage than has been hypothesized to date. It is believed by this author that focusing on this stage of the disease process can potentially lead to the development of treatments that are likely to be significantly less toxic than current immunosuppressive treatments and have a much greater potential for preventing or significantly delaying symptom progression than is likely with treatments focused on stage two or stage four of the disease process.

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## ***Scleroderma and Blood Hyperviscosity Research***

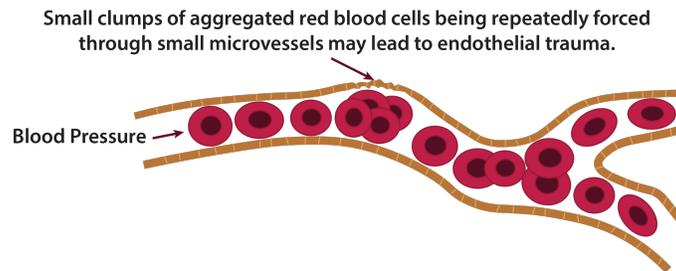
A series of research studies beginning with a paper in 1975 (Tietjen et al. 1975, McGrath et al. 1977, Weber et al. 1985, Jacobs et al. 1987) have consistently shown abnormally elevated blood viscosity (hyperviscosity) in the majority of systemic Scleroderma patients. This finding has been replicated in a number of studies with one of the more recent findings documented in 2006 (Volkov et al.). The specific type of blood hyperviscosity documented in these research articles (when reported) is red blood cell hyperaggregation (red blood cells clumping together abnormally). These studies have used a number of different methods to measure the type of and degree of blood hyperviscosity, both in vivo (actually monitoring the red blood cells circulating in the patients' bodies) and in vitro (blood samples withdrawn from the patient and measured separately).

Beyond the research showing blood hyperviscosity, many Scleroderma patients that were blood donors note anecdotally that at the point that they started to develop Scleroderma-related symptoms, the lab techs drawing blood commented that their blood was noticeably thicker than normal and that it took much longer than expected to do the blood draw. (It is worth noting that diagnosed Scleroderma patients cannot be blood donors. However, as it can sometimes take years for patients to finally receive a correct diagnosis, patients will often continue to be blood donors during that pre-diagnostic period.) In addition, some patients were told that their blood was clogging the filters in the equipment used to extract blood products from the donated blood. According to a well-known Scleroderma researcher and clinician, the hyperviscosity “actually can be visualized as sluggish flow through a nailfold video capillarscopy”.

Why is this significant? The average size of a micro-capillary is about 8 microns in diameter, with a normal range of 5 to 10 microns. A normal red blood cell is 6 to 8 microns in diameter. This means that some red blood cells have to fold in order to fit through the smallest capillaries. As red blood cells start to clump together, it becomes increasingly difficult for the “clump” of red blood cells to make it through the smallest micro-capillaries.

Normal blood pressure is very strong, and at least for a while, the pressure will be strong enough to force the clump through the micro-capillaries. However, at some point, this may potentially start to cause damage to the single layer of endothelial cells that line the micro-capillaries. The research literature on the effects of red blood cell hyperaggregation on micro-capillaries documents most of the early symptoms seen in Scleroderma, including tortuous capillaries that are seen in nail beds and glomerular damage (kidneys) caused by hemodynamic mechanisms (Anderson et al. 1989, Neuman et al. 1991, Vicaut 1995). Basically, this disease model assumes that the damage to the endothelial cell layer is directly from biomechanical damage, not a more complex biochemical process as has frequently been assumed in the research literature.

**Figure 1**



Although entirely speculative, a reasonable and testable hypothesis would be that with limited Scleroderma, either the degree of red blood cell hyperaggregation or the “stickiness” of the aggregated red blood cells may be lower than in the more rapidly progressing diffuse forms of the disease. There is some support for this hypothesis in the research literature (see discussion below in the section below: *Hyperviscosity Characteristics for Different Antibody Types*). Even if the degree of clumping is low by constant, and the overall hyperviscosity of the blood is only slightly elevated, over a long period of time damage to the endothelial lining could still occur resulting in the development and progression of Scleroderma symptoms.

Red blood cell clumping can also potentially explain one of the common very early symptoms experienced by many Scleroderma patients – severe fatigue. This is more commonly seen as an initial symptom in patients with diffuse Scleroderma than it is in limited Scleroderma. The reasoning behind this speculation is straight forward: tightly clumped red blood cells could cause functional anemia, even though this would not be obvious with normal blood tests such as hematocrit and hemoglobin. The clumped red blood cells would be functionally similar to sickle cells seen in patients with sickle cell anemia – the clumping of sickle cells results in greatly impaired blood flow. Notably, some of the common symptoms of sickle cell anemia include fatigue, shortness of breath, and cold hands and feet. These are common early symptoms, especially in patients with diffuse Scleroderma.

It is worth noting that the specific mechanism of action for how Scleroderma-related antibodies lead to red blood cell hyperaggregation has not been addressed in the research literature. This may be a fruitful area for research that could potentially lead to additional treatments that target this specific biochemical process.

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## ***Hyperviscosity: Implications for Treatment***

### **Plasmapheresis (aka Therapeutic Plasma Exchange or TPE)**

If the hyperviscosity / biomechanical damage model for microcapillary endothelial damage is correct, then this presents an opportunity for a stage 3 intervention. In theory, *any* treatment that can reduce or eliminate the red blood cell aggregation should reduce or prevent the development of scleroderma-related symptoms. While this could potentially be a drug-based treatment, it could also be non-drug treatment approach, for example plasmapheresis.

Plasmapheresis (also called therapeutic plasma exchange and often abbreviated TPE) is considered the “gold standard” in treating blood hyperviscosity disorders (Piccini & Nilsson 2006). Basically, plasmapheresis is a procedure that mechanically replaces most of the plasma while preserving the red and white blood cells. Specifically, the procedure involves removing blood from one arm, running it through a machine that centrifuges out and keeps the red and white blood cells and most of the platelets, discards the plasma (the liquid part of the blood), and replaces it with either new plasma or, more commonly, sterilized albumin. The combined albumin and the original red and white cells plus platelets are remixed and returned to the other arm. Typically, this takes about 90 minutes and is done in an outpatient hospital environment. The effect of a single one blood volume plasmapheresis treatment is to remove about 65% of blood components except for almost all of the red and white blood cells and about 70% to 75% of the platelets. This includes beneficial things like clotting factors but also potentially harmful things such as autoantibodies.

In the mid 1980s through the early 1990s a series of investigational research studies (Weber et al. 1985, von Rhede Van der Kloot et al. 1985, Jacobs et al., 1987, Jacobs et al. 1991) were done in the Netherlands that examined red blood cell aggregation in patients with primary Raynaud’s (not related to an underlying autoimmune condition) and patients with secondary Raynaud’s associated with Scleroderma. They developed a way of measuring blood viscosity in vivo (live circulation) rather than in vitro (in a test tube). In vivo testing is considered a more reliable way of measuring viscosity.

The initial studies looked first at the difference in blood viscosity between these two groups of Raynaud’s patients. The researchers then tried using plasmapheresis on both the primary and secondary Raynaud’s patients. The treatment protocol in these early studies mostly involved doing four plasmapheresis treatments – one per week for four weeks – and then studying the results of this intervention.

Here is a summary of some of the key findings from this series of research studies:

- While overall blood viscosity was slightly elevated in patients with primary Raynaud’s (versus normal controls) and even more elevated in secondary Raynaud’s patients, when they looked specifically at red blood cell aggregation, they found that red blood cell aggregation was the same for the control group and primary Raynaud’s patients, but highly elevated for the secondary Raynaud’s patient group. This finding suggests that different mechanisms are likely to be involved in the two different forms of Raynaud’s.
- The four-treatment intervention protocol (one treatment per week for four weeks) essentially eliminated the abnormal red blood cell clumping found in the secondary

Raynaud's patient group. What they also found was that this treatment protocol had almost no effect on Raynaud's symptoms in patients with primary Raynaud's. In contrast, this treatment regimen typically eliminated *all* of the Raynaud's symptoms in the Scleroderma patients with secondary Raynaud's for a number of months.

- The studies also reported significant improvement in other Scleroderma-related symptoms, including healing of digital ulcers. Patients were monitored for up to three years following this single series of treatments. After a varying number of months, red blood cell aggregation returned to pre-treatment levels and Raynaud's symptoms redeveloped, but none of the patients developed skin ulcers during the follow up period.
- One of the early studies noted that skin that had been "tough and tense" before the series of plasmapheresis treatments became "supple and pliable" on the face and hands after the treatments. Since this was not a primary outcome measure in this pilot study, this was noted in passing and was not measured objectively.

Since all of these studies were open label without control groups, any overall conclusion about the potential for treating Scleroderma symptoms using plasmapheresis needs to be tempered by the need to do well controlled research.

In a private communication in 1993 between the author of this paper and one of the lead researchers on this series of studies, it was learned that a number of Scleroderma patients in the Netherlands were being treated with long-term regular plasmapheresis treatments based on the results of these research studies. The researcher, who was also a clinician, indicated that a treatment protocol of one treatment per week for four weeks followed by a resting period of two months before repeating the treatment cycle (16 treatments per year) yielded significant improvement of existing clinical symptoms and no additional symptom progression when used with limited Scleroderma patients. There were no side effects experienced other than localized to the immediate treatment period. However, they found that this treatment protocol was not aggressive enough to completely stop symptom progression in rapidly progressing diffuse Scleroderma patients. By switching to a weekly plasmapheresis treatment schedule with these patients, they were able to stop symptom progression entirely. Unfortunately, after an average of about 1½ years of weekly treatments, these patients began to develop major complications from the plasmapheresis treatments themselves, including infections and other complications resulting from the immunosuppressive effects of constant weekly plasmapheresis treatments. Notably, the less-frequent treatment protocol used for the limited Scleroderma patients showed none of these side effects.

Other studies that have looked at the effects of plasmapheresis on systemic Scleroderma patients. In several of these studies the treatment protocol was a combination of a limited number of plasmapheresis treatments plus a standard drug, for example, d-penicillamine plus plasmapheresis versus d-penicillamine alone. In all cases, the group that added plasmapheresis to the treatment regimen showed significantly better outcomes than the drug group alone. Again, any interpretation of these results needs to be tempered by the limited design of these studies. It is also worth noting that in a couple of studies where plasmapheresis was tried with patients who had severe symptoms, there was no improvement in symptoms. Once major systemic organ damage has occurred in a patient with late-stage Scleroderma, it is unlikely that *any* treatment intervention can reverse the damage so this outcome is not surprising.

A recently published study (Weiss et al. 2015) reports the case of a male patient, originally diagnosed with anti-centromere positive limited systemic scleroderma in early 1990, who has been undergoing regular plasmapheresis treatments (16 treatments per year) as the sole systemic intervention for more than 22 years, beginning in late 1993. Prior to

commencing treatment, the patient exhibited symptoms including severe gastro esophageal reflux disease (GERD) with esophagitis, frequent Raynaud's attacks, reduced lung function, and chronic chilling. With the exception of mild residual Raynaud's, all of the patient's symptoms reversed after three years of regular PA treatments and he remains in complete remission. It is notable that when treatments were suspended in late 1996 to observe the natural progression of the disease, the patient complained of return of reflux symptoms about six months after the last treatment. The reflux symptoms again disappeared about a year after regular treatments were restored.

While the preliminary research findings on using plasmapheresis treatments to eliminate or reduce red blood cell clumping and potentially slow or prevent the development of Scleroderma-related symptoms is promising, there are a number of issues that need to be considered with this treatment approach:

- The anecdotal reports from the Netherlands clinician (as well as clinicians in the US) suggest that while regular but limited plasmapheresis treatments may be a way to completely control and prevent symptom progression in patients with limited Scleroderma, it cannot be an effective treatment for rapidly progressing diffuse Scleroderma patients. However, it is likely that treating diffuse Scleroderma patients with plasmapheresis at a frequency that can be tolerated without clinically significant immunosuppressant effects will significantly slow down the symptom progression rate for this group of patients. This hypothesis clearly needs to be researched in order to verify that this will be the case.
- Plasmapheresis requires either good external venous access in both arms or an alternative way of having reliable long-term access to veins. Normal external venous access is the preferred method when it is an option, but only some patients will be able to maintain good venous access over a long period of time. Standard external venous access is often complicated by obesity, advanced age, or overall health problems. When external venous access is not an option, two alternative methods are available: a central venous catheter or a central venous fistula. While creating a fistula is a more involved surgical procedure, it carries lower risk than a venous catheter and is thus more suitable for the long-term access that would be needed for effective disease control.
- Plasmapheresis is a relatively expensive procedure. A recent study (Winters et al. 2011) put the fully loaded actual cost of a single TPE treatment at about \$1200 per treatment. This is about what Medicare reimburses for a single TPE treatment currently. Assuming a treatment frequency of about 16 treatments per year as suggested by the pilot studies and anecdotal reports, annual cost would be about \$20,000 per year. While this is a significant cost, it is slightly lower than the average annual cost for biologic drugs such as adalimumab (Humira), infliximab (Remicade), and etanercept (Enbrel) that are now commonly used to treat RA and other autoimmune diseases.

Current Medicare Guidelines (Centers for Medicare & Medicaid Services National Coverage Determination 110.14) indicate that plasmapheresis is covered for "*Treatment of life threatening scleroderma and polymyositis when the patient is unresponsive to conventional therapy*". Most insurance plans (but not all) follow Medicare coverage guidelines. For example, the current (May 2014) Aetna insurance guidelines for covering plasmapheresis treatments for Scleroderma is similar: "*Scleroderma and polymyositis, in persons who are unresponsive to conventional therapy*". This suggests that coverage for plasmapheresis treatments should be available for most patients but it may take some negotiation with Medicare or insurance companies to achieve this. Ultimately, since no conventional therapy is effective for treating Scleroderma, this should be a strong arguing point. The author of this paper is aware of one recent case

where Medicare readily approved plasmapheresis coverage for a patient that had been receiving long-term plasmapheresis therapy for Scleroderma without requiring additional documentation.

- Plasmapheresis treatments are usually performed in an outpatient hospital setting. While plasmapheresis is typically available in larger hospitals, smaller community and rural hospitals may not have the necessary equipment in-house, requiring some patients to travel to a larger hospital. However, with a limited treatment frequency such as 16 times a year, this may still be a reasonable treatment option for suitable patients even if they need to travel some distance to receive these treatments.

Overall, in contrast to standard immunosuppressive treatments that can have significant short-term side effects with the potential of major long-term complications, plasmapheresis treatments are generally very well tolerated with only minor side effects immediately during and after each treatment and no known long-term complications at the suggested treatment frequency of around 16 treatments per year. The preliminary research results as well as anecdotal reports indicating long-term efficacy of regular plasmapheresis treatments, suggests that this is a treatment option that should be considered, especially for early stage limited Scleroderma patients with suitable insurance coverage and local treatment availability.

## **Alternative Approaches to Treating Scleroderma Blood Hyperviscosity**

While plasmapheresis is the only specific intervention for Scleroderma-related red blood cell hyperaggregation that has been studied in a research setting, there are some alternative treatment approaches to reducing red blood cell clumping that should be studied. It is important to note that these alternative approaches to reducing red blood cell clumping have not been tested with Scleroderma patients, so even though research indicates that these treatments appear to reduce red blood cell aggregation, these findings may not generalize to Scleroderma-specific red blood cell hyperaggregation.

These alternative approaches are discussed in more detail later in this paper in the section titled *Directions for Further Research*:

- Nattokinase is a fibrinolytic enzyme (compound that dissolves blood clots) that is extracted from natto – a vegetable cheese-like food made from fermented soybeans that has been popular in Japan for more than 1000 years. A recent study (Pais et al. 2006) showed dose dependent decrease in red blood cell aggregation as well as overall plasma viscosity.
- Purified poloxamer 188 is an FDA approved drug that lowers blood viscosity, decreases red blood cell aggregation, and decreases friction between red blood cells and vessel walls to increase microvascular blood flow and decrease cell injury. While it has mostly been researched as a potential treatment for sickle cell disease, it may have potential to treat Scleroderma by reducing red blood cell aggregation.
- Trental (Pentoxifylline) is a drug known to improve red blood cell flexibility. It is also known to improve tissue oxygenation and also reduce RBC aggregation.
- There is some data that suggests that Intravenous Laser Blood Irradiation Therapy can temporarily reduce red blood cell aggregation.

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## ***Hyperviscosity: Directions for Future Research***

### **General Issues**

#### ***Endothelial Damage – Biomechanical or Biochemical***

One of the major unanswered questions in Scleroderma research is whether the antibodies associated with systemic Scleroderma actually cause the development of Scleroderma related symptoms (pathogenic theory) or instead are merely a marker of the underlying disease (epiphenomena theory). The above-mentioned research on hyperviscosity, endothelial damage, and plasmapheresis is consistent with the pathogenic theory but is not definitive. A single plasmapheresis treatment removes 80% to 85% of *everything* in the blood except for red blood cells, white blood cells, and most of the platelets. This includes Scleroderma-related antibodies (as well as beneficial antibodies) but also might include cofactors also circulating in the blood that are actually the direct cause of the red blood cell hyperaggregation.

The hypothesis advanced in this paper is that the cascade of events leading to Scleroderma symptom development and progression starts from repeated biomechanical damage to the endothelial layers of the microcapillaries resulting from clumped groups of red blood cells being forced by blood pressure through microcapillaries that are sized to barely pass a single red blood cell at a time. The basic research that shows that plasmapheresis eliminates red blood cell hyperaggregation for a significant period of time following a series of treatments does not directly answer the question as to whether or not this hypothesis is correct. The fact that this treatment approach leads to symptom improvement is definitely consistent with the red blood cell hyperaggregation/biomechanical damage hypothesis. However, it can also be argued that the fact that plasmapheresis also eliminates a significant percentage of circulating (potentially pathogenic) autoantibodies or some other cofactor is also consistent with an alternative hypothesis that it is simply the reduction in the number of circulating antibodies that is somehow leading to the symptom improvement.

#### ***Support for Biomechanical Endothelial Damage Hypothesis***

While certainly far from definitive, some of the results from the series of early plasmapheresis treatment studies do, in fact, support the red blood cell hyperaggregation / biomechanical damage hypothesis directly. One of the more striking findings in these pilot studies was that a series of four weekly plasmapheresis treatments quickly eliminated Raynaud's symptoms from almost all of the test subjects, including patients with long standing Raynaud's secondary to Scleroderma. With alternative treatments that reduce the number of circulating antibodies, e.g., immunosuppressant therapies, this rapid elimination of Raynaud's does not occur.

Raynaud's symptoms occur from the blood vessels spasming and dramatically restricting blood flow for a period of time. The early research on plasmapheresis and Raynaud's demonstrated that there is almost no red blood cell clumping in patients with primary Raynaud's (in contrast to those with Raynaud's secondary to Scleroderma). It also showed that plasmapheresis had little effect on primary Raynaud's symptoms but was very effective in reducing secondary Raynaud's symptoms. This suggests very different mechanisms of action in the two different forms of Raynaud's. It is entirely reasonable that if the endothelial damage is caused by clumped red blood cells, this could easily lead to hyper-sensitive blood vessels that spasm and restrict blood flow, resulting in classic Raynaud's symptoms. However, if red blood cell clumping is eliminated, you would expect

that healing would occur fairly rapidly since there would be no more trauma to the endothelium and this would be likely to reduce or eliminate the Raynaud's symptoms. In contrast, any treatment that gradually reduces the number of circulating autoantibodies might result in a corresponding gradual improvement in Raynaud's symptoms, but would not occur as rapidly. One of the earliest studies of using plasma exchange for treating patients with Raynaud's syndrome (O'Reilly et al. 1979) could not explain why short-term reduction in presumed circulating pathogenic factors from one series of four weekly plasmapheresis treatments could lead to long-term symptomatic and quantitative improvements. Since later research has shown that a single four week plasmapheresis treatment series results in elimination of RBC aggregation for a typical six-month or longer time period, this lends support to the hypothesis that direct mechanical elimination of RBC clumping accounts for long-term systemic improvements rather than the more indirect effective of temporarily reducing possible circulating pathogenic factors.

There is, in fact, a fairly easy way to do the research needed to determine if the improvement in Scleroderma-related symptoms seen with plasmapheresis treatments is a function of the mechanical separation of clumped red blood cells or more a result of reduced antibody levels. Basically, any alternative treatment approach that: 1) reduces or eliminates red blood cell hyperaggregation for a period of time, *and* 2) leads to an objective reductive in Scleroderma-related symptoms *without* reducing overall circulating antibody levels, then this would lend strong support to the hypothesis that the endothelial cell damage is, in fact, a direct result of biomechanical damage from clumped red blood cells – not because of the reduction of circulating autoantibody levels.

Normal plasmapheresis treatments, as mentioned above, work as follows:

- Blood is continuously extracted and run through a centrifuge that separates out red blood cells and white blood cells from the plasma. This results in separation of the aggregated red blood cells. This disaggregation may be a direct result of the mechanical process of separating out the red and white cells, or this could potentially be the result of the removal of a plasma component that is leading to the clumping, thus allowing the red blood cells to separate.
- The plasma (containing the Scleroderma-related and other antibodies) is discarded.
- The extracted red and white blood cells are combined with either sterilized albumin or donated plasma and re-circulated back to the patient.

Instead of discarding the patient's plasma and replacing it with new autoantibody-free donated plasma or sterilized albumin, it is actually very easy to modify the plumbing on the equipment used to perform plasmapheresis to instead recombine the patient's original plasma with the red and white blood cells that have been separated out by the centrifugal process. These red cells would no longer be clumped, but since the original plasma is maintained, the level of circulating autoantibodies will not be reduced, as is the case with normal plasmapheresis treatments. This procedure is known as autologous plasmapheresis. If Raynaud's symptoms are eliminated or significantly improved using this altered plasmapheresis intervention, then this would definitely show that the improvement was a result of the elimination of red blood cell clumping rather than a reduction in circulating antibodies.

It turns out that a very early pilot study that used plasmapheresis to treat a small group of patients with scleroderma and Raynaud's used autologous plasma exchange as a control group with the expectation that it would act as a placebo control treatment (McCune et al. 1983). However, the researchers were surprised that improvements in pulse volume, digital blood pressure, and skin temperature occurred in some of the patients with both the

standard plasma exchange procedure and also with the "placebo" plasma exchange. Since both regular and autologous plasmapheresis disaggregate the red blood cells, this result is not surprising if RBC disaggregation is the primary mechanism of action from plasmapheresis treatments rather than reduction in circulating antibody levels.

One other area of research on scleroderma treatments lends strong indirect support to the disaggregation hypothesis. A number of studies (Di Spaltro et al. 1993, Krasagakis et al. 1998, Rook et al. 1992, Wollina et al. 1999, and Knobler et al. 2006) have looked at using extracorporeal photopheresis (ECP) as a way to treat systemic scleroderma patients. Extracorporeal photopheresis is used primarily to treat T-cell lymphomas or diseases such as graft-versus-host disease that can occur with organ transplants. The process is similar to plasmapheresis in that the patient's blood is withdrawn intravenously, cells are separated out by mechanical centrifuging, and the extracted white blood cells are treated with a drug called psoralen which makes T-lymphocytes more sensitive to ultraviolet light. The treated cells are then treated with UV light, causing death of diseased cells, and then returned to the patient's body. In almost all studies of ECP for treating patients with systemic scleroderma, photopheresis has been found to be beneficial, although many of the studies were pilot studies done with patients having differing specific scleroderma diagnoses or at differing stages of the disease process and the outcome measures varied widely among the various studies.

What is particularly noteworthy is that in *all* of these studies, you will see a statement such as "the mechanism by which photopheresis is of therapeutic benefit in scleroderma is unclear at present" (Knobler et al. 2006). While a few of these studies offer theoretical explanations that might account for some of the beneficial effects, it is very clear from reviewing these studies that the researchers really have no idea why ECP seems to lead to symptom improvements. However, if the beneficial effects of ECP are viewed from the perspective of disaggregating clumped red blood cells, the observed therapeutic benefits make complete and logical sense since photopheresis, like plasmapheresis, uses a centrifugal blood separation process that should lead to the same type of mechanical disaggregation of red blood cells that has been documented with plasmapheresis. More important, photopheresis does *not* reduce circulating antibody levels, in essence making ECP analogous to the proposed autologous plasmapheresis approach outlined above and adding support to the hypothesis that most of the beneficial effects of plasmapheresis are from RBC disaggregation instead of antibody reduction.

### ***Potential Additional Benefit from Reduction of Circulating Antibody Levels?***

While a major hypothesis advanced in this paper is that the primary benefit seen from treating scleroderma patients with plasmapheresis is from RBC disaggregation rather than from antibody reduction, it is important to note that if the circulating antibodies are in fact pathogenic, it would be expected that reducing antibody levels using standard plasmapheresis would result in a longer-lasting beneficial therapeutic effect than would be expected using autologous plasmapheresis or similar procedures which do not reduce circulating antibody levels. Early plasmapheresis studies that studied the impact of plasmapheresis treatments on Raynaud's symptoms indicated that the Raynaud's symptoms generally did not reappear for at least several months following a series of four weekly standard plasmapheresis treatments. It would be reasonable to expect that the red blood cell clumping would reoccur more rapidly with autologous plasmapheresis treatments, resulting in a return of Raynaud's symptoms in a shorter period of time. However, since the primary goal of a controlled study comparing the effects of standard versus autologous plasmapheresis treatments on RBC aggregation would be to determine whether or not the elimination of Raynaud's symptoms is mostly a function of eliminating red blood cell clumping or instead reducing circulating antibody levels, the duration of the

response is not of paramount importance in separating out the exact mechanisms of action with plasmapheresis. However, if it can be shown that there is a clear benefit to using a procedure that temporarily reduces antibody levels in addition to disaggregation clumped RBCs, then the higher cost of standard plasmapheresis as compared to autologous plasmapheresis may be justified in some cases.

### ***Hyperviscosity Characteristics for Different Antibody Types***

Basic initial research needs to be done to look at the blood hyperviscosity characteristics of scleroderma patients with different antibody variants, including the degree of red blood cell clumping (if abnormal) as well as the “stickiness” of the aggregated cells. It is possible that different antibody subtypes might have different aggregation properties. For example, even if two different subtypes show a similar degree of clumping when the heart is resting (diastolic blood pressure), if one subtype is more “sticky” than the other subtype, then when the heart is pumping (systolic blood pressure), those clumped red blood cells would be less likely to break apart - potentially causing more damage to the endothelial cells.

A recent paper (Muangchan et al. 2012) lends support to the hypothesis that different variants of scleroderma may have different degrees of RBC aggregation. While the main focus of this article was on the association between C-Reactive Protein (CRP) and disease activity in scleroderma, it included data showing that patients with diffuse scleroderma had much higher erythrocyte sedimentation rates (ESR) than patients with limited scleroderma. ESR is highly correlated with RBC aggregation and can thus be considered to be strong proxy measure of RBC aggregation (Baskurt et al. 2011). While speculative at this point, this could potentially explain some of the differences in symptom development between limited and diffuse scleroderma patients. If RBC aggregation consists of smaller and fewer clumps in patients with limited scleroderma, then this could lead to slower rates of endothelial damage and subsequent slower development of Raynaud’s and other symptoms. In contrast, with diffuse scleroderma, early symptoms often include severe fatigue and pain. If the RBC aggregation in diffuse scleroderma is characterized by larger clumps of red blood cells, this could directly lead to capillary blockage similar to what is seen in sickle cell anemia crisis, which shares similar symptoms of anemia-based fatigue and pain from the directly impaired circulation.

The Muangchan paper also documents that ESR (RBC aggregation by proxy) is higher in early stages of diffuse scleroderma (< 3 years) than it is in later stages (> 3 years). One of the commonly observed characteristics of diffuse scleroderma is an improvement in skin scores after the first few years. Assuming the overall model advanced in this paper is correct – that RBC aggregation directly leads to the vascular damage seen in scleroderma – then this later reduction in ESR/RBC aggregation could provide one possible explanation for this phenomenon.

Any studies of hyperviscosity characteristics should be separated into antibody specific patient subsets. Initially, anti-centromere (ACA) positive and anti-Scl70 patient groups are the most easily identified for research purposes. Ideally, patients with anti-RNA Polymerase III antibodies should also be studied as an additional subtype since research suggest that this antibody profile occurs with a similar frequency to ACA and anti-Scl70 antibodies.

A company called Health Onvector makes a new generation blood analyzer (SCV-200) that can measure both viscosity and stickiness. This would be ideal for this type of study. Alternative systems may well exist that can be considered as well.

## ***Is Hyperviscosity Universal in Systemic Scleroderma?***

The question as to whether all patients with systemic scleroderma have increased blood viscosity, specifically red blood cell aggregation, is key to determining if the disease pathogenesis model proposed in this paper is valid or not. If the triggering mechanism for endothelial damage and later systemic vascular damage is RBC aggregation, then all systemic scleroderma patients would need to have at least a small degree of RBC aggregation. As is noted earlier, even small clumps of RBCs could cause damage over time while larger clumps could lead to much more rapid symptom development.

Currently, physicians do not routinely test directly for blood viscosity in scleroderma patients, much less red blood cell aggregation, but they do frequently test for ESR. The ESR value is usually interpreted as a marker of disease activity. But since ESR is also a direct proxy measure for RBC aggregation, monitoring ESR may be useful way to see if treatments lead to reduced ESR and (presumably) reduced RBC aggregation.

However, it is important to note that ESR does *not* need to be above the normal range to indicate potential RBC aggregation. To illustrate, in the case study discussed above (Weiss et al. 2015), Table 1 below shows the patient's ESR values before starting plasmapheresis treatments and over the next several years of continuous regular treatments.

**Table 1**

Date	ESR (mm/h)
11/20/91	13
Post-Treatment	
9/13/94	10
6/23/95	6
8/27/03	4
11/9/06	4
6/18/09	4

The initial ESR value of 13 is well below the top of the normal range (< 20). However, note the marked reduction in ESR value during treatments. By implication, these results suggest that even though the ESR value was always in the normal range, the patient did have some degree of RBC aggregation before starting treatments and that regular plasmapheresis treatments appears to have lead to a reduction to a “true” ESR value of 4 with completely or mostly disaggregated red blood cells.

## ***Replication of Earlier Plasmapheresis Research***

While plasmapheresis is far from an ideal treatment option even for limited Scleroderma patients for reasons cited above (venous access, cost, availability), nevertheless it still may be the best treatment option for some patients. For this reason, it is important to redo some of the earlier research using modern plasmapheresis equipment (which has fewer side effects than earlier generations of equipment) and better methods of measuring changes in viscosity. In order to make the interpretation of initial research results as clean and unambiguous as possible, patients should be ANA and antibody positive and results should be separated out by antibody type.

## ***Pharmaceutical Approaches to Treating Scleroderma Hyperviscosity***

It is very important to understand that Scleroderma-related blood hyperviscosity is very different than the more common forms of blood hyperviscosity that result from excess plasma proteins, white blood cells, or platelets instead of the red blood cell

hyperaggregation that research suggests appears to be the case with Scleroderma patients. Because of this, initial drug trials need to focus on drugs that specifically have been shown to reduce red blood cell aggregation, for example, nattokinase, poloxamer 188, and Trental (pentoxifylline):

### **Nattokinase**

Nattokinase is a fibrinolytic enzyme (compound that dissolves blood clots) that is extracted from natto – a vegetable cheese-like food made from fermented soybeans that has been popular in Japan for more than 1000 years. Nattokinase was isolated from Natto in 1980 by Dr. Hiroyuki Sum at the University of Chicago Medical School. A number of studies, both in vivo and in vitro, have consistently shown that nattokinase has strong pro-fibrinolytic beneficial effects, including delaying clotting, platelet aggregation and the development of blood clots. In addition, a recent study (Pais et al. 2006) looked at the effects of nattokinase on red blood cell aggregation. This study showed dose dependent decrease in red blood cell aggregation as well as overall plasma viscosity. Nattokinase is sold as an over-the-counter supplement in the US but is considered a drug and less available in some other countries. While nattokinase is generally considered very safe, it can interact with anticoagulant drugs such as warfarin and heparin and should only be used under medical supervision by patients with bleeding disorders.

Since nattokinase is generally considered very safe and is widely available in the US as an over-the-counter supplement at a very low price, it would be relatively easy and inexpensive to conduct an initial research study to look at its safety and efficacy in a small group of Scleroderma patients. An open label pilot study could look at the effects of daily administration of two different dosage levels over a limited time period (3 to 6 months). The primary dependent measure would be changes (if any) in total blood viscosity and red blood cell aggregation. Additional dependent measures would be changes in modified Rodnan skin scores as well as self-reported changes in Raynaud's and GI symptoms. If no reduction of red blood cell aggregation is evident after an initial 3-month trial period, then nattokinase is likely to be ineffective in treating Scleroderma.

### **Vepoloxamer (Purified Poloxamer 188)**

Vepoloxamer (Mast Therapeutics, Inc.) is an investigational agent with potential utility in a wide range of diseases that are characterized by impaired blood flow. The active ingredient in Vepoloxamer is a purified form of poloxamer 188. Research has shown that poloxamer 188 lowers blood viscosity, decreases red blood cell aggregation, and decreases friction between red blood cells and vessel walls to increase microvascular blood flow and decrease cell injury. While it has mostly been studied as a potential treatment for sickle cell disease, it may also have potential to treat Scleroderma by reducing red blood cell aggregation, inflammation, and restoring cell membrane integrity. A Phase 3 study of using Vepoloxamer to treat sickle cell disease is currently underway.

Poloxamer 188 is considered safe and is widely used in many over the counter products such as toothpaste, laxatives, and mouthwash. The FDA approved it more than 50 years ago as a therapeutic reagent to reduce viscosity of blood before it is used in transfusions. However, similar safety studies have not been specifically done on Vepoloxamer and it does not currently have FDA approval outside the scope of the current Phase 3 clinical trial mentioned above.

### **Trental (Pentoxifylline)**

Pentoxifylline is a drug primarily used for treating chronic occlusive peripheral vascular disorders by lowering blood viscosity and improving red blood cell flexibility. It is known to improve tissue oxygenation and also reduce RBC aggregation.

## ***Non-pharmaceutical Approaches to Treating Scleroderma Hyperviscosity***

### **Laser Blood Irradiation Therapy**

There is some data that suggests that Intravenous Laser Blood Irradiation Therapy can temporarily reduce red blood cell aggregation. Since this effect only lasts for a few hours, it would presumably require frequent treatments in order for this approach to potentially be effective in treating Scleroderma patients. While this is clearly not practical with an intravenous approach, a Canadian company (Vielight, Inc.) has developed an intranasal device that they claim can achieve the same results using a device which irradiates circulating blood through the inside of the nasal cavity.

Because of the uncertainty of whether the short documented duration of decreased red blood cell aggregation is likely to be effective in Scleroderma patients, an initial pilot study should be limited to a small group of patients in order to determine if this approach will be effective.

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## ***Summary***

The focus of this paper is to propose a new disease pathogenesis model for Scleroderma that, if correct, can potentially lead to the development of new treatments that are likely to be significantly less toxic than current immunosuppressive treatments and have a much greater potential for safely preventing or significantly delaying symptom progression than occurs with current treatment approaches.

- In 1979, researchers proposed that essentially all of Scleroderma symptom development and progression can be accounted for as a cascade of events that starts with repeated trauma to the endothelial layer of cells that line blood vessels. No specific cause of the repeated trauma was proposed in this initial paper. This theory is now generally accepted, but there is no clear explanation for the mechanism of endothelial damage.
- A number of research studies, beginning with an initial study in 1975, have consistently shown abnormally elevated blood viscosity in the majority of systemic Scleroderma patients. When reported, the specific type of blood hyperviscosity documented in these studies is red blood cell hyperaggregation, not the more common platelet aggregation that is seen in most other hyperviscosity disorders.
- The smallest micro-capillaries are on average about 8 microns in diameter (range 5 to 10 microns). Normal red blood cells are 6 to 8 microns in diameter. This means that only one red blood cell at a time can pass through micro-capillaries. This paper hypothesizes that the endothelial damage seen in Scleroderma is a direct result of biomechanical damage that occurs when clumped red blood cells are repeatedly forced through the microcapillary system by hydraulic force from normal blood pressure.
- If this disease model is correct, then *any* treatment that can either prevent red blood cell hyperaggregation or break it apart once it has already occurred should lead to an effective way to prevent Scleroderma symptoms from progressing, potentially allowing the body to heal over time. If a treatment can be started early enough in the disease process, it should be able to prevent symptoms from developing at all. However, since this treatment approach does not prevent the development of additional autoantibodies, it would need to be continued on a permanent basis. A good analogy would be the use of the insulin to prevent or delay diabetes complications from developing over time.

- Plasmapheresis is considered the “gold standard” treatment for hyperviscosity syndromes. A series of early research studies have demonstrated that a series of plasmapheresis treatments can eliminate the red blood cell clumping and associated hyperviscosity in Scleroderma patients for a number of months. These studies also indicated that secondary Raynaud’s symptoms were typically completely eliminated following a series of treatments and that patients experienced no digital ulceration during the follow-up period.
  - The results of these plasmapheresis studies directly support the hypothesis that Scleroderma symptoms are triggered by endothelial damage resulting from biomechanical trauma. However, since plasmapheresis treatments also significantly reduce circulating autoantibody levels in addition to eliminating red blood cell clumping, this does not eliminate the possibility that symptom improvement following plasmapheresis treatments could instead be a result of the reduced circulating antibody levels. These competing explanations can easily be tested using a modified plasmapheresis procedure that can eliminate RBC clumping without reducing circulating antibody levels.
  - While plasmapheresis treatments may be an appropriate treatment option for some Scleroderma patients, they are not ideal for treating other patients because of cost, venous access issues, availability, and limited effectiveness in diffuse Scleroderma. This suggests that alternative approaches to eliminating red blood cell clumping need to be researched, including pharmaceutical approaches.
  - Three drugs have been shown in preliminary research studies to reduce red blood cell aggregation: nattokinase, Poloxamer 188, and Trental (pentoxifylline). These drugs merit further study to see if they reduce scleroderma-related red blood cell aggregation and might lead to pharmaceutical alternatives to plasmapheresis.
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## ***Annotated Reference List***

1. Kahaleh MB, Sherer GK, LeRoy EC. Endothelial injury in scleroderma. *Journal of Experimental Medicine*. 1979; 149(6); 1326-1335.

This was the first study that speculated that many, if not all, of the manifestations of scleroderma could be explained on the basis of damage to the endothelium resulting from repeated trauma.

2. Tietjen GW, Chien S, Leroy EC, Gavras I, Gavras H, Gump FE. Blood viscosity, plasma proteins, and Raynaud syndrome. *Arch Surg*. 1975; 110(11); 1343-6.

This was the first study that looked at several different measures of blood viscosity with a group of 20 patients that had Raynaud’s syndrome, almost all of which also had a documented autoimmune disease. Sixteen of the 20 patients had scleroderma. The results showed that both whole blood viscosity and specifically red blood cell aggregation were significantly elevated ( $p < .01$ ) vs. a control group.

3. Anderson S, King A J, Brenner B M. Hyperlipidemia and Glomerular Sclerosis: An Alternative Viewpoint. *The American Journal of Medicine* 1989; 87; 5-34N - 5-38N.

This paper suggests a direct causal link between progressive glomerular injury and hyperviscosity syndrome, as noted in several disorders.

4. Jacobs M J H M, Breslau P J, Slaaf D W, Reneman R S, Lemmens J A J. Nomenclature of Raynaud's phenomenon: A capillary microscopic and hemorheologic study. *Surgery* 1987; 101; 136-145.

This study verified that a key characteristic of secondary Raynaud's phenomenon is increased blood viscosity. It suggests that the increased viscosity may be the causative factor in secondary Raynaud's phenomenon, while it is absent in primary Raynaud's phenomenon.

5. Jacobs M J H M, Jorning P J G, von Rhede van der Kloot E J H, Kitslaaar P J E H M, Lemmens, H A J, Slaaf D W, Reneman R S. Plasmapheresis in Raynaud's phenomenon in systemic sclerosis: a microcirculatory study. *International Journal of Microcirculation: Clinical and Experimental* 1991; 10; 1-11.

This is a very important paper that directly measured a variety of microcirculatory and hemorheological parameters before and after plasmapheresis in patients with Raynaud's secondary to SSc. They performed 4 plasmapheresis treatments with 2 liters of plasma weekly. Follow-up was done 3, 9, 24, and 36 months following treatment. All (18) patients claimed explicit improvement of their complaints. Raynaud's phenomenon had disappeared and skin ulcers healed. RBC velocity increased significantly, resulting in significantly reduced RBC aggregation and plasma viscosity.

After 3 years four patients were still free of complaints, but in 14 patients the systems had reappeared between 6 and 9 months after the last treatment, although no ulcers had reoccurred. RBC aggregation and plasma viscosity returned to their pre-treatment abnormal levels after about 9 months, while skin capillary blood flow remained significantly enhanced for 24 months.

6. Neumann H A M, Van den Broek M J T B. Increased collagen IV layer in the basal membrane area of the capillaries in severe chronic venous insufficiency. *VASA* 1991; 20; 26-29.

While this paper focuses on chronic venous insufficiency, it suggests a mechanism wherein increased capillary hypertension (which could result from chronic increased plasma viscosity) induces dilatation and a tortuous course of the capillaries. This is followed by capillary leakage allowing water and other plasma components to accumulate in the interstitium (the swelling stage in scleroderma?). The study found significant increase of the collagen IV layer in and/or around these capillaries.

7. von Rhede van der Kloot E J H, Jacobs M J H M, Weber H, Lemmens H A J. Plasma Filtration in Patients with Raynaud's Phenomenon. *Clinical Hemorheology* 1985; 5; 79-84.

This is the report of the pilot study that preceded the 1991 study. The findings were similar to the later paper, although no long-term follow-ups were done. One additional finding was reported - the skin that had been tough and tense before filtration became supple and pliable on the face and hands.

8. Weber H, Schmid-Schonbein H, Lemmens H A J. Plasmapheresis as a Treatment of Raynaud's Attacks: Microrheological Differential Diagnosis and Evaluation of Efficacy. *Clinical Hemorheology* 1985; 5; 85-97.

This was an early paper that documented the differences in plasma hemorheology between primary and secondary Raynaud's phenomenon and a control group, as well as the differential effect of plasmapheresis on the different groups. They found that primary Raynaud's patients had slightly but significantly higher overall plasma viscosity than normal controls and that secondary Raynaud's patients had

slightly but significantly higher overall plasma viscosity than the primary Raynaud's patients. However, when they looked specifically at RBC aggregation, they found that RBC aggregation was the same for the control group and primary Raynaud's patients, but highly elevated ( $p < .0015$ ) for the secondary Raynaud's.

9. Cozzi F, Marson P, rosada M, DeSilverstro G, Bullo A, Punzi L, Todesco S. Long-term Therapy with Plasma Exchange in System Sclerosis: Effects on Laboratory Markers Reflecting Disease Activity. *Transfusion and Apheresis Science* 2001; 25; 25-31.

This more recent paper looked at changes in a number of laboratory markers following relatively long term plasmapheresis. The study has several flaws, including the fact that the control group was not a good match for the treatment group. Also, the treatment group combined plasmapheresis with D-penicillamine vs the control group that was treated with D-penicillamine alone. The treatment group was significantly worse from a symptom and laboratory marker than the control group. Nevertheless, there were significant improvements in laboratory markers and clinical scores in the treatment group while markers and clinical scores remained or worsened in the D-penicillamine only group.

10. Volkov S, Utset TO, Schmitz A, Ellman M, Cao D, Sweiss N. Whole Blood Viscosity (WBV) and Digital Ulcers in Scleroderma Patients. *Fellow's Abstract F56, 2006 ACR/AHRP Annual Scientific Meeting, Washington, DC.*

This recent study showed highly significant increased whole blood viscosity (WBV) in a randomly selected group of scleroderma patients, including 19 with diffuse scleroderma and 14 with limited scleroderma. It also documented that the WBV was also very significantly elevated in the patients with active digital ulceration vs. those with no history of digital ulceration.

11. M A McGrath, R Peek, R Penny. Blood hyperviscosity with reduced skin blood flow in scleroderma. *Annals of the Rheumatic Diseases* 01/1978; 36(6):569-74.

This was an early study that measured hyperviscosity in a randomly selected group of scleroderma patients. In addition to detecting significant overall blood hyperviscosity in 70% of the patients at low shear rates, there was also a significant increase in plasma viscosity suggesting increased fibrinogen and immunoglobulins as the cause of the hyperviscosity.

12. Kahaleh B. (2008). The microvascular endothelium in scleroderma. *Rheumatology (Oxford)*. 47 Suppl 5:v14-5.

This article notes that vascular endothelial injury in scleroderma leads to a host of pathological changes in the blood vessels that adversely impact the physiology of many organ systems and eventually results in a state of chronic state of reduced blood flow to the tissues.

13. Pais E, Alexy T, Holsworth RE Jr, Meiselman HJ. Effects of nattokinase, a pro-fibrinolytic enzyme, on red blood cell aggregation and whole blood viscosity. *Clinical Hemorheology and Microcirculation* 2006: 35(1-2); 139-42.

This study showed a dose-dependent reduction in red blood cell aggregation and low-shear overall blood viscosity when in vitro blood samples were treated with nattokinase. This replicated previous findings from in vivo animal trials.

14. Haak AJ, Tsou P, Amin M, Ruth JH, Campbell P, Fox, DA, Khanna D, Larsen, SD. Targeting the myofibroblast genetic switch: inhibitors of MRTF/SRF-regulated gene transcription

prevent fibrosis in a murine model of skin injury. *The Journal of Pharmacology and Experimental Therapeutics* 2014; 114.213520.

This study explored a novel antifibrotic drug that appears to inhibit development of skin thickening and collagen formation and may have potential as a new therapeutic approach to treating scleroderma and other fibrotic disorders. This is very early research and needs to be replicated and tested for safety and efficacy in scleroderma patients.

15. Toth K, Wenby RB, Meiselman HJ. Inhibition of polymer-induced red blood cell aggregation by poloxamer 188. *Biorheology*. 2000; 37(4); 301-12.

Previous studies have suggested that certain non-ionic poloxamers can reduce red blood cell aggregation in whole blood and in RBC-plasma suspensions. This study showed dose dependent reduction in both the degree of and the strength of red blood cell aggregation when combined with red blood cells that were artificially aggregated. Previous studies done in vivo have shown that sickle cell patients receiving Poloxamer 188 had decreased pain and decreased hospitalization, compared to controls.

16. Dodds AJ, O'Reilly MJ, Yates CJ, Cotton LT, Flute PT, Dormandy JA. Haemorrhological response to plasma exchange in Raynaud's syndrome. *British Medical Journal*. 1979; 2(6199): 1186–1187.

Eight patients with Raynaud's syndrome were treated by weekly plasma exchange for four weeks. The mean whole-blood viscosity was significantly lower after treatment, and the mean index of red-cell deformability was significantly improved. All patients noticed symptomatic improvement including healing of digital ulcers.

17. Vicaut E. Opposite effects of red blood cell aggregation on resistance to blood flow. *The Journal of Cardiovascular Surgery*. 1995 Aug; 36(4): 361-8.

This study showed that hyperaggregation of RBC is responsible for a decrease in arteriolar blood flow velocity and for a decrease in density of perfused capillaries which may have important deleterious consequences for tissue oxygenation.

18. Piccini JP, Nilsson K. *The Osler Medical Handbook: Mobile Medicine Series, 2e*. Johns Hopkins Hospital. 2006 Aug;36(4):361-8.

19. McGrath MA, Peek R, Penny R. Blood hyperviscosity with reduced skin blood flow in scleroderma. *Annals of the Rheumatic Diseases*. 1977; 36(6): 569–574.

This study was carried out to determine whether abnormalities of blood viscosity occur in scleroderma. A unique finding was a delayed recovery of the blood flow after cooling. These observations suggest that the increased resistance to blood flow in skin affected by scleroderma may be caused by an interaction between the occlusive vascular lesion and blood hyperviscosity. In addition, blood flow patterns and hyperviscosity could help distinguish scleroderma from primary Raynaud's disease.

20. Słoczyńska K, Kózka M, Pękala E, Marchewka A, Marona H. In vitro effect of pentoxifylline and lisofylline on deformability and aggregation of red blood cells from healthy subjects and patients with chronic venous disease. *Acta Biochimica Polonica*. 2013; 60(1): 129-35.

This study showed that pentoxifylline improved RBC deformability and also significantly reduced RBC aggregation in patients with chronic venous disease.

21. O'Reilly MJ, Talpos G, Roberts VC, White JM, Cotton LT. Controlled trial of plasma exchange in treatment of Raynaud's syndrome. *British Medical Journal*. 1979 Apr 28;1(6171):1113-5.

This pioneering study compared plasmapheresis against heparin treatment and placebo in a total group of 27 patients with Raynaud's syndrome. Only the plasmapheresis group should improvement in symptoms and improvement of blood vessels. Improvements were maintained for six months in the treatment group. Notably, the researchers were surprised that the improvements were maintained for this long period of time from a short-term reduction in presumed circulating pathogenic factors.

22. McCune MA, Winkelmann RK, Osmundson PJ, Pineda AA. Plasma exchange: a controlled study of the effect in patients with Raynaud's phenomenon and scleroderma. *Journal of Clinical Apheresis*. 1983;1(4):206-14.

This early study of plasmapheresis treatments with a small group of scleroderma patients is notable in that it attempted to compare standard plasma exchange with a sham version of plasma exchange. The researchers were surprised to find that both standard plasma exchange and sham plasma exchange had a positive effect on improving circulation in some of the patients.

23. Krasagakis K, Dippel E, Ramaker J, Owsianowski M, Orfanos CE. Management of severe scleroderma with long-term extracorporeal photopheresis. *Dermatology*. 1998. 309-15.

This study looked at the effects of photopheresis on a group of 16 patients with severe scleroderma, with treatment periods ranging from 6 to 45 months. The study indicated that best results were obtained with patients that had symptoms for less than 2 years.

24. Di Spaltro, FX, Cottrill, C, Cahill, C, Degnan, E, Mulford, GJ, Scarborough, D, Franks, AJ, Klainer, AS, Bisaccia, E. Extracorporeal photochemotherapy in progressive systemic sclerosis. *Int J Dermatol*, 1993 32(6), 417-421.

This early, open label pilot study included a group of 9 patients that were treated with ECP for 2 days each month for 6 to 21 months. Significant improvements were noted in the skin, musculoskeletal system, functional index, and symptoms including Raynaud's, dyspnea, fatigue, dysphagia, as digital ulcers.

25. Rook AH, Freundlich B, Jegasothy BV, Perez MI, Barr WG, Jimenez SA, et al. Treatment of systemic sclerosis with extracorporeal photochemotherapy. Results of a multicenter trial. *Arch Dermatol*. 1992 Mar; 128(3): 337-46.

In this randomized, parallel-group, single-blinded study comparing photopheresis with D-penicillamine, overall results showed significantly greater improvement in the photopheresis group as compared with the D-penicillamine group. Improvements in skin score, percent skin involvement and oral aperture were significantly improved only in the photopheresis group.

26. Wollina U, Oelzner S, Looks A, Hipler UC, Knoll B, Lange D, et al. [Progressive systemic sclerosis - treatment results of extracorporeal photopheresis]. *Hautarzt*. 1999. 637-42.

In a non-randomized, uncontrolled study of 26 patients with systemic scleroderma treated over an 8 month period, the researchers reported that 18 patients experienced partial remission, 8 remained stable, and 1 patient showed slight progression.

27. Knobler RM, French LE, Kim Y, Bisaccia E, Graninger W, Nahavandi H, Strobl FJ, Keystone E, Mehlmauer M, Rook AH, Braverman I. A randomized, double-blind, placebo-controlled trial of photopheresis in systemic sclerosis. *J Am Acad Dermatol*. Volume 54. 2006. 793-799.

This recent well-designed double-blinded, placebo controlled study showed a statistically significant improvement in skin scores at 6 months and 12 months following a one-year series of photopheresis treatments in the active treatment group but not in the sham-treatment placebo group.

28. Weiss J, Roy M, Harris E S. Benefit of Long-Term Therapeutic Plasma Exchange Treatment in a Patient with CREST Syndrome (Limited Systemic Scleroderma): A 21-year Success Story. *Transfusion* September 2015. 55(S3): 103A. Presented as a poster at the AABB Annual Meeting Anaheim, CA, October 2015

This case study is the only known example where very long term plasmapheresis (21 plus years) has been used as the sole systemic treatment for a patient with anti-centromere positive limited scleroderma. The treatment frequency is 16 times per year. After three years of treatments, the patient achieved complete reversal of all symptoms, including early lung damage and severe GERD, except for mild residual Raynaud's. Temporarily stopping the treatments in 1996 lead to the return of GERD symptoms after six months but this was again reversed following resumption of regular treatments. The patient remains in remission and is in overall excellent health more than 25 years after being diagnosed with limited scleroderma.

29. Muangchan, Chayawee, et al. "Association of C-reactive protein with high disease activity in systemic sclerosis: Results from the Canadian Scleroderma Research Group." *Arthritis care & research* 64.9 (2012): 1405-1414.

This paper primarily looked at the correlation between CRP and disease activity in a pool of 1043 patients. They found that it is correlated with disease activity, severity, poor pulmonary function, and shorter survival. What is relevant to this paper is that they also documented erythrocyte sedimentation rates (ESR) and showed that ESR is noticeably higher in patients with diffuse scleroderma than limited scleroderma and this it is also higher in early stage diffuse scleroderma than later stage diffuse scleroderma. ESR is highly correlated with RBC aggregation, suggesting a greater degree of RBC aggregation in patients with diffuse scleroderma than in limited scleroderma.

30. Baskurt O, Björn N, Meiselman HJ. *Red Blood Cell Aggregation*. Boca Raton: CRC Press 2011.

"The first book to offer a comprehensive review of the subject, *Red Blood Cell Aggregation* tackles these and other questions related to red blood cell (RBC) aggregates. The book covers basic, clinical, and physiological aspects of this important biophysical phenomenon and integrates these areas with concepts in bioengineering. It brings together state-of-the-art research on the determinants, mechanisms, and measurement and effects of RBC aggregation as well as on variations and comparative aspects."

31. Winters JL, Brown D, Hazard E, Chainani A, Andrzejewski C. Cost-minimization analysis of the direct costs of TPE and IVIg in the treatment of Guillain-Barré syndrome. *BMC Health Serv Res*. 2011;11:101.

While this recent paper was focused on comparing the cost of TPE versus IVIg for treating Guillain- Barré syndrome, it did a detailed analysis of the actual costs for TPE treatments. Fully loaded costs were approximately \$1200 per treatment. This is almost exactly what Medicare reimbursement rates are for TPE treatments.

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## ***Revision History***

- 7/2014:** Initial version published
- 1/2015:** Added pentoxifylline as a proposed investigational drug for reducing RBC aggregation in scleroderma. Added mention of two early papers on plasmapheresis and scleroderma that lend support to the hypothesis that reduction in RBC aggregation from plasmapheresis may be the primary mechanism of action rather in improving scleroderma symptoms rather than reduction of possible circulating pathogenic factors.
- 3/2015:** Added additional research that lends support to hypothesis that RBC disaggregation is the primary mechanism of action from plasmapheresis treatments rather than antibody reduction.
- 11/2015:** Added additional research that lends support to the hypothesis that RBC aggregation is greater in diffuse scleroderma than limited scleroderma, potentially accounting for typical different presenting symptom profiles. This research also suggests a possible explanation for why there is often some symptom improvement seen after a few years in many patients with diffuse scleroderma. Added discussion of a recently published very-long-term case study showing successful treatment of a patient with limited scleroderma using plasmapheresis alone.
- 1/2016:** Updated the information on the average cost of plasmapheresis.