Pediatric connective tissue diseases

Looking through the skin and beyond

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PEDIATRIC CONNECTIVE TISSUE DISEASES

Looking through the skin and beyond

DIENEKE SCHONENBERG-MEINEMA

Pediatric connective tissue diseases:

looking through the skin and beyond

Dieneke Schonenberg-Meinema

The work in this thesis was performed at the Department of Pediatric Immunology, Rheumatology and Infectious Diseases at Amsterdam University Medical Centers, at the location of AMC/Emma Children's Hospital from the University of Amsterdam, The Netherlands. This thesis contains collaborative projects with Department of Rheumatology from Ghent University in Belgium and Department of Pediatric Rheumatology from Sophia Children's Hospital, Erasmus Medical Center, Rotterdam, The Netherlands.

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Pediatric connective tissue diseases:
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General introduction, aims and outline of thesis

GENERAL INTRODUCTION AND AIMS

Autoimmunity in children

Autoimmunity is a pathological phenomenon in the body when the immune system reacts with an inflammatory reaction to its own cells or organs, as if it were foreign. The word ‘auto-immune’ is a combined word from two ancient Greek and Latin words, meaning immunity (Latin) to itself (auto from Greek). Autoimmune diseases in children are fortunately rare but, when present, these diseases are often chronic and a lifelong burden. They may give limitations in normal growth and development during childhood, leading to invalidating disabilities in adult life. Moreover, these diseases often give limitations in normal daily activities such as school, sports and social activities.

In these last decades there has been an enormous growth in international collaborations between physicians and researchers in the field of pediatric rheumatology. Existing networks are expanding and cross-linking, leading to larger cohort studies. These collaborations are important in rare rheumatic diseases in children, for which new scientific insights and therapeutic developments are only implemented until long investigating and approving procedures are completed in adult patients. Much improvement has been made during the last decades in terms of early diagnoses and treatment options for (systemic) autoimmune diseases in children. This has led to a higher quality of life for patients with childhood-onset of chronic autoimmune diseases and led to better outcomes for these children in adulthood. Still, there are a lot of unmet needs as will be described below.

The autoimmune diseases that are studied in this thesis are childhood-onset systemic lupus erythematosus (cSLE), juvenile mixed connective tissue disease (jMCTD) and localized scleroderma (LS). The focus of this thesis is the identification of novel disease biomarkers, mainly in imaging, which may help to correctly classify these (systemic) autoimmune disease at an early stage of disease. The value of these different prosperous biomarkers are examined in terms of monitoring disease activity and risk for damage. Novel biomarkers have to lead to adapted personalized treatment choices and better outcomes for individual pediatric patients.

Systemic Lupus Erythematosus (SLE)

Incidence, prevalence and demographics

SLE is a systemic autoimmune disease which is chronic and thus lifelong. It often leads to severe inflammation with multi-organ involvement but there is a huge variety of clinical presentations. These presentations vary not only in type of organ involvement but also in severity of the inflammation from mild to very severe, and are sometimes even life-threatening (see figure 1). SLE is characterized by the presence of one or more auto-antibodies against nuclear antigens of cells, such as anti-nuclear antibodies (ANA) and anti-double stranded DNA (anti-ds-DNA), or against cell-surface antigens, for instance to red blood cells leading to haemolytic anaemia. The auto-antibodies in SLE lead to formation of immune complexes and a cascade of inflammatory responses leading to low complement levels of C3 and C4.

The incidence of adult-onset SLE (aSLE) is estimated up to 25 per 100,000 and the prevalence is 20-150 per 100,000 (1-5). SLE with a childhood onset (before the age of 18 years) is called childhood-onset SLE (cSLE). Fortunately, cSLE has a lower incidence than aSLE, of 1-2 per 100,000 and a prevalence of 2-25 per 100,000 children worldwide (5-7). Approximately 10-20% of all SLE cases start during childhood, and those childhood patients have a mean age of onset at 11-12 years (8). SLE occurs predominantly in women, the female:male ratio is approximately 7-15: 1 (1, 7).

The prevalence of SLE, in both adults and children, is higher *and* more severe in people with Asian, African-American, African-Caribbean and Hispanic background compared to white individuals (1, 9). In a recent epidemiologic study, black females had the highest prevalence of SLE (230 per 100,000) (3). It has also been shown that non-white patients are diagnosed at a significant younger median age (10, 11).

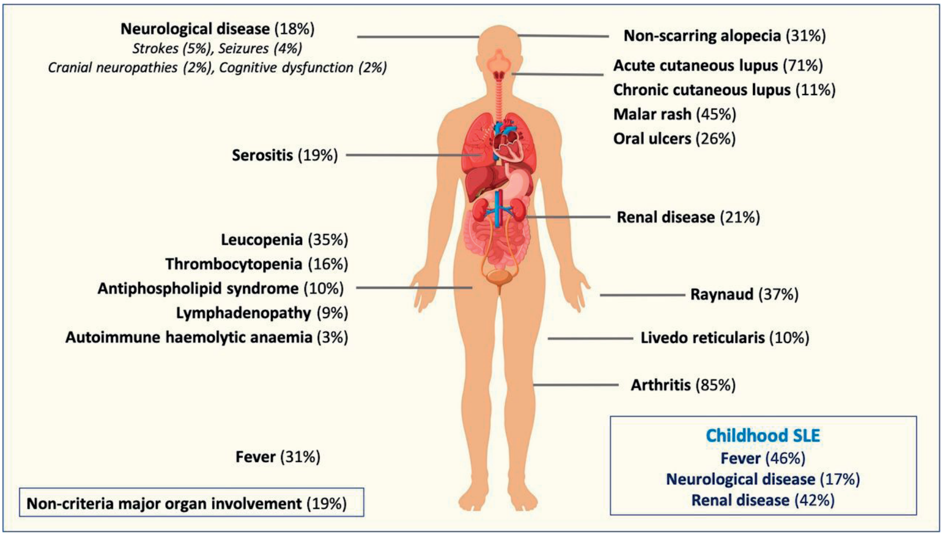

Figure 1. Multi-organ involvement In SLE (with consent from Fanouriakis et al. (12))

Classification criteria and disease activity scores

As mentioned above, SLE is a heterogeneous disease with many different presentations of (multi-)organ involvement. For high quality studies, a homogeneous study population is needed, or homogeneous subgroups. Over the years classification criteria for SLE have evolved and been adapted (figure 2). The Systemic Lupus International Collaborating Clinics (SLICC) 2012 criteria were proposed because of some limitations in the earlier ACR 1997 criteria (13). The SLICC 2012 criteria defined 17 criteria of which a minimum of 4 should be present with a minimum of 1 (of 11) clinical and 1 (of 6) immunological criteria (14). Presence of lupus nephritis with presence of ANA or anti-double-stranded DNA (anti-ds-DNA) antibodies without other criteria was also qualified for SLE classification. The SLICC 2012 criteria were validated and showed a higher sensitivity than ACR 1997 criteria (97 versus 83%) but a lower specificity (84 versus 96%) (14). In 2019, the new weighted EULAR/ACR criteria for SLE were published (15). These EULAR/ACR classification criteria start with the presence of ANA and thereafter other criteria (7 clinical and 3 immunological) must be met with each weighted points between 2 and 10. Patients are classified with SLE if they have a score of ≥ 10. Classification criteria were developed to improve detection of new/early SLE in order to prevent delay in diagnosis. In a validation cohort, these EULAR/ACR 2019 criteria had a sensitivity of 96 percent and a specificity of 93% (15).

Recently, all classification criteria were compared for their performance, including the SLICC 2012 criteria, adapted with weighing factors for clinical manifestations with scores between 1-26. There was overall agreement for the diagnosis of aSLE by physician-rated patient scenarios without any statistically significant differences between the ACR 1997, SLICC 2012 and EULAR 2019 criteria (17). It was concluded that both (unweighted) SLICC 2012 or the more complex (weighted) EULAR/ACR 2019 can be used in the research field of SLE, although performance is always dependent on the type of patient population.

In a subsequent study, the performance of these three different classification criteria were also assessed in cSLE. SLICC 2012 criteria showed highest sensitivity (95%) and ACR 1997 the highest specificity (95%) (18). These high numbers for sensitivity and specificity show that all current available classification criteria seem sufficient for studies in cSLE-patients. In this thesis, the SLICC 2012 criteria were used.

Multiple SLE disease activity scoring systems have been developed, validated and improved over the last decades. Two of the most used disease activity scores are Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) and British Isles Lupus Assessment Group (BILAG) (19, 20). These disease activity scores are mostly used in clinical trials to evaluate patient outcomes and treatment responses but can also be used in daily clinical practice for follow-up. In clinical setting the SLEDAI is easy to use in daily practice, although this score needs some blood results and therefore it often takes a week after the patient was seen. The scoring of BILAG is more time consuming because to scoring list is longer than SLEDAI and scoring items need to be compared with the last visit (in terms of: improving, same or worse). Comparing these two scoring systems it was concluded by Yee et al. that SLEDAI is reliable when compared to BILAG but some patients, requiring increased treatment, were less likely to be picked up by SLEDAI (21).

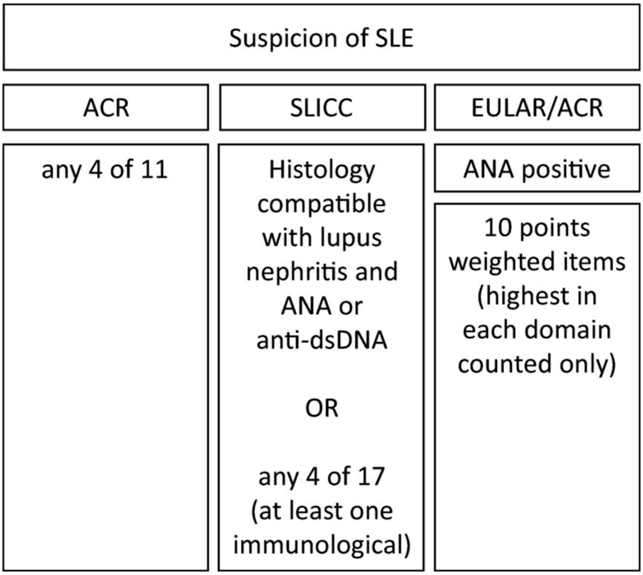

Figure 2. Comparison of different classification criteria for SLE over the last decades (with consent from Aringer et al. (16))

Childhood-onset versus adult-onset SLE: disease damage and mortality

In general, cSLE patients suffer from more severe disease than aSLE patients. Longitudinal studies show that this is reflected in higher disease activity, not only at disease onset but also during follow-up and by higher disease damage scores (22, 23). These same studies show that cSLE-patients are more prone for renal involvement, which can ause chronic kidney insufficiency leading to dialysis or kidney transplantation (10, 22, 23). Neuropsychiatric involvement is also higher in cSLE compared to aSLE (10). Large studies show that especially renal and neuropsychiatric involvement (in cSLE) is associated with a higher risk for irreversible disease damage (25, 26). In a long-term study in adults with cSLE (with a median disease duration of 20 years), 62% of patients had disease damage, predominantly in musculoskeletal, neuropsychiatric and renal systems. Specific disease damage occurred at a low median age of 20 years (cerebrovascular accidents), 24 years (renal transplants), 34 years (replacement arthroplasties) and 39 years (myocardial infarctions) (24). All of these data show that the damage in cSLE patients already occurs at a relatively early age when their peers are in the bloom of their life. Children with SLE differ from the aSLE patients in other types of damage (such as growth failure and puberty delay) but also in terms of disease burden in daily life, with different indicators for quality of life and educational goals. Health related quality of life (HRQoL) in adults with a childhood-onset of SLE is impaired in most domains, compared to the general population. The overall HRQoL score was irrespective of disease related organ damage by SLICC damage index (SDI) (24). After the educational phase of their life, SLE has a great impact on employment as an adult independent individual. Groot et al. showed that in adults with SLE, with a childhood-onset of disease, 50% of patients had adjusted their vocational choice, 44% of patients did not have a paid job and 51% had been declared work disabled. Disease related organ damage was equally prevalent in patients with and without paid employment (27).

Over the last decades, mortality rates decreased significantly from a 5-year survival rate of 50% in 1955 to >95% in the early 2000s (28, 29). The mean age of death has risen from 42 years in 1970s to 58 years in 2013. Nevertheless, the age-specific standard mortality *ratio* (compared to healthy age-matched peers) was still found to be the highest for SLE-patients in the age group of 25-34 years when compared to older age groups (28). In a more recent study was shown that mortality in patients with cSLE still is still 4.8% only 5 years after diagnosis, and in all age groups comorbidities attributed to 16.3% all-cause mortality (30).

It is necessary to lower the mortality rate in SLE further but also to lower risks of irreversible disease damage. Besides that, drug toxicity also needs to be considered constantly because of lifelong dependence on immunosuppressive medication, which is needed to halt disease progression and prevent disease flares. In particular for adolescent patients, which will have to live their whole life with this disease, it is needed to improve the daily quality of life in all domains, and to support their ability to pursuit their goals in life. Considering all of the above, it means that although a lot of progress has been made in the last decades, there are still many unmet needs for more disease biomarkers in SLE to improve treatment decisions and to predict risk for damage.

New techniques in the assessment of novel SLE disease biomarkers

Nailfold videocapillaroscopy

Nailfold capillaroscopy is an attractive imaging tool to use in daily clinical practice because is it non-invasive and easy to use with direct imaging results during the nailfold investigation of the patient. Over the last years, the quality of imaging of the nailfold microvascular bed has greatly improved with x200 magnification videocapillaroscopy. The main reason for us to further investigate capillary abnormalities in cSLE was because of the observation that many of our cSLE patients showed nailfold capillary hemorrhages (figure 3). Initially, Raynaud's phenomenon or acro-cyanotic complaints were an indication in these patients to look at their nailfold capillaries. Because SLE is a disease with intravascular inflammation, some studies had already used this technique to observe the capillary bed in the nailfolds of SLE patients, also without outspoken acro-cyanotic complaints. Recently, a systematic literature review showed that *adult* SLE patients have significantly more abnormal capillary shapes and -hemorrhages, which were associated with disease activity (by SLEDAI) (31). This systemic review also indicated that most data were cross-sectional of nature and less is known about longitudinal follow-up of these capillary abnormalities. Data on nailfold abnormalities in patients with cSLE were not investigated in this review.

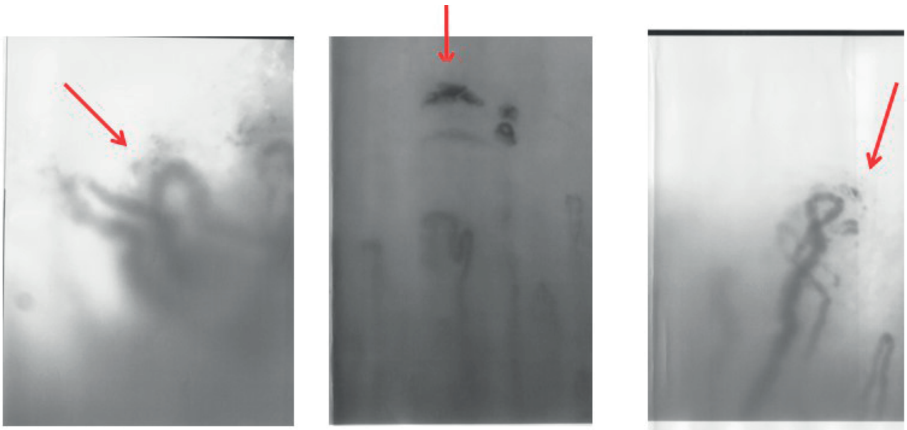

Figure 3. Our first observations of capillary hemorrhages (red arrows) in cSLE patients by light microscopy

A possible relation of these nailfold capillary hemorrhages with the observed vascular problems in SLE seems logical. Hypothetically, these capillary hemorrhages might have diagnostic and/or prognostic value or might be a biomarker to monitor disease activity. It is already known that one of the negative long-term effects in SLE is a higher risk for cardiovascular disease. Studies in adults with SLE have shown that the overall risk of cardiovascular disease is increased up to 17-fold, but even >50-fold in female patients between 35-44 years (32). In the earlier mentioned study of Groot et al., it was shown that cardio- and cerebrovascular complications occur in 5-10% of adults with cSLE, with the majority of events occurring before the age of 40 (24). Hersh et al. found that, although the percentage of cardiovascular complications was similar between adult- and childhood-onset patient groups, the cSLE-patients were (much) younger (mean age of 32 compared to 48 years) at presentation of the myocardial infarction (22). Clearly, in these patients premature coronary-artery atherosclerosis is prevalent and occurs more frequent and at a much lower age than in healthy age-matched controls (33). This is worrisome, specifically since these studies only included survivors of cardiovascular events and most likely the numbers found on coronary artery disease is an underestimation of the real prevalence and incidence. Traditional risk factors for cardiovascular disease (such as diabetes, obesity, hypertension) do not fully explain this increased risk for cardiovascular disease in SLE-patients: non-traditional and more SLE-specific risk factors such as chronic/cumulative corticosteroid use, vascular injury, high levels of pro-inflammatory immune complexes and decreased levels of endothelial progenitor cells (EPC) for vascular repair and/or remodeling, also seem to play an important role (34). The pathophysiology of premature atherosclerosis in SLE is not yet completely

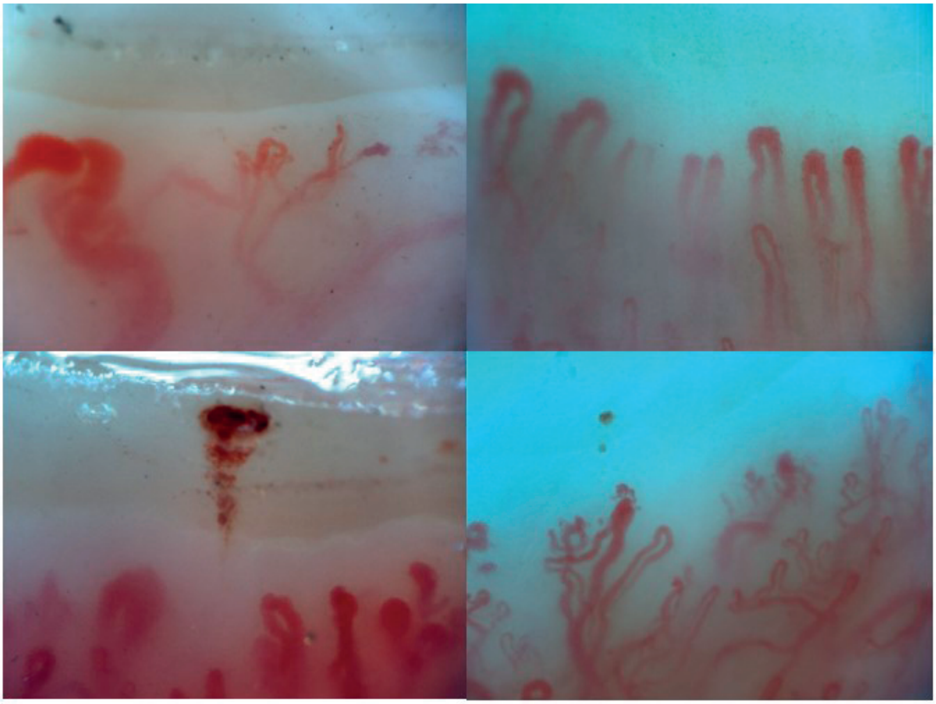

Figure 4. Nailfold capillary scleroderma pattern with: giant capillaries (upper lift/right and lower left), capillary hemorrhages (lower left), avascular areas (upper left) and ramifications (lower right)

understood, but endothelial dysregulation due to endothelial cell damage combined with lower endothelial progenitor cells and continuous inflammation seem to play an important role (35, 36). Capillary abnormalities in SLE might be a mirror for the sick endothelium and may be important to detect in follow-up to monitor vascular health in SLE over time.

Besides capillary hemorrhages and abnormal capillary shapes, a so-called capillary scleroderma pattern (figure 4) has been observed in a subgroup of SLE patients (37, 38). In general, a capillary scleroderma pattern in SLE has been suggested to be a sign that these patients are at risk for of possible overlap disease and these patients should be monitored for clinical features of SSc (39). Although it was suggested to be a useful diagnostic marker, the actual value of the observation of this scleroderma pattern in SLE has not yet been thoroughly investigated longitudinally.

All of these above observations on the nailfold capillary bed of SLE patients leads to the question whether these pathological capillary changes reflect overall disease severity in SLE, are related to specific organ involvement, or show different subgroups of patients correlated with a specific type of capillary abnormalities. In short, if capillary abnormalities prove to be a biomarker of SLE, reflecting disease activity or damage, they will be important for use in clinical practice.

Interferon gene signatures and phenotyping of patients

Interferon (FN) is produced by plasmacytoid dendritic cells, induced by (viral) infections, but also in response to immune complexes in rheumatic diseases (40). IFNs, especially type 1 IFN, play an important role in the pathogenesis of SLE (41). An IFN gene signature is a description of a group with the same upregulated genes. In SLE, a chronic and high ‘type 1 IFN signature’ has been associated with more severe SLE disease activity and with specific type of organ involvement such as nephritis and (muco)cutaneous involvement (42). It has been shown in cSLE that the type of organs that are involved mostly do not change after the first two years after diagnosis (24). This could mean that treatment regimens might be chosen or rapidly adapted to specific phenotypes in those first years after diagnosis.

It has also been suggested that high levels of IFN type 1 have a modulating effect on the endothelium via plaque-residing macrophages, potentiating foam cell and extracellular trap formation, inducing endothelial dysfunction and lead to exacerbated atherosclerosis outcomes in patients suffering from inflammatory diseases and risk for atherosclerosis (36). This links to the paragraph above on premature coronary-artery atherosclerosis in SLE whose pathophysiology is important to unravel further.

There is a high complexity in different types of IFN signatures in (c)SLE, but new insights point to some stratification in different disease subgroups (43, 44). Possibly, these new stratification of IFN signatures can predict (future) severity and treatment responses better than the known disease activity scores such as SLEDAI and BILAG, by defining more specific SLE subgroups. This might be very helpful in new treatment strategies to become more steroid-sparing and lower toxic side effects. This is extra important because damage in SLE is also partially related to steroid toxicity.

New biomarkers and importance for implementation in treatment choices

Newer treatments for children with SLE, such as belimumab, are now more widely available, and other new treatments are being developed. Other new treatments for this disease targeting IFN, JAK, IL-12 and IL-23 are being investigated, as well as the combined use of rituximab and belimumab as anti-B cell therapy (45). Nevertheless, it is still unknown *which patients* need *which therapy* at *which time point* to prevent irreversible disease damage. It is necessary to further define SLE subgroups that need different personalized treatment approaches. This thesis aims to provide some of many steps to the new insights that are still needed to reach this goal.

A broader spectrum of connective tissue diseases (CTD's)

Overlap disease in systemic connective tissue diseases

There is a group of patients (children and adults) that show clinical *overlap* features between the diseases SLE, systemic sclerosis (SSc) and/or dermatomyositis (DM) (see figure 5). These patients often have anti-ribonucleoprotein (RNP) antibodies combined with Raynaud's phenomenon, arthritis and/or myositis (46, 47). In the past, there has been a lot of debate whether to call this phenomenon of symptoms an 'overlap disease' or a disease entity of its own (48, 49). This overlapping group of symptoms in systemic autoimmunity has been defined as mixed connective tissue disease (MCTD), or as undifferentiated CTD (UCTD) when classification criteria are not (yet) met. For MCTD, different sets of classification criteria have been proposed, such as Sharp, Alarcon-Segovia, Kasukawa, and Kahn, of which most have good specificity but sometimes lower sensitivity (50). The existence of four sets of diagnostic criteria shows that there is still lot of debate on the best classification of this disease. UCTD is considered to be a diagnosis of exclusion when a patient with an autoimmune disease does not fulfil the criteria for other specific systemic autoimmune diseases such as SLE, SSc, DM and Sjögren's syndrome (or MCTD). Although juvenile dermatomyositis (JDM) is not considered as a lifelong disease, MCTD and UCTD are considered to be chronic and lifelong, just like SLE and SSc. Approximately one quarter of MCTD patients have a juvenile-onset (jMCTD) of the disease, i.e. onset of disease before the age of 18 (51). Treatments for MCTD or UCTD are chosen according the most severe symptoms. In contrast with SLE, MCTD patients are in general not at risk for the development of nephritis but they do seem to be at risk for pulmonary involvement with severe vascular complications such as pulmonary hypertension (52). Recently, it was highlighted that there are still no clinical practice guidelines for MCTD patients, not only in terms of standard of care but also in consensus of classification criteria (53).

Literature on jMCTD is scarce, but a recent longitudinal study showed that during follow-up of jMCTD, SLE-like and myositis-like symptoms decreased over time while SSc-like symptoms increased. This study was a cohort of 55 patients with a mean disease duration of 16 years, and presence of rheumatoid factor (RF) was a predictor for ongoing active disease (54). These findings are in line with a study (in adults) that showed that 58% of MCTD-patients did not change their initial clinical presentation and 17% of patients evolved into SSc (48). In a large cohort of adult- and jSSc patients, it was shown that the juvenile group showed more overlap disease and relatively more musculoskeletal involvement. Looking at organ-specific damage, another longitudinal study (median 15 years after disease onset) showed that patients with jMCTD showed impaired right- and left-ventricle cardiac function compared to healthy controls, although no patients had signs of pulmonary hypertension (55). In terms of survival, children with MCTD did better than adults (55, 56).

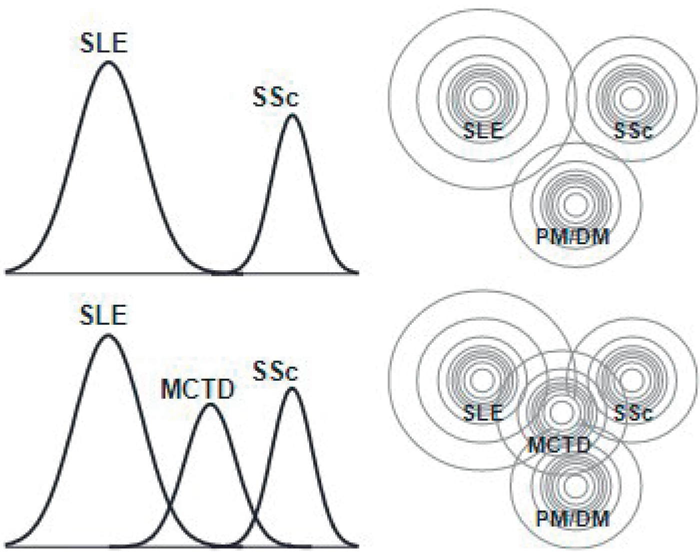

Figure 5. Visualization of overlapping disease in the CTD-spectrum (with consent from Aringer et al.(49))

It is important to have more predictive markers to know which patients are at risk for cardiac, pulmonary and kidney inflammation because of screening protocols in daily clinical practice. The optimal frequency for urine-analysis, pulmonary function tests (for detection of restriction and interstitial lung disease) and cardiac ultrasound (to screen for pulmonary hypertension) is still unknown. In (j)MCTD/UCTD, capillary abnormalities such as a scleroderma pattern might be helpful in detecting patients with risk for SSc-like symptoms.

Furthermore, if children with MCTD, with or without RF-positivity, are more prone for musculoskeletal disease, this is important to realize in screening and treatment of these children. Their young bodies are growing with risks for growth deformities and bone or cartilage erosions. In JIA, it has been established that RF-positive patients have higher risk for erosive damage than RF-negative patients (57). It seems that MRI, as imaging modality without the radiation side effects, has a better performance of detecting bone erosions than X-ray (58). There is scarcity in studies that investigate musculoskeletal disease activity or -damage by MRI in patients with systemic autoimmune diseases.

Localized scleroderma (LS)

Whereas SSc in the young is ultra-rare, the entity of localized scleroderma (LS) , also called morphea, is a slightly more common autoimmune disease, mostly limited to the skin. LS is predominantly diagnosed in childhood with a mean age of onset at 6-8 years (59, 60). Although LS is a part of the scleroderma spectrum, it is a pathological process of *local* and chronic inflammation of a part of the skin, in contrast to the systemic involvement in SSc. LS leads to localized fibrosis and/or loss of subcutaneous tissue. The pathogenesis of this rare disease is probably multifactorial with a possible genetic predisposition combined with environmental factors leading to microvascular injury. An inflammatory cascade of pro-fibrotic cytokines is present, combined with activation T-lymphocytes and expression of vascular adhesion molecules (61-63). Subsequently, this chronic inflammation of the affected skin can lead to severe damage with growth deformities, functional disabilities and cosmetic mutilation. The incidence of LS is rare and estimated at 3.4 cases per million children per year (64, 65).

Defined subtypes in LS are linear scleroderma (majority), circumscribed morphea, generalised morphea, pan-sclerotic morphea and a mixed subtype (65, 66). Progressive hemofacial atrophy, deep morphea and bullous morphea are other subtypes (61). The affected skin areas can be anywhere on the body including the scalp and face, which is a subgroup that is called localized scleroderma ‘en coup de sabre’ (figure 6). New insights in the disease show that patients with LS also can have extra-cutaneous manifestations such as arthritis, uveitis, dental abnormalities and, although extremely rare, even neurological involvement in patients with LS en coup de sabre (67, 68).

LS can be mild and mono-phasic for which only topical anti-inflammatory treatment is needed, mostly prescribed by a dermatologist. Nevertheless, many patients need early and aggressive systemic treatment. This is supported by long-term studies which showed that 25-44% of the patients developed significant disability, 56% of patients had permanent damage, and 31-89% of patients had ongoing active disease in their adult life (69, 70). In this latter, more severe, subgroup of patients with LS the disease was chronic or had a course with exacerbations and remissions.

Figure 6. Localized Scleroderma en coup de sabre (with consent from Kreuter et al. The Lancet Rheum May 2022; 4 (5); E374)

Due to its rarity, there is often a diagnostic delay in LS, and even after diagnosis not all physicians are aware of the possibilities of effective *systemic* treatment options. Patients in need of systemic anti-inflammatory treatment regimens are frequently treated jointly by paediatric rheumatologists and dermatologists with experience in prescribing those treatments (71-74). According to a recent international consensus guideline, given the rarity of the disease, it is advised that children with suspected LS are referred to a specialised paediatric rheumatology centre for clinical assessment and treatment (71).

Disease activity in LS can be measured clinically by the Localized Scleroderma Cutaneous Assessment Tool (LoSCAT). LoSCAT gives measurements for severity of disease activity with Skin Severity Index (LoSSI) and provides a Skin Damage Index (LoSDI) (75, 76). LoSSI describes body surface area involvement, degree of erythema, skin thickness and appearance of new lesion or old lesion extension, each graded from 0 to 3, at 18 anatomic sites. LoSDI measures damage by a similar scoring system describing three domains: skin atrophy, subcutaneous tissue loss and hypo-hyperpigmentation, also graded from 0 to 3 (75, 76). The outcome of LoSCAT is a quantitative parameter and it describes the most severe part of the lesion. In practice, it is an adequate measurement when used by the same person and it is correlated with measurements of physician global assessments (PGA's) (77). In LoSCAT, skin scores need to be given for the scoring items dermal atrophy, subcutaneous atrophy and skin thickness. There is risk for some variability in the inter-observer scoring of these items, especially for scores 1 and 2 in the range of 0-3 (78).

As LS can be a slowly smouldering disease, it is highly recommended to use frequent photography in longitudinal follow-up, because over the years subcutaneous fat atrophy can be slowly progressive, even in absence of skin redness. This means that disease progression can be missed with consequence risk for damage. An unmet need is the lack of objective but also accessible biomarkers that more quantitatively reflect disease activity. Currently, it is difficult to decide when to increase treatment or when it is possible and safe to taper treatment and stop. In this thesis, a pilot study will evaluate multiple imaging techniques in the search of new biomarkers in LS.

Outline of thesis

In **chapter 1** the background and aspects in diagnosis, follow-up and treatment of the autoimmune diseases cSLE, jMCTD and LS are highlighted with the current problems in treating these patients in the daily practice a pediatric rheumatologist.

Part I. Nailfold capillaroscopy in cSLE

The focus in part I is on detailed microvascular descriptions of the nailfold capillaries in cSLE and in healthy controls.

In **chapter 2** the results of a systematic literature review on nailfold capillary abnormalities in cSLE are reported and discussed.

Next, in **chapter 3** the results are shown from our cross-sectional study on capillary abnormalities in cSLE, compared to matched healthy controls.

In **chapter 4** a world-wide multicenter study compares of the capillary findings in pediatric patients with different rheumatic diseases and healthy controls.

In **chapter 5** the longitudinal data are described from our cSLE-cohort with follow-up nailfold videocapillaroscopy, specifically looking at capillary scleroderma patterns and disease damage as primary outcome.

Part II. New biomarkers in (systemic) connective tissue diseases

In part II other disease biomarkers are discussed which can be used to improve the clinical care of patients with autoimmune diseases such as cSLE, jMCTD/UCTD and LS.

In **chapter 6** subtypes of different interferon signatures are discussed and their role with neutrophil- and plasmablast signatures in stratifying cSLE patients to specific fingerprints with different (milder or severe) disease phenotypes.

In **chapter 7** unique quantitative data are described investigating skin thickness (by high frequency ultrasonography), skin hardness (by durometer) and dynamics of microcirculation (by laser speckle contrast analysis (LASCA)), in skin lesions of patients with LS, compared to contra-lateral healthy skin.

In **chapter 8** various MRI abnormalities in children with CTD are reported in relation to the clinical severity of arthritis according to their treating physicians.

Part III. Discussion, summary and appendix

In **chapter 9** the results from this thesis are discussed in a broader perspective. This leads to a recommendations on the future needs for research projects and needs in patient care for cSLE, jMCTD and LS.

In **chapter 10**, a summary is given in English and Dutch of chapter 2 until 8.

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Part I

Nailfold videocapillaroscopy in cSLE

2

Capillaroscopy in childhood-onset Systemic Lupus Erythematosus (cSLE): a first systematic review

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ABSTRACT

BACKGROUND: Recently, a systematic review indicated that, compared to healthy controls, adult patients with systemic lupus erythematosus (SLE) show significant more abnormal capillary morphology and hemorrhages in nailfold capillaroscopy and that these capillary changes are associated with disease activity. As yet, no systematic literature evaluation of capillaroscopy in childhood-onset SLE (cSLE) has been performed.

OBJECTIVE: To systematically review the literature on nailfold capillary characteristics in cSLE.

METHODS: Search terms "SLE or Lupus", "Capillaroscopy" and "Juvenile or Childhood or Pediatric or Child" were used in PubMed, Embase and Web of Science. Capillary findings were evaluated according to the current international consensus-based definitions for analysis of capillaroscopic characteristics from the European League against Rheumatism (EULAR) Study Group on Microcirculation in Rheumatic Diseases (SG MC/RD).

RESULTS: After screening eighty search hits, six articles were retained, of which two case-control studies and four case series. For capillary density, no difference was found between cSLE and healthy controls (one study). Differences in capillary diameter, capillary morphology, hemorrhages and semi-quantitative score were inconclusive or non-interpretable. A scleroderma pattern was not detected in case control studies but was reported in a minority of cSLE patients in three out of four case series.

CONCLUSIONS: Literature on nailfold capillary findings in cSLE is scarce and inconclusive. To evaluate capillary characteristics in cSLE, prospective longitudinal studies are needed. Future studies should use uniform definitions for capillary characteristics and findings should be compared with healthy controls, matched for age and ethnicity. The EULAR SG MC/RD is stepping forward to this need.

INTRODUCTION

Nailfold capillaroscopy is a noninvasive magnification method to visualize the capillaries in the fingertips. Capillary changes in adult and pediatric patients with Raynaud's phenomenon (RP) are characterized by scleroderma patterns when associated with a scleroderma spectrum disease [1-5]. Notably, a scleroderma pattern is one of the 2013 classification criteria for systemic sclerosis (SSc) developed by American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR). The severity of this scleroderma pattern seems associated with (the risk of) severe organ inflammation and fibrosis in SSc [6, 7].

Recently, Cutolo et al. concluded in a systematic review on capillaroscopic characteristics in adults with systemic lupus erythematosus (SLE) that a significantly higher number of tortuous capillaries, abnormal capillary morphology and hemorrhages are detected in SLE-patients when compared to healthy controls [8]. The "semi-quantitative nailfold capillaroscopic score (NFC)", rating capillary changes from zero to three or as "normal/minor/major/severe pattern", is also higher in adult SLE-patients than in healthy controls and seems to correlate with disease activity [8]. In his review, Cutolo focused only on adult patients with SLE. He used standardized definitions for uniformity and global interpretability, from the EULAR Study Group on Microcirculation in Rheumatic Diseases (SG MC/RD), to summarize capillaroscopic literature data [7].

As capillaries from healthy children quantitatively differ from adults in terms of capillary density, -diameter, morphology and presence of microhemorrhages, capillary changes should be separately described in adult and pediatric cohorts [9-11]. Moreover, patients with childhood-onset SLE (cSLE) have been found to show a more severe disease presentation and disease course than adult-onset SLE patients [12-15]. These reports in children suggest that SLE-patients should be studied in separate cohorts for children and adults.

For that reason, the aim of our study was to systematically review the literature on nailfold capillary changes in cSLE with the standard terminology of the EULAR SG MC/RD.

METHODS

Search strategy and process

To identify all original research articles that reported on the nailfold capillaroscopic assessment of cSLE, literature was systematically searched, with the latest update on the 2nd of April 2019, with search terms “SLE or Lupus”, “Capillaroscopy” and “Juvenile or Childhood or Pediatric or Child” in PubMed, Embase and Web of Science. The search was not restricted in publication date.

Two reviewers (DS and AN) screened the search hits for relevance by title and abstract. An overview of the search process is shown in figure 1. SLE should be diagnosed according to the international classification criteria from American College of Rheumatology (ACR) or Systemic Lupus International Collaborating Clinics (SLICC). Articles with a different topic, objective or patient population (for instance Raynaud’s phenomenon) mentioning capillaroscopy as a secondary outcome were included if they described capillaroscopic details from a sub-cohort of cSLE patients. There was no minimum count for study population. Reference lists of the retained manuscripts were checked for additional relevant articles.

Quality appraisal and level of evidence

The articles underwent quality appraisal by authors DS and MvdB, using a standardized scoring sheet from the National Institutes of Health (NIH) Quality Assessment tool for Observational Cohort and Cross Sectional Studies [16] which contains 15 questions, resulting in a score ranging between 0-15 (see supplementary file 1). In a second phase, DS and AN reached consensus on the scores. No articles were excluded based on their quality, as the aim of this review was to provide a description of literature. As such, case-control studies as well as case series were considered.

Evaluation of capillaroscopic variables

The capillaroscopic findings were evaluated by quantitative, semi-quantitative and qualitative assessment according to the parameters and definitions from the EULAR SG MC/RD [1, 17, 18].

The following quantitative capillary variables were extracted from the included studies: density (as number of capillaries per linear mm), dimension of a capillary limb (apical, afferent/efferent width or loop length in micrometer (µm), normal morphology as hairpin (stereotype hairpin shape), crossing (once or twice) and tortuous (limbs bend but do not cross) and abnormal morphology as all other shapes and lastly hemorrhages (over the top or around the whole length of the capillary) [17-19].

The reported quantitative variables that defined semi-quantitative assessments in the reviewed articles were translated by the current definitions from EULAR SG MC/RD as described above.

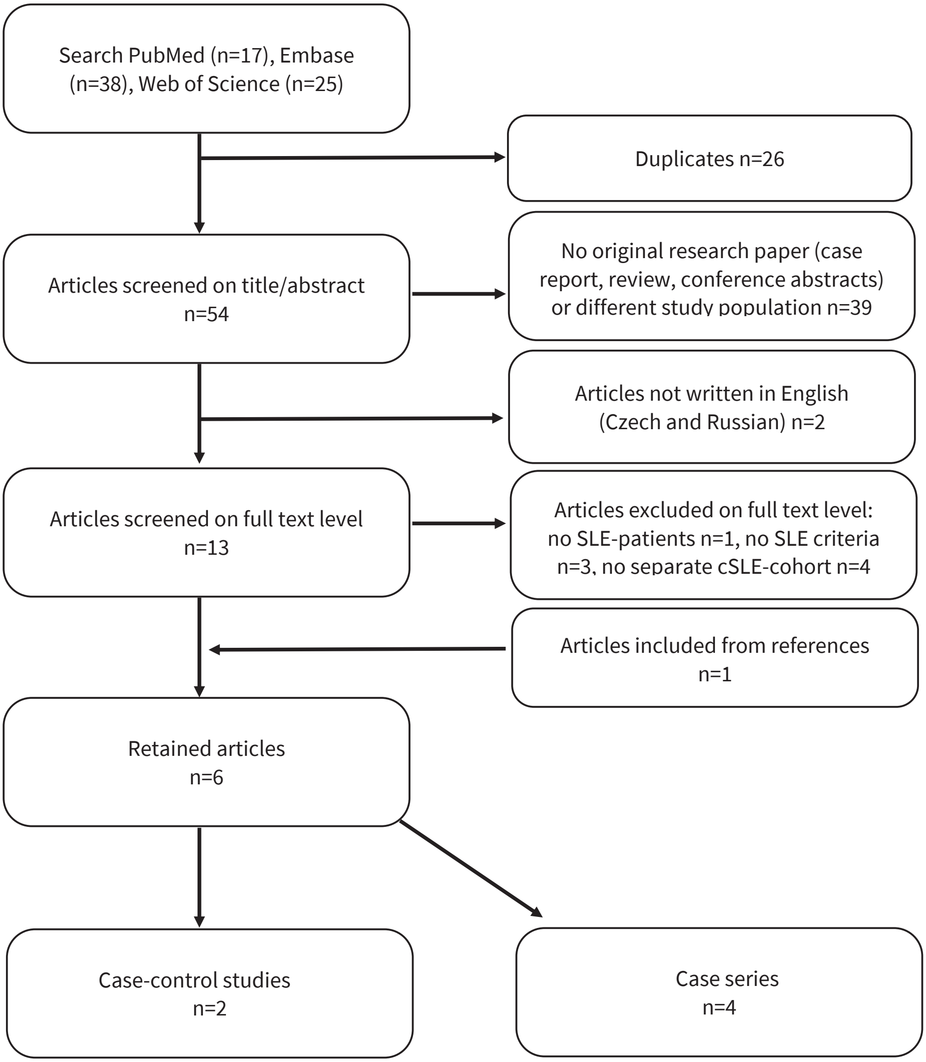

Figure 1. Flow chart search process and selection

Qualitative assessment was reported as detection of the scleroderma pattern. Scleroderma pattern was defined as by Cutolo et al. (2000) or Maricq et al. (1981) with "enlarged/giant capillaries, hemorrhages and a-vascularity" as main observations with possible detection of "ramified/bushy capillaries" as a sign of neo-angiogenesis [20, 21].

RESULTS

The literature search provided a total of eighty hits (see figure 1). Duplicates (n=26) and articles that were not an original research paper (i.e. case report, review or conference meeting, n=39) were excluded. Articles written in other languages than English, French, German, Dutch or Italian were also excluded (n=2). One extra article was found by screening references.

Subsequently, fourteen original articles remained for review on a full text level by DS, AN, KM and VS. One article, with left ventricular function as primary outcome, was included as it described a well-defined cohort of cSLE and capillary abnormalities. Another eight articles were excluded for the following reasons: no capillaroscopy performed in patients with SLE (n=1), no separate pediatric cohort of SLE patients (n=3) or SLE was not diagnosed (or this was not reported) according to the ACR or SLICC criteria (n=4).

Finally, six articles were retained for this review. An overview of the retained studies with their sample sizes, demographics, (different) used capillaroscopic techniques and magnification scales is shown in table 1.

Quality appraisal and level of evidence

Supplementary file A shows the quality appraisal of the included articles. The retained articles consisted of two case-control studies and four case series. Two articles have an NIH score ≤7/15 (low to moderate quality), which concerned the two case-control studies. Reasons for these scores are the limitations of the study design (cross-sectional cohort descriptions), small sample sizes with no/limited follow-up period or the absence of details on the disease duration.

Quantitative assessment of capillaroscopy in cSLE

In supplementary file B (tables B.1-B.4), capillary characteristics are described in detail according to the quantitative assessment. An overview of significant capillary characteristics in cSLE patients compared to healthy controls is shown in table 2. Details on the quantitative assessment of nailfold capillaries were reported in only one case control study. For density per millimeter, no significant difference was observed between cSLE patients and healthy children [22]. The same research group did not find significant differences in capillary length between cSLE patients and healthy controls [22]. For capillary morphology, Ingegnoli (2005) observed that cSLE patients show significantly increased ‘elongated, tortuous loops’ compared to healthy controls (p =0.002), although the term ‘tortuous’ was not defined in this study [22]. The presence of microhemorrhages was not observed in the cSLE-patients from this case-control study.

Table 1. Overview of included studies (n=6): sample size and used techniques

Author	Sample size cSLE patients	Sex M/F	Average age (years)	Average disease duration at inclusion (years)	Disease specific serologic abnormalities (%)	Concomitant therapies (%)	Raynaud's phenomenon (%)	Technique	Magnification	Number of fingers
Chung et al. 2015 [23]	92	13/79	12.4	3.6	Anti-ds-DNA (75) Anti-Sm (6.5) Anti-RNP (25)	Steroids (98.9) Cyclophosphamide (51.1) Hydroxychloroquine (17.4) Azathioprine (37) Mycophenolate mofetil (3.3)	23.9	NR	200x	10
Ingegnoli et al. 2013 [25]	35	2/33	13.2	2.3	NR	NR	34.3	NVC	200x	10
Piotto et al. 2012 [27]	30	5/25	14.4	4.4	NR	NR	36.6	Optical microscopy	10x-16x	8[a]
Ingegnoli et al. 2005 [24]	123[b]	4/119	29.9, range 5-69	7.9	Anti-ds-DNA (77.2) Anti-Sm (20.3) Anti-RNP (26.8)	NR	54	NVC	100x-200x	10
Ingegnoli et al. 2005 [22]	34	3/31	14.5	NR	NR	NR	NR	NVC	200x	10
Spencer-Green et al. 1983 [26]	7	0/7	15.3	NR	NR	NR	NR	Stereo microscopy	25-40x	4[c]

[a] thumbs excluded [b] including adults, separate size of cSLE sub-cohort is not reported; childhood-onset SLE data are reported in percentages of capillary characteristics [c] 2nd-3rd finger bilaterally

NR=not reported, NVC = nailfold video capillaroscopy, Anti-ds-DNA = anti-double-stranded DNA antibodies, Anti-Sm = anti-Smith antibody, Anti-RNP = anti-ribonucleoprotein antibody

Table 2. Significance of capillaroscopic changes in cSLE compared to healthy age-matched controls (data from 2 case control studies)

			Significant	Non-significant	Conclusion
Quantitative evaluation	**Density**	**Mean density**	0 studies	1 study [22]	**No significant difference in density between cSLE and healthy controls**
	Dimensions	**Diameter (mean width)**	0 studies	0 studies	No data
		Length	0 studies	1 study [22]	Inconclusive
	Morphology	**Normal morphology Tortuous**	1 study [22][a]	0 studies	Suggestive that cSLE show more 'tortuous capillaries' compared to healthy controls
		Abnormal morphology	0 studies	0 studies	No data
	Hemorrhages		0 studies	0 studies	No data
Qualitative evaluation	**Semi-quantitative score**		0 studies	0 studies	No data
	Other patterns		0 studies	2 studies [22, 26][b]	**"Scleroderma pattern" is not significantly more detected in cSLE patients compared to healthy controls**

[a] 'tortuous' on itself was not defined, though rather included in the author's definition of 'major abnormalities', defined as: "normal or decreased density", "a high presence of alterations in dimensions (>10% of longer loops or presence of enlarged loops)", presence of normal variations according to the EULAR SG MCRD (">50% tortuous") or presence of abnormal shapes ("meandering or branched") or "presence of hemorrhages" [b] according to Maricq (1981) or Cutolo (2000)

Semi-quantitative assessment of capillaroscopy in cSLE

Compared to healthy controls, a significantly increased detection of ‘major morphologic loop abnormalities’ was reported by Ingegnoli. It remained unclear if the authors assessed this capillary characteristic semi-quantitatively or quantitatively, since they specified the finding further as the presence of ‘elongated and tortuous capillaries’ (see paragraph 3.2) [22].

In case series of (c)SLE patients, the reported percentages of ‘major capillary abnormalities’ varied from 23 to 36% (see supplementary file C). These percentages are interpreted with caution as in these studies the term ‘tortuous’ was part of the definition for ‘major capillary abnormalities’, but was not further defined [23-25].

Qualitative assessment of capillaroscopy in cSLE

Both Spencer-Green (1983) and Ingegnoli (2005) did not detect a scleroderma pattern in cSLE (including follow-up) nor in healthy controls (see supplementary file D) [22, 26]. A scleroderma pattern was detected in 2.7% (n= 5/188) of the patients from three case series (see supplementary file D) [24, 25, 27].

DISCUSSION

This systematic review shows that there is a major lack of studies of capillaroscopy in cSLE, especially in the current light of the standardized definition for abnormal capillary morphology from the consensus of the EULAR SG MC/RD [17, 18]. After screening, only two-case control studies and four case series were retained. The main reasons for excluding articles were the lack of reporting the definition for diagnosing SLE or the fact that the pediatric patient cohort was not separately described.

Based on the results of our systematic review, we cannot make any solid conclusions on the nailfold capillary changes that might be present in cSLE-patients. As in adults with SLE, the capillary density of cSLE patients seems to be preserved. Unfortunately, because of the limited number of studies, we cannot draw any conclusions on the dimension of capillaries and the presence of hemorrhages. The observation from Ingegnoli that cSLE-patients have significantly more ‘major morphologic loop abnormalities’ compared to healthy controls could not be adopted in our conclusion because it was unknown if the authors meant ‘tortuous’ as being a variant of normal morphology, such as described by Andrade and adopted with the EULAR SG MC/RD or ‘tortuous’ as abnormal morphology (15,16). The latter being capillaries that also have other shapes, such as multiple crossing, branching or a non-convex tip. Using the same standardized definition for abnormal capillary morphology, as proposed by EULAR SG MC/RD, will simplify the comparison and interpretation of data between future studies that are ongoing in this field world-wide. From literature, it seems that tortuous capillary morphology, as described by the EULAR SG MC/RD, is a frequent capillaroscopic finding, also in the healthy pediatric/adolescent population and as such considered a-specific and as a normal morphological variation of the nailfold capillary [10]. On the other hand, if tortuous loops form the majority of the capillaries (>50% of the capillaries) this seems to be more characteristic for SLE in adults [8, 22].

Compared to healthy controls, adult SLE-patients show significantly more abnormal capillary morphology as well as a higher semi-quantitative score, as was shown by Cutolo in his review from 40 studies on adults with SLE [8]. From our systematic review of the pediatric literature, it is still unknown if these findings could also apply to cSLE-patients.

Whereas it was not detected in case-control studies, a scleroderma pattern was detected in a minority of cSLE-patients from case series [24, 25, 27]. Although definitions for a scleroderma pattern differed in these case series, the described definitions seem to match our pre-defined criteria for a scleroderma pattern according to Maricq and Cutolo [20, 21] (see supplementary file D). A scleroderma pattern in a minor percentage of SLE patients was also reported in the review of capillaroscopy findings in adults with SLE [8]. Future studies, explicitly longitudinal data, will further elucidate if the (pathological) detection of a scleroderma pattern in (c)SLE-patients might be correlated with a specific clinical (overlap) phenotype.

Noteworthy, one case control study indicates that capillaries from healthy children show differences according to age. Density increased progressively in relation to age although this trend was not significant ($r=0.226$, $p=0.15$) but capillary length differed significantly according to age ($r=0.485$, $p=0.001$) [22]. These findings emphasize that capillaroscopic abnormalities in SLE-patients should be studied in separate cohorts for adults and children. Even the transition period from child to adult should be considered as a separate age category in studies. Besides age, ethnicity should also be investigated as a confounder in capillary characteristics, definitely in SLE, as it is very well known that ethnicity has a major impact on severity of the disease [10, 28].

CONCLUSION

Studies on nailfold capillary findings in cSLE are scarce, have small sample sizes and are inconclusive. In order to better evaluate capillary findings in (c)SLE, prospective, longitudinal studies are needed that use uniform definitions. Results from these (larger) cohort-studies should be compared with healthy controls, matched for age and ethnicity.

Supplementary file A: Quality appraisal (n=6)

Questions (according to NIH quality assessment tool*)	Chung et at. 2015 [23]	Ingegnoli et al. 2013 [25]	Piotto et al. 2012 [27]	Ingegnoli et al. 2005 [24]	Ingegnoli et al. 2005 [22]	Spencer-Green et al. 1983 [26]
1. Was the research question or objective in this paper clearly stated?	Yes	Yes	Yes	Yes	Yes	Yes
2. Was the study population clearly specified and defined?	Yes	Yes	Yes	Yes***	Yes	Yes
3. Was the participation rate of eligible persons at least 50%?	NR	NR	NR	Yes	NR	NR
4a. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? [d]	Yes	Yes	Yes	Yes	Yes	Yes
4b. Were inclusion and exclusion criteria for being in the study pre-specified and applied uniformly to all participants? [d]	Yes	Yes	Yes	Yes	Yes	Yes
5. Was a sample size justification, power description, or variance and effect estimates provided?	NR	NR	NR	NR	NR	NR
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	Yes	Yes	Yes	Yes	No	No
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	Yes	NR	NA	NR	Yes	No
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	Yes	Yes	Yes	Yes	No	No
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	NR	Yes	Yes	NR	No
10. Was the exposure(s) assessed more than once over time?	NR	Yes	No	NR	NR	No
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	No	Yes	Yes	Yes	Yes	Yes
12. Were the outcome assessors blinded to the exposure status of participants?	NR	NR	NR	Yes	Yes	Yes
13. Was loss to follow-up after baseline 20% or less?	NR	NR	NR	NR	NR	NR
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	Yes	NR	NR	Yes	No	No
Total score **	9	8	8	11	7	6

http://www.nhlbi.nih.gov/health-pro/guidelines/in-develop/cardiovascular-risk-reduction/tools/cohort.
NR = not reported: this item was not reported in the manuscript. NA = not applicable. No = item was reported, answer was No. **NR, NA, No's were scored as zero. Yes equals 1 point. ***including adults, separate size of cSLE sub-cohort is not reported

Supplementary file B: Summary quantitative capillary characteristics

Table B.1 Capillary density

In all tables, the results from case-control studies and case series without healthy controls are separated by a bolder black line.

Author	Study type	Sample size	Description of capillaroscopic characteristic	Result in SLE patients	Result in healthy subjects	Significance
Ingegnoli et al. 2005 [22]	Cross-sectional and prospective[a]	34 cSLE 50 healthy children 20 healthy adults	Mean density per mm (range[b])	No exact results given	Healthy children 6.1 (5-8) Healthy adults 7.3 (6-8)	Not significant (p-value not mentioned)

[a] one follow-up in a selected sub-cohort. [b] normal: 6-8 capillaries per mm.

Table B.2 Capillary dimensions

Author	Study type	Sample size	Description of capillaroscopic characteristic	Result in SLE patients	Result in healthy subjects	Significance
Ingegnoli et al. 2005 [22]	Cross-sectional and prospective[a]	34 cSLE 50 healthy children 20 healthy adults	Length in µm (range)[b]	No exact results given	Healthy children 341.04 (159-500) Healthy adults 442.77 (359-500)	No significant difference in capillary length between HC and cSLE (p-value not mentioned)
Piotto et al. 2012 [27]	Cross-sectional	30 cSLE	Capillary elongation and dilatation[c]	No elongated capillaries No exact results given on dilatations	Not applicable	Not applicable

[a] one follow-up in a selected sub-cohort. [b] normal length 200-500 µm, elongated defined as >10% longer than normal. [c] according to Andrade 1990 [29]: dilated when widened in afferent, transitional and efferent branches with calibers ranging from 4-9 times normal dimension. Giant loops if calibers >10 greater than those of normal adjacent loops.

Table B.3 Capillary morphology

Author	Study type	Sample size	Description of capillaroscopic characteristic	Result in SLE patients	Result in healthy subjects	Significance
Ingegnoli et al. 2005 [22]	Cross-sectional and prospective[a]	34 cSLE 50 healthy children 20 healthy adults	Tortuous, meandering and branched loops[b]	No exact results given	No exact results given	Significantly increased elongated, tortuous loops in SLE (p=0.0002)
Piotto et al. 2012 [27]	Cross-sectional	30 cSLE	Crossed, bushy and bizarre capillaries[c]	No tortuous and crossed capillaries	Not applicable	Not applicable

[a] one follow-up in a selected sub-cohort. [b] ‘tortuous’ is not defined. [c] defined according to Andrade 1990 [29]: “atypical capillaries, such as crossed, bushy and bizarre capillaries”.

Table B.4 Capillary hemorrhages

Author	Study type	Sample size	Description of capillaroscopic characteristic	Result in SLE patients	Result in healthy subjects	Significance
Ingegnoli et al. 2005 [22]	Cross-sectional and prospective[a]	34 cSLE 50 healthy children 20 healthy adults	Presence of pathologic hemorrhages[b]	No exact results given	10% (7/70 total healthy controls including adults)[c]	Not stated

[a] one follow-up in a selected sub-cohort. [b] defined as: “over the top or around the whole length of capillary”. [c] extra note reported in text: “microhemorrhages grouped within limited areas”, though ‘microhemorrhages is not defined.

Supplementary file C: Summary semi-quantitative analysis

Author	Study type	Sample size	Description of capillaroscopic characteristic	Result in SLE patients	Result in healthy subjects	Significance
Ingegnoli et al. 2005 [22]	Cross-sectional and prospective[a]	34 cSLE 50 healthy children 20 healthy adults	Normal Minor abnormalities Major abnormalities[b]	Approximately 30% Approximately 30-40% Approximately 25%[c]	28/70 (incl adults) 39/70 3/70	Significantly increased major morphologic loop abnormalities (p=0.0002)[d]
Chung et al. 2015 [23]	Cross-sectional capillaroscopy	92 cSLE 50 control (for echocardiography only)	Normal Major abnormalities[b]	No exact results given 32/92 (34.8%)	Not stated	Not applicable
Ingegnoli et al. 2013 [25]	Cross-sectional with minimal 6 months follow-up[e]	35 cSLE 27 adult onset SLE	Normal Minor abnormalities[f] Major abnormalities[g]	12/35 (adult-onset 7/27) 14/35 (adult-onset 10/27) 8/35 (adult-onset 9/27)	Not applicable	No significant differences in pattern between childhood-onset and adult-onset SLE patients (p-value not mentioned)
Ingegnoli et al. 2005 [24]	Retrospective	123 SLE [h]	Normal Minor abnormalities Major abnormalities[b]	35/123 41/123 44/123	Not applicable	Not applicable

[a] one follow-up in a selected sub-cohort. [b] according to Ingegnoli 2013 [25] defined as: "normal or decreased density", "a high presence of alterations in dimensions (>10% of longer loops or presence of enlarged loops)", presence of normal variations according to the EULAR SG MCRD (">50% tortuous") or presence of abnormal shapes ("meandering or branched") or "presence of hemorrhages". [c] detailed numbers not mentioned; estimated data from figure. [d] defined as: "characterized by elongated, tortuous loops, in SLE". [e] no details on follow-up period. [f] defined as: "density 6-8/mm, elongated <10%, tortuous <50%, parallel rows, no pathologic hemorrhages".
[g] defined as: "density ≤6-8/mm, elongated >10%, tortuous > 50%, enlarged, meandering, branched, disarrangement, -/+ pathological hemorrhages". [h] size of cSLE sub-cohort not reported.

Supplementary file D: Summary qualitative scoring patterns

Author	Study type	Sample size	Description of capillaroscopic characteristic	Result in SLE patients	Result in healthy subjects	Significance
Ingegnoli et al. 2005 [22]	Cross-sectional and prospective[a]	34 cSLE 50 healthy children 20 healthy adults	Scleroderma pattern[b]	0/34[c]	0/70	No difference
Spencer-Green et al. 1983 [26]	Cross-sectional	7 cSLE 34 healthy children	Normal[d] Abnormal pattern[e]	7/7 0/7	34/34 0/34	No difference
Ingegnoli et al. 2013 [25]	Cross-sectional with minimal 6 months follow-up[f]	35 cSLE 27 adult onset SLE	Scleroderma pattern[b]	1/35 1/27	Not applicable	No significant differences in pattern between childhood-onset and adult-onset SLE patients[g]
Piotto et al. 2012 [27]	Cross-sectional	30 cSLE	Normal[g] Unspecific microangiopathy [i] Scleroderma pattern[J]	28/30 1/30 1/30	Not applicable	Not applicable
Ingegnoli et al. 2005 [24]	Retrospective	123 SLE[k]	Scleroderma pattern[b]	3/123	Not applicable	Not applicable

[a] one follow-up in a selected sub-cohort. [b] defined as: "density <6-8/mm, elongated >10%, tortuous, enlarged, meandering, branched, disarrangement with or without vascular areas, ++ pathological hemorrhages". [c] none of the cSLE patients developed a scleroderma pattern during follow-up period. [d] defined as: "uniform, homogeneous distribution and appearance of loops". [e] defined as: "large, dilated loops with capillary a-vascularity and non-uniform appearance". [f] no details on follow-up period
[g] p-value not mentioned. [h] defined as: "presence of parallel non-dysmorphic capillaries and lack of deletion areas". [i] defined as: "dilated capillaries and other morphological changes in absence of deletion areas". Deletion areas defined as: "absence of two or more successive capillaries". [J] defined as: "dilated or giant capillary loops and vascular deletion areas". Deletion areas defined as: "absence of two or more successive capillaries". [k] including adults, the size of cSLE sub-cohort is not reported.

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Nailfold videocapillaroscopy in cSLE

3

Nailfold capillary abnormalities in childhood-onset systemic lupus erythematosus: a cross-sectional study compared with healthy controls

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ABSTRACT

OBJECTIVES: For selection of high-risk systemic lupus erythematosus (SLE) patients it is necessary to obtain indicators of disease severity that predict disease damage. As in systemic sclerosis, nailfold capillary abnormalities could be such a biomarker in SLE. The primary objective of this cross-sectional study is to describe capillary abnormalities in childhood-onset SLE (cSLE) cohort (onset < 18 years) and compare them with matched healthy controls. The secondary objective is to correlate the observed capillary abnormalities with demographical variables in both cohorts and with disease-specific variables in cSLE patients.

METHODS: Healthy controls were matched for ethnic background, age and gender. Videocapillaroscopy was performed in eight fingers with 2-4 images per finger. Quantitative and qualitative assessments of nailfold capillaroscopy images were performed according to the definitions of the EULAR study group on microcirculation in Rheumatic Diseases.

RESULTS: Both groups (n=41 cSLE-patients and n=41 healthy controls) were comparable for ethnic background (p=0.317). Counted per mm, cSLE-patients showed significantly more 'giants' (p=0.032), 'abnormal capillary shapes' (p=0.003), 'large capillary hemorrhages' (p<0.001) and 'pericapillary extravasations' (p<0.001). Combined 'abnormal capillary shapes and pericapillary extravasations' (in the same finger) were detected in 78% (32/41 patients). By qualitative analysis, 'microangiopathy' was detected in 68.3% (28/41) and a 'scleroderma pattern' in 17.1% (7/41) of the cSLE-patients (without scleroderma symptoms). The difference of percentage positive anti-RNP antibodies in the group with or without a scleroderma pattern was not significant (p=0.089). The number of 'abnormal capillary shapes per mm' was significantly correlated with treatment-naivety. The number of 'large pathological hemorrhages per mm' was significantly correlated with SLEDAI score and presence of nephritis. Compared to healthy controls, 'pericapillary extravasations' were found in significantly higher numbers per mm (p<0.001) as well as in percentage of patients (p<0.001).

CONCLUSIONS: Our observations confirm that giants, abnormal capillary morphology and capillary hemorrhages are also observed in cSLE, as was already known for adults with SLE. Number of capillary hemorrhages in cSLE was significantly correlated with disease activity. A high frequency and total amount of "pericapillary extravasations" was observed in cSLE patients, possibly revealing a new subtype of capillary hemorrhage that might reflect endothelial damage in these pediatric patients.

INTRODUCTION

Nailfold capillaroscopy (NFC), a non-invasive magnification method, is used to visualize the capillaries of the fingertips. NFC is a diagnostic instrument, used in patients with Raynaud's phenomenon: a capillary scleroderma pattern is associated with systemic sclerosis (SSc) [1-3].

Systemic Lupus Erythematosus (SLE) patients can also show capillary abnormalities in NFC. As concluded in a recent review, adults with SLE show a significantly higher number of tortuous capillaries, abnormal capillary morphology, hemorrhages and "semi-quantitative NFC score", when compared to healthy controls [4]. Additionally, the NFC-score (by rating severity of capillary changes) also seems to correlate with disease activity [4]. Studies on nailfold capillary findings in children with SLE are scarce and inconclusive. In our recently published systematic review, data from six published studies on this topic were not comparable as different definitions for abnormal morphology were used [5]. Moreover, the definition for abnormal capillary morphology was recently further specified and revised by the European League Against Rheumatism (EULAR) Study Group on Microcirculation in Rheumatic Diseases (SG MCRD) [6, 7].

The diagnosis of childhood-onset (c)SLE is often delayed due to heterogeneity of presenting symptoms, and is dependent on recognition by and experience of the clinical physician. To prevent organ damage, it is important to prevent delay in diagnosis. Delay in diagnosis is specifically mentioned as one of the patients' unmet needs in a recent publication of 'state of the art on clinical guidelines' [8]. Prevention of delay in diagnosis is especially important for cSLE-patients, because it was shown that they have more severe symptoms at presentation and a more severe disease course compared to patients with adult onset SLE [9-13]. Heterogeneity is not only applicable for disease symptoms but also for disease severity with mild to severely affected patients and, depending on type of organ manifestations, a higher risk of mortality [12]. SLE is associated with progressive (irreversible) organ damage, which has shown to be a predictor of additional morbidity and early mortality [14]. A recent international recommendation for treatment in SLE is based on the treat-to-target principle: 'since damage predicts subsequent damage and death, prevention of damage accrual should be a major therapeutic goal in SLE' [15]. Steroid-related damage is an important factor in SLE and has become an outcome parameter for damage in long-term SLE follow-up studies [16, 17]. Selection of patients who need aggressive and steroid-sparing treatment in early phases of the disease will lead to less organ damage and lower cumulative steroid-use. For selection of high-risk patients it is necessary to obtain indicators of disease severity that predict (severe) future disease damage. Nailfold capillary abnormalities could be such an indicator or biomarker in SLE. For systemic sclerosis (SSc), multiple studies have shown that capillary abnormalities (by qualitative description) can be of use as a prognostic biomarker [18-22].

This study was conducted by the EULAR SG MCRD. The primary objective of this cross-sectional study is to describe possible capillary abnormalities in cSLE patients and compare them with healthy controls, matched for skin pigmentation, age and gender. These demographic variables have been described as confounding factors in healthy controls in interpreting capillary characteristics, such as density [23, 24]. The secondary objective is to correlate the observed capillary abnormalities with demographical variables in both cohorts and with disease-specific variables in cSLE patients.

METHODS

Patients and controls

Consecutive patients with (suspected) cSLE were cross-sectional included during a visit at the (outpatient) clinic. Criteria for inclusion were SLE diagnosis according to the 2012 Systemic Lupus International Collaborating Clinics (SLICC) classification criteria [25] and age of disease onset < 18 years old. Patients were excluded if they did not fulfil a minimum of four SLICC criteria, if they declined capillaroscopy examination/analysis of their capillaroscopy images, if it was impossible to collect images with good quality (due to nailfold skin thickness) or when a patient was too sick to undergo capillaroscopy examination. Demographical and clinical data were collected from patient charts. For cSLE-patients with one-time cross-sectional capillaroscopy, informed consent was waived by our ethical committee. Nevertheless, most cSLE-patients were part of a longitudinal cohort study for which an informed consent by patients (from 12 years of age) and/or both parents (for patients below 16 years) was signed. If capillaroscopy was performed longitudinally, images from the first capillaroscopy were used.

For healthy controls, children and adolescents from schools around the Amsterdam University Medical Centers (AUMC) and via personal contacts of the authors were approached for one-time capillaroscopy. This project was approved, combined with our longitudinal cohort study (Dutch trial register registration no. NL60885.018.17) by the ethical committee from the AUMC. Inclusion followed if they did not suffer from a chronic disease and had signed informed consent (child from 12 years of age and/or both parents for children below 16 years old). Age, gender, ethnic background, Raynaud symptoms and periungual trauma were noted. Disease activity was measured by Systemic Lupus Erythematosus Activity Index (SLEDAI) score. Patients and healthy controls were coded with an unique study number.

Nailfold capillaroscopy technique and image collection

NVC was performed with a x200 magnification lens from Optilia. All images were collected by one investigator (DS). The patients/healthy children stayed in a room of 20-22^{0}C for a minimum of 15-20 minutes. During capillaroscopy they were in sitting position with the hand on a table at the level of their heart. A drop of oil was applied to the fingers before examination. In total, eight fingers per cSLE-patient (excluding the thumbs) were examined. Per finger, four images were stored. From November 2017 until June 2018, a cohort of healthy children were included. In healthy children, eight fingers were examined and two images per finger were stored (according to the EULAR SG MRCD study protocol). From this larger healthy pediatric study cohort, healthy controls were matched with our cohort of cSLE-patients according to ethnic background, age and gender (in that order).

Image analysis

Post-examination, the following quantitative capillaroscopy characteristics were evaluated by primary investigator (DS) with a grid per millimeter: density (number of capillaries in distal row per mm), number of abnormal shapes (as defined by EULAR SG MCRD as all other shapes than hairpin (stereotype hairpin shape), crossing (once or twice) and tortuous (limbs bend but do not cross)) [6, 7], number of giant capillaries (if apical diameter >50 µm), maximum apical diameter (in µm, by Optipix software version 1.7.6), and number of capillary hemorrhages [3]. Hemorrhages were defined in two subtypes: 'large pathological hemorrhages' as large deposit of hemosiderin with a cap-like appearance [1] and 'pericapillary extravasations' as small point-shaped hemorrhages surrounding the capillary loop (image 1). Examined subjects were asked for finger trauma and manicure treatment in the 2-3 weeks prior to examination.

Qualitatively, three capillary patterns were described. A scleroderma pattern was defined by presence of giant capillaries, possibly combined with large pathological capillary hemorrhages, loss of capillaries and abnormal capillary shapes, according to the 'Fast Track Algorithm' [26]. If the observed capillary pattern showed abnormal capillary morphology or hemorrhages, but did not match the criteria for scleroderma, it was called 'microangiopathy', referring to non-specific abnormalities. A normal pattern showed no capillary abnormalities.

Capillaries from images of low visible quality were excluded and not analyzed.

Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics type 26. Descriptive statistics were reported in terms of percentages, means and standard deviations or medians and inter-quartile ranges depending on distribution of outcome data. Demographical differences between both study groups were calculated with a paired t-test (in case of normal data distribution), McNemar test (for binary and nominal outcome variables) and Wilcoxon signed rank test (in case of no normal data distribution). Linear regression by ANOVA and logistic regression were used for respectively numerical and categorical outcome data. Demographic and clinical variables (only for the cSLE-cohort) were tested as co-variate factors for the amount (per mm) of 'abnormal capillary shapes', 'large hemorrhages' or 'pericapillary extravasations'. Type of ethnic background was analyzed as an ordinal variable for three types of skin pigmentation: white/white-mixed, Asian/North-African/Middle-eastern and African/Afro-Caribbean. P-values <0.05 were considered as statistically significant.

RESULTS

Inclusion and demographics

Fifty-two patients with (suspected) cSLE were eligible for inclusion between April 2016 until September 2019. After revising SLICC-criteria, seven patients did not fulfil a minimum of four criteria and were excluded. Two patients were excluded because it was not possible to obtain clear capillaroscopy images due to skin thickness around their nailfold. One patient, with circulatory insufficiency admitted on intensive care unit, was too sick to undergo capillaroscopy examination. Therefore, forty-one patients were included for analysis.

The same number of healthy controls (n=41) were matched from a cohort of healthy children (n=140) with capillaroscopy images, first by matching for ethnic background (p=0.317). The cSLE-cohort had significantly more female patients (36 (87.8%) versus 29 (70.7%), p=0.039) and higher median age (median 17 versus 12 years, $p<0.001$) compared to healthy controls (see table 1).

In total, 8055 capillaries from 1147 images could be analyzed from 41 cSLE-patients. From healthy controls (n=41), 4253 capillaries were analyzed from 656 images. Disease characteristics of the cSLE-cohort are shown in table 1. Fifty six percent (56.1%) of patients were treatment naive and investigated at time of diagnosis.

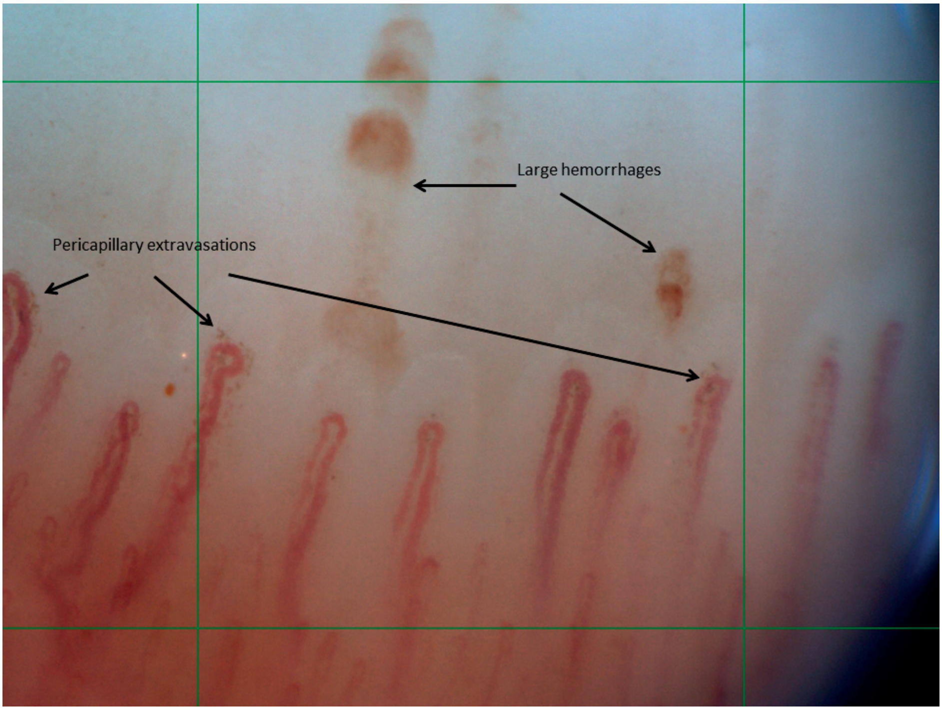

Image 1. 'Large hemorrhages' versus 'pericapillary extravasations'.
Magnification 200x; green lines show 1 mm grid

Quantitative capillary variables

Compared to healthy controls and counted per millimeter, cSLE-patients showed significantly more giant capillaries (p=0.032), abnormal capillary shapes (p=0.03), more large pathological hemorrhages (p<0.001) and more pericapillary extravasations (p<0.001) (table 2). In total, large pathological hemorrhages and pericapillary extravasations were significantly more observed in respectively 75.6% (31/41) and 87.8% (36/41) of cSLE-patients compared to healthy controls (resp. in 17.1% (7/41) and in 36.6% (15/41), McNemar test; resp. p<0.001 and p<0.001)).

Qualitative capillary patterns

Compared to healthy controls, cSLE-patients showed significantly more abnormal capillary patterns (Z= -5.291, p<0.001) (table 2). In total, thirty-two patients (32/41, 78%) showed a specific combination of 'pericapillary extravasations' and 'abnormal capillary shapes' combined in the same finger (as shown in image 2), including all patients (n=7) with a scleroderma pattern. Looking at frequency and localization of these two combined capillary abnormalities, three patients (3/32, 9.4%) showed this combination of capillary abnormalities in all eight examined fingers, 59.4% (19/32) in four or more fingers and 78.1% (25/32) in three or more fingers. Three other patients with high number of ‘pericapillary extravasations’ (with a total count of 96, 71 and 128 extravasations) were also qualitatively analyzed as ‘microangiopathy’, but these three patients did not show the specific combination with ‘abnormal capillary shapes’.

Clinical details of cSLE-patients with capillary scleroderma pattern (n=7/41, 17.1%) are shown in supplementary file 1. Five out of seven patients (71.4%) with a scleroderma pattern had positive anti-RNP antibodies versus 32.4% (n=11/34) of patients without a scleroderma pattern, this difference was not significant (p=0.089). None of these patients showed any signs of sclerodactyly nor other classification criteria for SSc. This was also not detected at follow-up (range 1-9 years). Two healthy controls showed one giant capillary (per person) with diameters of 54.7 and 62.4 µm. As no other capillary abnormalities were found in these healthy individuals, this was not scored as a scleroderma pattern in these two healthy controls. Both giants were observed in the second fingers (with more frequent use and risk for trauma) while the giants in cSLE-patients were observed in the fourth/fifth fingers.

Correlations with demographic and clinical variables

Capillary morphology

In cSLE-patients, the amount of ‘abnormal shapes per mm’ was significantly correlated with periungual trauma (p=0.049) and treatment-naivety (p=0.022). In healthy controls, no correlations were found for the amount of abnormal shapes per mm (supplementary file 2).

Apical diameter

cSLE patients showed no significant correlation between the presence of giants and Raynaud’s phenomenon (OR 2.3, 95% CI 0.48 – 11.08, p=0.299). There was also no significant correlation between the amount of ‘giants per mm’ and presence of anti-RNP antibodies (supplementary file 3).

Large pathological hemorrhages

In cSLE-patients, ‘large pathological hemorrhages per mm’ showed a significant correlation with SLEDAI scores (at diagnosis (p=0.009) and at moment of capillaroscopy (p=0.002)) and nephritis (p=0.012). In healthy controls, the amount of ‘large pathological hemorrhages per mm’ was significantly correlated with periungual trauma (p=0.004) (see table 3).

Table 1. Demographical variables and clinical characteristics of study groups

	cSLE-patients, n=41	Healthy controls, n=41	p-value
Female, n (%)	36 (87.8)	29 (70.7)	**0.039[a]**
Ethnicity, n (%)			0.317[b]
African/Afro-Caribbean	18 (43.9)	15 (36.6)	
White	15 (36.6)	14 (36.6)	
North-African/Middle-Eastern	3 (7.3)	4 (9.8)	
Asian	3 (7.3)	5 (12.2)	
Mixed/other	2 (4.9)	2 (4.9)	
Age at capillaroscopy in years, median (IQR)	17 (14-18)	12 (11-16.5)	**<0.001[c]**
Raynaud's phenomenon / acro-cyanotic symptoms, n (%)	14 (34.1)	2 (4.9)	**0.002[a]**
Age at onset in years, median (IQR 25-75)	14 (12.5-16)		
Disease duration in months, median (IQR)	12.9 (0.1-44.5)		
Prednisone naive, n (%)	23 (56.1)		
ANA at diagnosis, n (%)	41 (100)		
ANA + anti-ds-DNA	26 (63.4)		
ANA + anti-RNP	16 (39)		
ANA + anti-Sm	14 (34.1)		
Cutaneous involvement, n (%)	27 (65.9)		
Nephritis, n (%)	13 (31.7)		
Neuropsychiatric involvement, n (%)	6 (14.6)		
Antiphospholipid antibodies, n (%)	5 (12.2)		
SLEDAI score at diagnosis, median (IQR)	12 (8-16)		
SLEDAI score at capillaroscopy, median (IQR)	5 (3-10.5)		

Bold indicates statistically significant p values (<0.05). [a] McNemar test. [b] Wilcoxon signed rank test: ordinal variables (3 groups: white/mixed/other, Asian/North-African/Middle-Eastern and African/Afro-Caribbean). [c] paired t-test. ANA= Anti-Nuclear Antibodies, anti-ds-DNA= anti-double stranded DNA antibodies, anti-RNP= anti-Ribonucleoprotein, anti-Sm= anti-Smith antibodies, SLEDAI= Systemic Lupus Erythematosus Disease Activity Index, IQR= interquartile range.

Table 2. Capillary characteristics

Quantitative parameters	cSLE-patients, n=41	Healthy controls, n=41	p-value
Density per mm, mean (SD)	6.83 (1.06)	6.53 (0.86)	0.117[a]
Max apical diameter in µm, median (IQR)	37.7 (35.2-45.9)	38.6 (32.8-41.2)	0.206[b]
Giant capillaries per mm, mean (SD)	0.04 (0.13) [c]	0.003 (0.013)	**0.032[b]**
Abnormal shapes per mm, median (IQR)	0.31 (0.13-0.73) [c]	0.21 (0.06-0.38)	**0.003[b]**
Hemorrhages per mm, median (IQR)	1.1 (0.39-2.34) [c]	0 (0-0.16)	
Large pathological hemorrhages per mm	0.07 (0-0.24)	0 (0-0)	**<0.001**
Pericapillary extravasations per mm	1.11 (0.28-2.15)	0 (0-0.13)	**<0.001**
Qualitative patterns			
Normal capillary pattern, n (%)	6 (14.6)	37 (90.2)	**<0.001[b]**
Microangiopathy, n (%)	28 (68.3) [c]	4 (9.8)	
Scleroderma pattern, n (%)	7 (17.1) [c]	0 (0)	

Bold indicates statistically significant p values (<0.05). Mm= millimeter, SD= standard deviation, µm =micrometer. [a] paired t-test. [b] Wilcoxon signed rank test. [c]nailfold capillary abnormalities which are also described in adult-onset SLE (4).

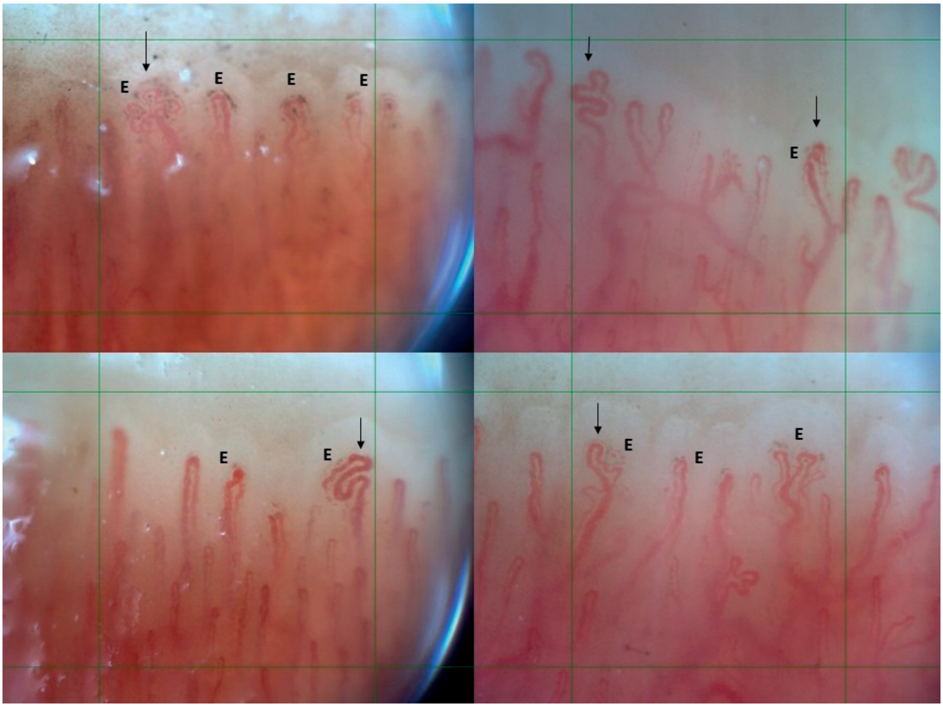

Image 2. Combination of pericapillary extravasations (E) and abnormal shapes (black arrows).
Magnification 200x; green lines show 1 mm grid; images from four different patients

Pericapillary extravasations

In both cSLE-patients ($p<0.001$) and in healthy controls ($p=0.001$), the amount of 'pericapillary extravasations per mm', was significant positively correlated with darker skin pigmentation (see table 4). In healthy controls, the presence of 'pericapillary extravasations' (observed or not) was significantly correlated with darker skin pigmentation (logistic regression, OR 14.33, 95% CI 3.30-62.32, $p<0.001$).

Table 3. Correlations between clinical and demographical variables and amount of "large hemorrhages per mm"

Variable	Regression coefficient β (95% CI) cSLE-patients	p-value	Regression coefficient B (95% CI) healthy controls	p-value
Skin pigmentation (ordinal)	0.103 (-0.001 – 0.207)	0.052	0.000 (-0.051 – 0.052)	0.990
Trauma	-0.068 (-0.376 – 0.239)	0.656	0.163 (0.054 – 0.271)	**0.004**
Raynaud/acrocyanosis	-0.087 (-0.298 – 0.124)	0.410	-0.037 (-0.247 – 0.174)	0.726
Treatment-naivety	-0.061 (-0.264 – 0.141)	0.543		
Disease duration	-0.002 (-0.006 – 0.001)	0.241		
SLEDAI at diagnosis	0.019 (0.005 – 0.032)	**0.009**		
SLEDAI at capillaroscopy	0.021 (0.008 – 0.035)	**0.002**		
Anti-RNP	-0.017 (-0.224 – 0.189)	0.866		
Cutaneous involvement	-0.128 (-0.337 – 0.081)	0.222		
Neuropsychiatric involvement	0.121 (-0.162 – 0.404)	0.392		
Nephritis	0.260 (0.06 – 0.460)	**0.012**		
Antiphospholipid antibodies	0.000 (-0.071 – 0.072)	0.989		

Table 4. Correlations between clinical, demographical variables and amount of "pericapillary extravasations per mm"

Variable	Regression coefficient β (95% CI) cSLE-patients	p-value	Regression coefficient B (95% CI) healthy controls	p-value
Skin pigmentation (ordinal)	0.846 (0.536 – 1.157)	**<0.001**	0.146 (0.063 – 0.230)	**0.001**
Trauma	-0.587 (-1.740 – 0.567)	0.310	-0.028 (-0.253 – 0.197)	0.803
Raynaud/acrocyanosis	0.193 (-0.612 – 0.997)	0.631	-0.129 (-0.521 – 0.262)	0.507
Treatment-naivety	0.265 (-0.501 – 1.031)	0.489		
Disease duration	0.011 (-0.002 – 0.024)	0.109		
SLEDAI at diagnosis	0.034 (-0.021 – 0.090)	0.216		
SLEDAI at capillaroscopy	0.025 (-0.31 – 0.081)	0.372		
Anti-RNP	0.518 (-0.249 – 1.284)	0.180		
Cutaneous involvement	-0.423 (-1.218 – 0.373)	0.289		
Neuropsychiatric involvement	-0.855 (-1.901 – 0.192)	0.106		
Nephritis	0.564 (-0.237 – 1.366)	0.163		
Antiphospholipid antibodies	-0.011 (-0.283 – 0.261)	0.934		

Bold indicates statistically significant p values (<0.05)

DISCUSSION

Our observations confirm that giants, abnormal capillary morphology and capillary hemorrhages are also observed in cSLE, as was already known for adults with SLE [4]. The uniqueness of our cohort is that more than half of the patients (23/41, 56.1%) were treatment-naive at the moment of capillaroscopy examination. This is the first study to describe abnormal capillary morphology in cSLE since the new published definitions for abnormal capillary shapes from EULAR SG MCRD in 2016 [6]. In this cross-sectional study and compared to healthy controls, cSLE-patients show significantly more giant capillaries, abnormal capillary morphology and capillary hemorrhages, both in absolute numbers (per mm) as well as in percentage of patients. The high number (median 1.1 per mm) of capillary hemorrhages in cSLE patients and the observation of two different subtypes of capillary hemorrhages are the other prominent findings of our study. Large hemorrhages were also observed in healthy controls but these were significantly correlated with trauma, which seems a logical explanation.

We found a significant correlation between the amount of large hemorrhages and SLEDAI score (at diagnosis and at capillaroscopy). Significantly higher SLEDAI scores in adult SLE with major capillary changes (defined by abnormal shapes and capillary hemorrhages) have been described before, further specified by a correlation between more capillary hemorrhages in the patient group with a SLEDAI score of >12 [27]. Ingegnoli also showed a linear correlation with SLEDAI score and severity of capillary abnormalities, by semi-quantitatively scoring patterns between 0-2 [28]. Approximately half of patients (56%) in our cohort were analyzed at the moment of diagnosis (treatment naïve). Improvement of abnormal capillary changes due to therapeutic intervention has been described in SSc [29, 30]. In our cohort, the median SLEDAI score of 5 at the moment of capillaroscopy is interpreted as a low disease activity score which may underestimate our results. Our significant correlation between the amount of abnormal capillary shapes and treatment-naivety confirms this. Presence of nephritis was significantly correlated with large pathological hemorrhages, while no other disease manifestations showed correlations with capillary abnormalities. An explanation could be that the found capillary abnormalities are representative for SLE in general and not specific for certain clinical symptoms of this severe disease.

A novel finding in this study was the observation of ‘pericapillary extravasations’: small point-shaped hemorrhages surrounding the capillary apex. These extravasations were observed in significantly higher frequency and count per mm in cSLE-patients, as compared to healthy controls. The ‘pericapillary extravasations’ seem a distinct subtype of capillary hemorrhage and were six times more often observed than ‘large pathological hemorrhages', when analyzed per mm. Interestingly, pericapillary extravasations were not correlated with periungual trauma (table 4), suggesting a pathophysiological origin such as endothelial wall damage. To our knowledge, such extravasations have been sporadically described in adult SLE-patients (and never in children), as "pearl necklaces of extravasates" or "extravasations of red blood cells, with the impression of punched out windows" [31, 32]. A possible explanation for this new observation could be that the quality and resolution of images from NVC have significantly improved in the last years. Hypothetically, this subtype of capillary hemorrhages might be small extravasations from a vulnerable capillary possibly due to endothelial activation and damage. It is possible that these 'pericapillary extravasations' are a reflection of endothelial dysregulation, as has been demonstrated in SLE-patients [33-35], leading to vasculopathy, which may be related to the pathogenesis of SLE. Pericapillary extravasations do not show migration towards the peripheral area (along with nail growth) as large hemorrhages do in a scleroderma pattern. Possibly, smaller hemosiderin deposits are cleared faster by phagocytic cells. The endothelial activation and damage, as described in SSc [36], does also seem to play a role in SLE [33-35]. SLE occurs 2 to 4 times more frequently among non-white populations [37] and this non-white population also seems to have a more severe disease course [11, 38]. It might be that the significant higher amount of extravasations, found in our non-white cSLE-patients, reflects this.

The combination of 'pericapillary extravasations and abnormal capillary shapes' were mostly observed in the fourth and fifth digits. These digits are less used in daily activities, suggesting that these capillary changes are less likely to be caused by trauma and further supporting a possible origin from a pathophysiological damage of endothelium. Multivariate analysis to determine correlations between detection of such 'specific microangiopathy pattern' with disease characteristics could not be performed due to small sample size of the group (< 10 subjects) that did not show this 'specific microangiopathy pattern' (n=9/41) [39]. All seven cSLE-patients with a scleroderma pattern also showed this specific combination of 'capillary abnormal shapes and pericapillary extravasations'. However, these patients did not have any other clinical criteria for SSc and positive anti-RNP antibodies were not significantly more detectable in these patients. Longitudinal studies are needed for clinical follow-up of these cSLE-patients with a capillary scleroderma pattern for a correct interpretation of this finding. The hypothesis for pathogenesis of a scleroderma pattern is that the capillary first typically enlarges due to endothelial damage forming a micro-aneurysmatic giant capillary, which might subsequently lead to a capillary microhemorrhage. These microhemorrhages are closely associated with the enlarged loops and have an obvious apical capillary genesis [1]. In our cohort we did not observe a correlation between the amount of large hemorrhages and the number of giant capillaries per mm (regression coefficient B 0.71, 95% CI -0.06

– 1.49, p=0.07). This observation also leads to the question if the pathogenesis of large capillary hemorrhages is different in SLE than in scleroderma, both distinct systemic autoimmune diseases with incidentally clinical overlap with other connective tissue diseases.

The limitations of this study include the relatively small sample size of this cSLE-cohort with 41 patients, due to the rarity of this disease. Our male/female ratio was 1/7, representing the general known male/female ratio of 1/8-10 in adults with SLE [37] and 1/5-6 in cSLE (14). Secondly, it is known that SLE occurs 2 to 4 times more frequently among non-white populations [37] which is also shown in our data (63.4% non-white). In cSLE, a median onset of 12.6 years (IQR 10.4-14.5) at diagnosis is described in the literature (14) which corresponds with our cohort with a median age of 14 years (IQR 12.5-16) at diagnosis. Although median age was significantly lower in the healthy cohort (12 versus 17 years), this difference will probably not make a difference for our outcome data, as it still concerned a pediatric (teenage) population. The same argument applies to matching of gender which was significantly different but with percentages of 87 versus 70% a majority of females in both cohorts.

CONCLUSION

This study confirms that children with SLE, like adult SLE-patients, also show significantly more giants, abnormal capillary morphology and capillary hemorrhages, when compared to healthy controls. In our study, these abnormal capillary findings were significantly correlated with SLEDAI scores, treatment-naivety and nephritis, thus making nailfold capillary abnormalities potentially interesting as disease biomarker(s). A prominent finding was the observation of a newly described subtype of capillary hemorrhage which we called “pericapillary extravasations”. By assessment of intra- and inter-observer variability we need to determine if these pericapillary extravasations are reproducible, to confirm if they are a distinct finding from large capillary hemorrhages.

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Supplementary file 1. Clinical characteristics of cSLE-cases with capillary scleroderma pattern, n=7.

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Demographics (age at presentation)	Girl, Caucasian, 12 years old	Girl, Caucasian, 13 years old	Boy, Asian, 13 years old	Girl, Afro-Caribbean, 15 years old	Girl, Afro-Caribbean 17 years old	Girl, Afro-Caribbean	Girl, Caucasian, 17 years old
Clinical symptoms	Butterfly rash of erosive ulcerative skin disease, extensive oral aphtous ulcers, chilblains, auto-immune hepatitis, splenomegaly with calcifications, leukopenia, thrombocytopenia	Butterfly rash and discoid skin lesions trunk, arms after sun exposure, apthous ulcers, leukopenia	Pleuritis, pericarditis, ascites, myositis, polyarthritis, fever, lymphadenopathy, deep venous thrombosis, hepatosplenomegaly, aphtous ulcers, leukopenia	Pleuritis, pericarditis, myositis, polyarthritis, fever, aphtous ulcers, leukopenia, thrombocytopenia	Aphtous ulcers, lymphadenopathy, skin rash (resulting in hyperpigmentation)	Vasculitis, arthritis, nephritis, mood disorder (intracranial hypertension/resorption)	Aphtous ulcers, leukopenia, thrombocytopenia
Skin biopsy	Vacuolar degeneration of the basal layer with abundant nuclear dust, also localized around the superficial blood vessels. Immunofluorescence: positive lupus band with (granular) staining of IgG, IgM, C1q and C3	The epidermis shows a hyperkeratotic basket-weave stratum corneum, with some vacuolar degeneration of the basal layer. The epidermis is atrophic with follicular plugging. Below the epidermis a mild perivascular and perifollicular, predominantly lymphocytic infiltrate	-	-	A-specific inflammation lymph node, lip biopsy inconclusive	Around superficial vessels, subcutaneous tissue and deep dermal plexus inflammation with lymphocytes, histiocytes, neutrophilic granulocytes en abundant nuclear dust. Also eosinophilic granulocytes around vessels and focal interstitial degranulation. Vessel wall is swollen with focal fibroid change. Immunofluorescence: depositions of complement factors (C1q>>C3c), combined with IgM (and some IgG)	-
ANA / anti-ds-DNA	positive / positive	positive / positive	positive / positive	positive / positive	positive / negative	positive / positive	positive / positive
Other auto-antibodies	anti-RNP, anti-Ro52, anti-SS-A, anti-Sm	anti-RNP, anti-SS-A, anti-Sm	anti-RNP, anti-SS-A, anti-Sm, rheumatoid factor	anti-RNP, anti-Sm, anti-Ro52, anti-ds-DNA, rheumatoid factor	none	anti-C1q antibodies	Anti-RNP, anti-Sm

Supplementary file 1. Clinical characteristics of cSLE-cases with capillary scleroderma pattern, n=7. (*continued*)

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Anti-phospholipid antibodies	negative	negative	positive	negative	positive	negative	negative
C3/C4	low	low	low	low	normal	low	normal
Coombs test	positive	positive	positive	positive	positive	positive	negative
SLEDAI at presentation	17	10	10	29	4	35	4
SLEDAI at capillaroscopy	17	10	8	6	4	35	4
Disease duration at capillaroscopy	at diagnosis	at diagnosis	4 years	5 years	at diagnosis	at diagnosis	at diagnosis
Discoloration of fingers	acrocyanosis in winter	no	biphasic Raynaud's phenomenon	acrocyanosis during whole year	no	no	Raynaud
Sclerodactyly	no	no	no	no	no	no	no
Pulmonary disease	no	no	restrictive pulmonary function	no	no	no	no
Nephritis	no	no	proteinuria: biopsy refused by parents/ patient	nephritis class V	no	nephritis class IV	no
Medication at capillaroscopy (and ever used)	None (prednisolone, hydroxychloroquine azathioprine, ritux-imab, mycophenolate mofetil, belimumab)	None (prednisolone, hydroxychloroquine azathioprine)	prednisolone, hydroxychloroquine, methotrexate (rituximab, mycophe-nolate mofetil)	prednisolone, hydroxychloroquine, mycophenolate mofetil, cyclophos-phamide (azathioprine, meth-otrexate, rituximab, belimumab)	Prednisolone (hy-droxychloroquine)	None (prednisolone, hydroxychloroquine, mycophenolate mofetil)	(hydroxychloro-quine, nifedipine, prednisolone)
Follow-up period	4 years	5 years	8 years	9 years	Lost to follow-up	2 years	1 year

Supplementary file 2. Correlations between clinical and demographical variables and amount of "abnormal shapes per mm"

Variable	Regression coefficient β (95% CI) cSLE	p-value	Regression coefficient B (95% CI) healthy controls	p-value
Skin pigmentation (ordinal)	0.037 (-0.1 – 0.173)	0.587	0.021 (-0.035 – 0.078)	0.454
Trauma	0.369 (0.001 – 0.737)	**0.049**	-0.012 (-0.146 – 0.121)	0.854
Raynaud/acrocyanosis	0.184 (-0.076 – 0.444)	0.160	-0.066 (-0.298 – 0.167)	0.572
Treatment-naivety	0.281 (0.042 – 0.519)	**0.022**		
Disease duration	0.005 (0.001 – 0.01)	**0.01**		
SLEDAI at diagnosis	0.009 (-0.009 – 0.028)	0.324		
SLEDAI at capillaroscopy	-0.01 (-0.029 – 0.008)	0.257		
Anti-RNP	0.226 (-0.023 – 0.475)	0.074		
Cutaneous involvement	-0.145 (-0.408 – 0.118)	0.271		
Neuropsychiatric involvement	0.065 (-0.293 – 0.422)	0.716		
Nephritis	-0.137 (-0.405 – 0.132)	0.309		
Antiphospholipid antibodies	-0.029 (-0.118 – 0.061)	0.518		

Bold indicates statistically significant p values (<0.05)

Supplementary file 3. Correlations between clinical and demographical variables and amount of "giant capillaries per mm"

Variable	Regression coefficient β (95% CI) cSLE	p-value
Skin pigmentation (ordinal)	-0.013 (-0.057 – 0.030)	0.543
Trauma	-0.026 (-0.149 – 0.098)	0.678
Raynaud/acro-cyanosis	0.054 (-0.030 – 0.137)	0.200
Treatment-naivety	0.013 (-0.068 – 0.095)	0.740
Disease duration	-0.001 (-0.002 – 0.000)	0.166
SLEDAI at diagnosis	-0.002 (-0.008 – 0.004)	0.487
SLEDAI at capillaroscopy	0.001 (-0.005 – 0.007)	0.629
Anti-RNP	0.052 (-0.029 – 0.134)	0.200
Cutaneous involvement	-0.022 (-0.107 – 0.063)	0.598
Neuropsychiatric involvement	-0.028 (-0.142 – 0.086)	0.619
Nephritis	-0.034 (-0.120 – 0.052)	0.430
Antiphospholipid antibodies	-0.002 (-0.031 – 0.026)	0.874

Bold indicates statistically significant p values (<0.05)

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4

Standardized nailfold capillaroscopy in children with rheumatic diseases: a worldwide study

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ABSTRACT

OBJECTIVE: To standardly assess and describe nailfold videocapillaroscopy (NVC) assessment in children and adolescents with juvenile rheumatic and musculoskeletal diseases (jRMD) versus healthy controls (HC).

METHODS: In consecutive jRMD children and matched HC from 13 centres worldwide, 16 NVC images per patient were acquired locally and read centrally for standard evaluation by international consensus definitions from the EULAR Study Group on Microcirculation in Rheumatic Diseases. Ninety-five patients with juvenile idiopathic arthritis (JIA), 22 with dermatomyositis (JDM), 20 with systemic lupus erythematosus (cSLE), 13 with systemic sclerosis (jSSc), 21 with localized scleroderma (lSc), 18 with mixed connective tissue disease (MCTD) and 20 with primary Raynaud's phenomenon (PRP) were included. NVC differences between juvenile subgroups and HC were calculated through multivariable regression analysis.

RESULTS: A total number of 6474 images were assessed from 413 subjects (mean age 12.1-years, 70.9% female). The quantitative NVC-characteristics were significantly lower (↓) or higher (↑) in the following subgroups compared to HC: For density: ↓ in jSSc, JDM, MCTD, cSLE and lSc; For dilations: ↑ in jSSc, MCTD and JDM; For abnormal shapes: ↑ JDM and MCTD; For haemorrhages: ↑ in jSSc, MCTD, JDM and cSLE. The qualitative NVC-assessment of JIA, lSc and PRP did not differ from HC, whereas the cSLE and jSSc, MCTD, JDM, cSLE subgroups showed more non-specific and scleroderma patterns respectively.

CONCLUSION: This analysis resulted from a pioneering registry of NVC in jRMD. The NVC-assessment in jRMD differed significantly from HC. Future prospective follow up will further elucidate the role of NVC in jRMD.

INTRODUCTION

Nailfold capillaroscopic examination has proven its worth in the field of rheumatology [1, 2]. In adults, together with the identification of autoantibodies, it allows discrimination between primary and secondary Raynaud's phenomenon (RP). The latter is mostly related to an underlying connective tissue disease (CTD) [3]. Moreover, its role was formalized, through the incorporation of "an abnormal nailfold capillaroscopy" as criterion (referring to the scleroderma pattern) in the systemic sclerosis (SSc) 2013 classification criteria [4]. Ever since its increased use, assembled efforts have led to the standardisation of the capillaroscopic technique and reading in adults [5-8]. In children and adolescents however, nailfold capillaroscopy is less used and studies are scarce and usually small, using non-standardised methods [9]. The European League Against Rheumatism (EULAR) Study Group of Microcirculation in Rheumatic Diseases found it thus timely to start an international collaborative to collect nailfold capillaroscopic data from children and adolescents with and without juvenile Rheumatic and Musculoskeletal Diseases (jRMD). We hypothesise that through nailfold videocapillaroscopy (NVC), as in adult disease, we may detect microvascular abnormalities in jRMD, presumably caused by systemic inflammatory immune responses.

This article presents the first data from a collaborative effort, in which the NVC-features of a large international cohort of children and adolescents are standardly described with international consensus definitions from the EULAR Study Group of Microcirculation in Rheumatic Diseases. The results could serve as a framework for paediatricians to interpret NVC-assessments in their rheumatology practice. For researchers, it could provide the prerequisites to further investigate NVC-characteristics and clinical correlations in disease specific studies.

PATIENTS AND METHODS

Study patients and healthy controls

Children and adolescents from 13 different centres worldwide were examined using NVC. All participating centres obtained approval from the local ethics committee (for Belgium: B670201627545) and written informed consents from all participants or representatives were obtained. Details on centre contributions and their approval numbers are found in the Supplementary file, table S1 [7].

Co-investigators were asked to include consecutive patients with RP and/or a definite diagnosis of a jRMD according to the physician's opinion and fulfilling the established classification criteria, irrespective of the disease duration or disease activity status [10-16]. RP was defined as the observation of at least a biphasic colour change after cold exposure [17]. Patients with an indefinite diagnosis or overlap (other than mixed connective tissue disease) were excluded. Additionally, the presence of antinuclear antibodies (ANA) and, if available, the specifications on extractable nuclear antigen antibodies, were documented, as per discretion of the local investigator. No records were taken on therapies, with the assumption that all patients were receiving a variety of therapies and the unawareness of a specific therapy that is clearly correlated with the presence of NVC-abnormalities.

Each jRMD patient with evaluable NVC images was manually matched to a healthy control child (HC) from the same gender and age group (groups with the following age ranges: < 5 years, 5-7 years, 8-10 years, 11-14 years and 15-18 years) [18]. If no exact age and gender matched HC was available, the authors selected an as close as possible available match, where priority was given to similar age over same gender. The HC were provided by different centres. They were mainly recruited at schools, some were siblings of patients or family members from the investigators. No reliable records were taken on the presence of RP in HC, recruited in circumstances where serological or clinical assessment was unavailable (e.g. in schools).

Capillaroscopic technique and reading method

Each child was examined with a standardised NVC-technique [7]. All fingers, except for the thumbs, were assessed with a x200-magnification contact lens (Videocap/Optilia/Inspectis/Dino-Lite microscope, depending on local equipment). Two adjacent central images per nailfold were captured, coded, and saved (set of 16 images per child) and the investigators were asked to place a grid on all images, corresponding to one-millimetre (mm) nailfold in real life, using centre-dependent image analysis software (DS Medica-Videocap, Italy; Optilia-Mediscope, Sweden; Inspectis, Sweden; Dino-Capture, Taiwan). The time to acquire the 16 images per child took about ten minutes. No specific training was given to the investigators, who were all operating in a capillaroscopic expert centre. The NVC-images were collected digitally through Web Share and read at the Ghent University by a trained observer, who was blind for healthy or disease status (KM) [19]. The graphic viewer "IrfanView" (Version 4.51) was used to correct for image sizes, which varied among centres, and to measure the dimension of the capillaries, using the one-mm grid as a reference. The reading and reporting method followed the capillaroscopic protocol from the EULAR Study Group of Microcirculation in Rheumatic Diseases (figure 1) [7, 20].

The quantitative NVC-assessment in the one-mm grid consisted of the following NVC-parameters:

- the “capillary density” (the number of capillaries in the distal row);
- the “capillary dimension” (the number of dilated capillaries (dilations) having an apical limb diameter between 20-50um and the number of giant capillaries (giants), having an apical limb diameter of >50um);
- the “capillary morphology” (the number of capillaries with a normal morphology -capillaries with a hairpin shape; once or twice-crossing shape; or tortuous shape i.e. limbs bend but do not cross; on the condition that the tip is convex- and abnormal morphology -all capillaries whose shape does not correspond to the definition of a normal shape) [21, 22] and;
- the presence of microhemorrhages (red or brown amorphous structures in the peri-capillary/periungual region).

Nailfold Video Capillaroscopic Protocol 	Left Hand				Right Hand			
	2nd	3rd	4th	5th	2nd	3rd	4th	5th
QUANTITATIVE ASSESSMENT *image level per linear mm*								
Capillary density	_ _	_ _	_ _	_ _	_ _	_ _	_ _	_ _
Number of dilations	_ _	_ _	_ _	_ _	_ _	_ _	_ _	_ _
Number of giants	_ _	_ _	_ _	_ _	_ _	_ _	_ _	_ _
Number of normal shapes	_ _	_ _	_ _	_ _	_ _	_ _	_ _	_ _
Number of abnormal shapes	_ _	_ _	_ _	_ _	_ _	_ _	_ _	_ _
Presence of microhaemorrhages	_ _	_ _	_ _	_ _	_ _	_ _	_ _	_ _
QUALITATIVE ASSESSMENT *subject level*								
Non-scleroderma pattern								
Normal pattern				____________				
Non-specific pattern				____________				
Scleroderma pattern				____________				

Figure 1. Standardized nailfold videocapillaroscopy (NVC) protocol.
Two images of the second, third, fourth and fifth digit of each hand are assessed according to the standard format to report on capillaroscopic characteristics, including the quantitative (per linear mm) and qualitative (the capillary pattern) assessment (image level). To report at subject level, the capillaroscopic parameters are deduced from all the obtained NVC-images from an individual subject:
- by calculating the mean of the capillary density, the mean of the number of dilations and the mean of the number of abnormal shapes (the sum of each NVC-parameter at image level divided by the number of assessed images per subject);
- by describing the presence or absence of the parameters ‘giants’ and ‘microhemorrhages’ in a dichotomous way and;
- by deriving the overall qualitative assessment (details in section 2.2).

To obtain the quantitative NVC-parameters at subject level, the means were calculated, except for the two NVC-parameters “giants” and “microhaemorrhages”, which were both reported in a dichotomous way at subject level, as being present or not.

The qualitative NVC-assessment consisted of categorizing the overall capillary pattern at subject level in a “scleroderma pattern” (a pattern with the presence of giants or the combination of abnormal shapes with an extremely lowered number of capillaries) or a “non-scleroderma pattern”. The latter included a “normal pattern” (capillaries of the distal row are normally shaped, homogenous in dimension and their density is ≥ 7 / linear mm) and a “non-specific pattern” [8]. To depict the overall capillary pattern at subject level, the following rules were applied: - as soon as one of the images was categorized as a “scleroderma pattern” the overall capillary pattern was a “scleroderma pattern”; -when no “scleroderma pattern” was present (none of the 16 images), the most dominant “non-scleroderma pattern” depicted the overall capillary pattern; - if both the “normal” and “non-specific” patterns were equally represented (eight “normal” patterns and eight “non-specific” patterns), the “non-specific pattern” was assigned as overruling capillary pattern. Examples of the quantitative and qualitative NVC-assessments at image level are given in figure 2.

Capillaroscopic characteristics	Non-scleroderma pattern		Scleroderma pattern		
	Normal	Non-specific abnormalities	Early	Active	Late
Density (mm)	≥7	↓	≥7	4-6	≤3
Dimension (μm)	Normal	20-50	>50 (giant)	>50 (giant)	-
Abnormal morphology	-	+	-	+	++

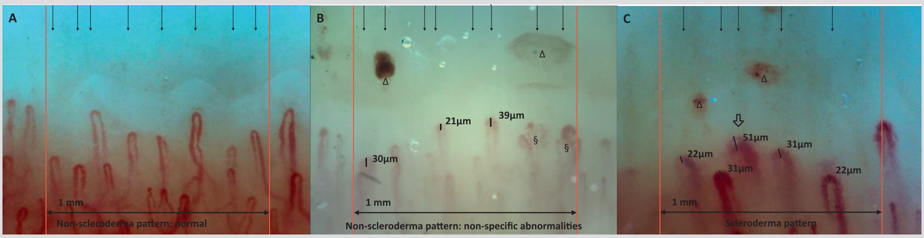

Figure 2. Standardized assessment of NVC-images according to the internationally consensus EULAR Study Group on Microcirculation in Rheumatic Diseases definitions.
A) An example of a stereotype “normal” pattern. Density: 8 capillaries per linear mm (↓). Dimension: no dilations, no giants. Morphology: no abnormal shapes. Microhemorrhages: absent. Interpretation: normal pattern (non-scleroderma pattern)
B) An example of a “non-specific” pattern. Density: 8 capillaries per linear mm (↓). Dimension: presence of 3 dilations per linear mm, no giants. Morphology: presence of 2 abnormal shapes (§). Microhemorrhages: present. Interpretation: non-specific abnormalities (non-scleroderma pattern)
C) An example of a “scleroderma” pattern. Density: 5 capillaries per linear mm (↓). Dimension: presence of a giant (⇓). Morphology: no abnormal shapes. Microhemorrhages: present. Interpretation: (active) scleroderma pattern.
Adapted from Smith V et al. *Standardization of nailfold capillaroscopy for the assessment of patients with Raynaud’s phenomenon and systemic sclerosis.* Autoimmunity reviews. 2020 [7].

Evaluability and handling of missing data

The evaluability was assessed per image based on the difficulty of the capillaroscopic reading. If all NVC-parameters were evaluable, the visibility of that image was considered as "good". On the other hand, an image was scored as "bad" if any NVC-parameter was not evaluable. A "bad" image was still included for analysis and the concerning unevaluable parameter was then encoded as a missing. A subject was excluded from further analysis if less than four out of the 16 images were scored as "good".

Statistical analysis

Analyses have been performed by IBM SPSS version 27 and R version 4.1.1. To assess the matched sample quality (jRMD vs HC), an Optimal Pair Propensity Score Matching method was used, that minimizes the overall distance (age and gender) between matched pairs [23]. Descriptive statistics were used to represent demographic features and to describe the NVC-parameters per subgroup, with means and standard deviations (SDs) for continuous variables and proportions for categorical variables. The following six NVC-parameters were analysed at subject level: the mean capillary density, the mean number of dilations, the presence of giants, the mean number of abnormal shapes, the presence of microhemorrhages and the capillary pattern. For comparison of these six NVC-parameters between disease subgroups and the overall HC-group, unmatched multivariable regression analysis was performed, adjusted for age and gender. Raw p-values were reported, and a Bonferroni correction was applied (α-level set at 0.007, as seven subgroups were compared to the overall HC-group) and corresponding 99.3% confidence intervals (CI) are reported. The mean differences (for continuous variables) and the odds ratios (for categorical variables) were presented, in comparison with the HC-subgroup. Additionally, exploratory subgroup analysis in the HC-group was done by calculating the Pearson correlation coefficient to quantify the linear relationship between age and the quantitative NVC-parameters density, dimension, and morphology.

RESULTS

A total number of 6474 NVC images were assessed from 413 subjects, consisting of 120 boys and 293 girls, with a mean age of 12.1 years (±3.72 SD). In some subjects, less than 16 NVC-images were assessed, due to erroneous storage of the images or because adjacent NVC-images were overlapping. Demographics per subgroup are shown in table 1 and details on ANA are found in the Supplementary file (table S2).

Table 1. Demographics (n=413)

	HC n=204	PRP n=20	cSLE n=20	JDM n=22	jSSc n=13	lSc n=21	MCTD n=18	JIA n=95				
								OA n=22	PA n=23	ERA n=20	PsA n=15	JIAS n=15
Age (years), mean +-SD	12.0 ±3.6	14.6 ±2.1	13.6 ±4.0	11.4 ±3.9	13.1 ±3.6	11.2 ±3.5	13.5 ±3.2			11.5 ±3.8		
								10.8 ±4.1	11.4 ±4.0	13.6 ±2.8	12.1 ±4.4	9.4 ±3.9
Female gender, n (%)	145 (71.1)	16 (80.0)	14 (70.0)	15 (68.2)	12 (92.3)	14 (66.7)	15 (83.3)			62 (65.2)		
								18 (81.8)	15 (65.2)	11 (55.0)	9 (60.0)	9 (60.0)
Race, n (%)												
White	161 (78.9)	17 (85.0)	8 (40.0)	17 (77.3)	5 (38.5)	15 (71.4)	12 (66.7)	19 (86.4)	20 (87.0)	17 (85.0)	13 (86.7)	8 (53.3)
Asian	6 (2.9)	0 (0.0)	3 (15.0)	0 (0.0)	3 (23.1)	1 (4.8)	1 (5.6)	2 (9.1)	0 (0.0)	0 (0.0)	2 (13.3)	6 (40.0)
Black	11 (5.4)	0 (0.0)	5 (25.0)	2 (9.1)	1 (7.7)	1 (4.8)	0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (6.7)
Other	26 (12.7)	3 (15.0)	4 (20.0)	3 (13.6)	4 (30.8)	4 (19.0)	5 (27.8)	0 (0.0)	3 (13.0)	3 (15.0)	0 (0.0)	0 (0.0)
Disease duration (years), mean ±SD	-	1.3 ±1.9	2.4 ±2.7	3.3 ±4.2	2.5 ±2.5	3.8 ±3.2	2.1 ±2.8	6.2 ±4.5	4.5 ±3.6	3.5 ±3.7	4.7 ±5.1	4.2 ±4.1
Raynaud's phenomenon, n (%)	4* (2.0)	20 (100)	4 (20.0)	0 (0.0)	13 (100)	2 (9.5)	10 (55.6)	2 (9.1)	1 (4.3)	2 (10.0)	0 (0.0)	1 (6.7)
ANA, n/total valid (%)	-	5/20 (25.0)	17/18 (94.4)	13/16 (81.2)	13/13 (100.0)	2/11 (18.2)	14/16 (87.5)	9/17 (40.9)	13/21 (59.1)	6/15 (40.0)	5/10 (50.0)	1/15 (6.7)

*4/204 (2.0%) of HC had RP, in which the cause remained undefined (primary or secondary), as no rheumatological work-out had been performed in volunteers at schools. ANA, antinuclear antibodies detected by indirect immunofluorescence-screening on Hep-2 cell substrates; cSLE, childhood onset systemic lupus erythematosus; ERA, enthesitis related arthritis; HC, healthy controls; JDM, juvenile dermatomyositis; JIA(S), juvenile idiopathic arthritis (systemic); jSSc, juvenile systemic sclerosis; lSc, localized scleroderma; MCTD, mixed connective tissue disease; OA, oligoarthritis; PA, polyarthritis; PRP, primary Raynaud's phenomenon; PsA, psoriatic arthritis.

Evaluability and matching

In 5912 NVC-images (91.3%) all NVC-parameters could be assessed. Most subjects had more than 12 images of "good" visibility (Supplementary file, table S3). From the 209 studied patients, five subjects had a bad general visibility (<4/16 "good" images): in four subjects (three with the systemic form of juvenile idiopathic arthritis (JIAS) and one with psoriatic arthritis), it was related to a technical problem with the focus of the lens, and in one patient with juvenile dermatomyositis (JDM) it was inherent to the capillaroscopic abnormalities, in which diffuse microhemorrhages made it impossible to assess other NVC-parameters. Those five subjects were excluded for further analysis (and were not matched).

As such, statistical analyses are performed on an age and gender balanced sample of 204 patients with jRMD and 204 HC. An Optimal Pair Propensity Score Matching method showed no imbalances in gender and a standardized mean difference in age below 0.1, which corresponds to a maximum age difference of three years within matched pairs.

Quantitative NVC-assessment per disease subset

Table 2 shows the NVC-parameters per subgroup, together with the mean differences and odds ratios per disease subset versus HC.

Capillary density

The mean capillary density in HC was 8.5 capillaries/linear mm (±1.2 SD), which was similar to the mean capillary density from patients with PRP and JIA. On the other hand, in patients with mixed connective tissue disease (MCTD), JDM and juvenile SSc (jSSc), a significantly lower capillary density was seen (7.1 ±1.3 SD, 6.3 ±2.0 SD and 5.2 ±1.9 SD respectively, p<0.001).

The density in childhood onset systemic lupus erythematosus (cSLE) (7.6 ±1.3 SD) and localized scleroderma (lSc) (7.7 ±1.2 SD) was significantly lower compared to HC as well (p=0.006 and p=0.005 respectively). However, the mean difference remained small (less than one capillary per linear mm). The results are visualized in figure 3A.

Capillary dimension

In HC, a mean of 0.5 (±0.6 SD) capillary dilations per linear mm was observed. In lSc and JIA patients, the capillary dimension did not differ from HC. An increased number of dilated capillaries was seen in PRP (1.0 ±0.9 SD, p=0.008) and in cSLE (0.9 ±1.1 SD, p=0.035), but was only statistically significantly different from HC in JDM, MCTD and in jSSc, in which a mean of respectively 1.5 (±1.2 SD), 1.8 (±1.0 SD) and 1.8 (±0.6 SD) capillary dilations per linear mm were observed (p<0.001) (table 2, figure 3B).

Table 2. Comparison of the quantitative and qualitative NVC-assessment in the different subgroups compared to HC (n=408).

	HC n=204	PRP n=20	cSLE n=20	JDM n=21	jSSc n=13	lSc n=21	MCTD n=18	JIA n=91
QUANTITATIVE ASSESSMENT								
Density								
Mean capillary density/linear mm, ±SD ***MD, (99.3CI)***	8.5±1.2	8.7±0.8 *0.2* *(-0.6;1.0)* *p=0.467*	7.6±1.3 ***-0.8*** ***(-1.6;0.0)*** ***p=0.006***	6.3±2.0 ***-2.1*** ***(-2.9;-1.4)*** ***p<0.001***	5.2±1.9 ***-3.2*** ***(-4.2;-2.2)*** ***p<0.001***	7.7±1.2 ***-0.8*** ***(-1.6;0.0)*** ***p=0.005***	7.1±1.3 ***-1.4*** ***(-2.3;-0.5)*** ***p<0.001***	8.7±1.2 *0.2* *(-0.2;0.6)* *p=0.229*
Dimensions								
Mean number of dilations/ linear mm, ±SD ***MD, (99.3CI)***	0.5±0.6	1.0±0.9 *0.4 (0.0;0.9)* *p=0.008*	0.9±1.1 *0.3* *(-0.1;0.8)* *p=0.035*	1.5±1.2 ***1.1*** ***(0.6;1.5)*** ***p<0.001***	1.8±0.6 ***1.3*** ***(0.7;1.8)*** ***p<0.001***	0.4±0.4 *-0.1* *(-0.5;0.4)* *p=0.675*	1.8±1.0 ***1.2*** ***(0.8;1.7)*** ***p<0.001***	0.4±0.6 *-0.1* *(-0.3;0.2)* *p=0.441*
Subjects with giants, n (%) ***OR (99.3CI)***	3 (1.5)	1 (5.0) *4.5 (0.1;63.1)* *p=0.185*	1 (5.0) *4.4 (0.1;58.5)* *p=0.188*	12 (57.1) ***71.8 (13.2;627.7)*** ***p<0.001***	11 (84.6) ***283.7 (33.2;5229.1)*** ***p<0.001***	0 (0.0) *1.3 (0.0;31.0)* *p=0.869*	10 (55.6) ***73.3 (12.0;701.3)*** ***p<0.001***	1 (1.1) *0.9 (0.0;11.7)* p=0.943
Morphology								
Mean number of abnormal shapes/linear mm, ±SD, *MD, (99.3CI)*	0.3±0.3	0.2±0.3 *0.0* *(-0.3;0.2)* *p=0.675*	0.3±0.4 *0.1* *(-0.2;0.3)* *p=0.452*	0.9±1.0 ***0.7*** ***(0.4;0.9)*** ***p<0.001***	0.5±0.4 *0.2* *(-0.1;0.6)* *p=0.043*	0.4±0.6 *0.2* *(-0.1;0.4)* *p=0.059*	0.6±0.4 ***0.3*** ***(0.1;0.6)*** ***p=0.001***	0.2±0.3 *0.0* *(-0.2;0.1)* *p=0.719*
Microhemorrhages								
Subjects with microhemorrhages, n (%), ***OR (99.3CI)***	80 (39.2)	11 (55.0) *1.6 (0.4;5.9)* *p=0.317*	16 (80.0) ***5.2 (1.3;29.8)*** ***p=0.001***	17 (85.3) ***6.2 (1.6;35.4)*** ***p<0.001***	11 (84.6) ***6.8*** ***(1.2;80.8)*** ***p=0.002***	8 (38.1) *1.0 (0.3;3.5)* *p=0.964*	14 (77.8) ***4.6 (1.1;26.8)*** ***p=0.003***	27 (29.7) *0.7 (0.3;1.4)* *p=0.134*

Table 2. Comparison of the quantitative and qualitative NVC-assessment in the different subgroups compared to HC (n=408). (*continued*)

	HC n=204	PRP n=20	cSLE n=20	JDM n=21	jSSc n=13	lSc n=21	MCTD n=18	JIA n=91
QUALITATIVE ASSESSMENT								
Scleroderma pattern								
Subjects with a scleroderma pattern, n (%), *OR (99.3CI)*	3 (1.5)	1 (5.0) *4.4 (0.1;61.3)* *p=0.189*	3 (15.0) ***11.4 (1.1;114.8)*** ***p=0.005***	13 (61.9) ***85.3 (15.8;748.4)*** ***p<0.001***	12 (92.3) ***465.9 (45.3;18977.3)*** ***p<0.001***	0 (0.0) *1.3 (0.0;31.3)* *p=0.861*	11 (61.1) ***85.7 (14.3;812.4)*** ***p<0.001***	1 (1.1) *1.0 (0.0;12.0)* *p=0.959*
Non-scleroderma patterns								
Subjects with a non-specific pattern, n (%), *OR (99.3CI)*	73 (35.8)	12 (60.0) *2.2 (0.6;8.5)* *p=0.096*	15 (75.0) ***4.5 (1.2;22.2)*** ***p=0.002***	4 (19.0) *0.4* *(0.1;1.8)* *p=0.131*	1 (7.7) *0.2* *(0.0;1.4)* *p=0.031*	7 (33.3) *1.0 (0.2;4.0)* *p=0.957*	7 (38.9) *1.1* *(0.2;4.0)* *p=0.901*	24 (26.4) *0.6 (0.3;1.4)* *p=0.118*
Subjects with a normal pattern, n (%)	128 (62.7)	7 (33.3)	2 (10.0)	4 (19.0)	0 (0.0)	14 (66.7)	0 (0.0)	66 (72.5)

CI: confidence intervals; cSLE, childhood onset systemic lupus erythematosus; HC, healthy controls; JDM, juvenile dermatomyositis; JIA, juvenile idiopathic arthritis; jSSc, juvenile systemic sclerosis; lSc, localized scleroderma; MCTD, mixed connective tissue disease; MD: mean difference (compared to HC); OR: odds ratio compared to HC; PRP, primary Raynaud's phenomenon; SD, standard deviation.
The mean differences (MD) (by multivariable regression analysis, adjusted for the matching factors age and gender) and odd's ratios per subgroup are compared to HC, showing the raw p-values and the Bonferroni corrected CI. When a significant difference ($p<0.007$, α-level set at 0.007, as seven subgroups were compared) between the subgroup and the HC group existed, these values are indicated in bold.

In jSSc, 37.09% of the capillaries were dilated and 15.96% of the capillaries were giants versus 5.60% dilations and 0.03% giants in HC ($p<0.001$). The same trends were observed in MCTD and JDM with respectively 26.33%, and 28.89% of dilated capillaries and 5.03% and 4.76% of giants ($p<0.001$).

Capillary morphology

The mean number of abnormal capillary shapes per linear mm in HC was 0.3 (±0.3 SD) and about the same values were observed in PRP, cSLE, lSc and JIA. JDM and MCTD subjects exhibited significantly more abnormal shapes compared to HC, being 0.9 (±1.0 SD) per linear mm and 0.6 (±0.4 SD) per linear mm ($p<0.001$) (table 2, figure 3C).

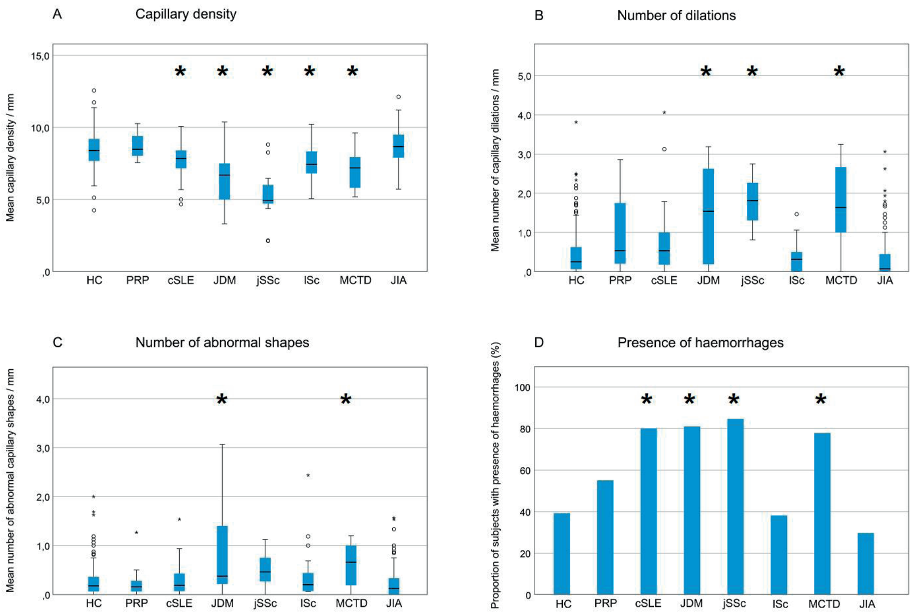

Figure 3. Quantitative NVC-assessment per subgroup, compared to the healthy subset (HC).
cSLE, childhood onset systemic lupus erythematosus; HC, healthy controls; JDM, juvenile dermatomyositis; JIA, juvenile idiopathic arthritis; jSSc, juvenile systemic sclerosis; lSc, localized scleroderma; MCTD, mixed connective tissue disease; PRP, primary Raynaud's phenomenon.
A) Box plot of the capillary density per linear mm;
B) Box plot of the number of dilations per linear mm;
C) Box plot of the number of abnormal shapes per linear mm. Variations in the subgroups are evidenced by the wide blue boxes and by the presence of outliers (° 1.5 x interquartile range) and extreme values (* 3 x interquartile range);
D) Bar graph of the proportion of subjects with presence of nailfold microhemorrhages.
The statistical significant differences between each subgroup and the overall HC subgroup are indicate with an asterix (*).

Of note, although the mean number of abnormal shapes in jSSc, being 0.5 (±0.4 SD) per linear mm, was not significantly different from HC (after Bonferroni correction), we found in a post-hoc analysis that the proportion of the mean number of abnormal shapes on the mean capillary density (number of abnormal shapes/total number of capillaries), did reveal a significant difference (12.15% in jSSc versus 3.25% in HC, p<0.001).

Presence of microhemorrhages

Figure 3D shows the proportions of subjects with microhemorrhages per subgroup. In 39.2% of the HC, microhemorrhages were found. The proportion increased in PRP to 55.0% (p=0.317) and was lower in JIA (29.7%, p=0.134). Significantly more microhemorrhages were observed in patients with CTD: cSLE 80.0%, JDM 85.3%, jSSc 84.6%, and MCTD 77.8%. The OR are represented in table 2.

Qualitative assessment

A “normal pattern” was observed in 62.7% of HC and was present in similar proportions in JIA and lSc. The “non-specific pattern” was the most dominant pattern in PRP (60.0%) and cSLE (75.0%), however, only in cSLE was it statistically significantly different from the proportion in HC (OR 4.5, CI 1.2-22.2, p=0.002). The odds to exhibit a “scleroderma pattern” was higher in the CTD-subsets compared to the odds in HC (table 2).

A “scleroderma pattern” was observed in three out of 204 HC (1.5%), in one out of 20 PRP (5.0%) and in one out of 91 JIA-patients (1.0%). In all five of them, clinical signs of an underlying CTD were absent. One of the HC with a “scleroderma pattern” had RP (a 16-year-old white girl). Another HC had a local trauma at the same finger in which giants were observed (a 6-year-old black boy). In the last “healthy” child, no explanation for this observed abnormality was at hand (a 14-year-old boy with mixed ethnicities (white/black)). The boy with PRP, whom had a “scleroderma pattern”, had no antinuclear antibodies at multiple occasions and no jRMD-related symptoms, but reported a compulsive habit of nail biting. The boy with JIA, whom had a “scleroderma pattern”, reported on nail biting as well.

Exploratory analysis of age-related nailfold capillaroscopic findings in HC

No age-related increase in capillary density was observed (R=-0.20, p=0.773) (figure 4A). On the contrary, the youngest age group (less than five-year-old children) exhibited a significantly higher capillary density compared to the other age groups. The Pearson’s correlation coefficient between the capillary density and age, by leaving out the youngest age group, resulted in a significant, though very weak positive correlation (R=0.14, p=0.046) (figure 4B).

There was a mild association between age and the number of capillary dilations, as reflected in a positive, but also small, correlation coefficient (R=0.18, p=0.011) (figure 4C). And there was no association with the number of abnormal shapes (R=-0.02, p=0.758) (figure 4D). No age-influence was found for the presence of microhemorrhages (data not shown).

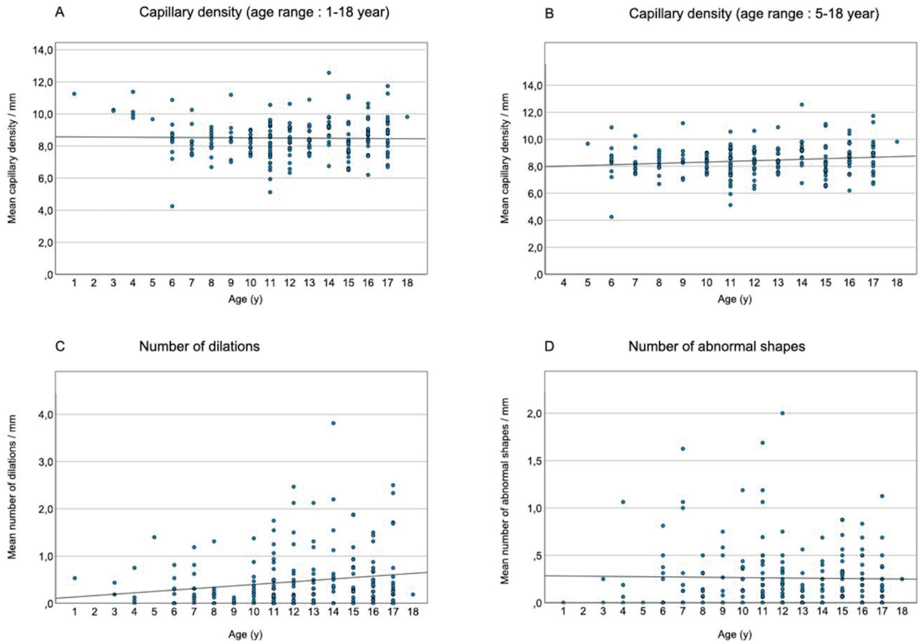

Figure 4. Exploratory analysis of the quantitative NVC-assessment in relation to age in the healthy subset. Scatter plot and fitted regression lines of: A) the capillary density in total healthy subset (n=204); B) the capillary density in healthy subset without the youngest group (n=197); C) the mean number of capillary dilations per linear mm (n=204); D) the mean number of abnormal capillary shapes per linear mm (n=204).

DISCUSSION

This study is a first multi-centre analysis of data from the international paediatric NVC-registry, set up by the EULAR Study Group of Microcirculation in Rheumatic Diseases. The NVC-characteristics of patients with varying jRMD and PRP are for the first time described in a standardised way and compared to a large group of HCs. The study reveals that the NVC-assessment in CTD such as jSSc, MCTD, JDM and cSLE differs significantly from HC. The NVC-characteristics from a large sample of JIA-patients are comparable with those from HC.

This study is one of the first of its kind, in that the NVC-images were assessed quantitatively and qualitatively, according to the standardised international consensus definitions from the EULAR Study Group on Microcirculation in Rheumatic Diseases [7, 20, 39]. The results are in accordance with the few previous publications on this topic in jRMD, in which a lowered capillary density is attested in jSSc, MCTD and JDM [24-29]. Additionally, we observed a lower capillary density in cSLE and lSc [28-30]. Interestingly, the mean capillary density in HC was 8.5/linear mm (±1.2 SD), which is in the normal range for density in adults (where the cut-off value is 7/linear mm) and may indicate that the same cut-off values for normality can be used in children [8].

Although Piotto *et al.* had previously found a strong correlation between density and age in 100 healthy children (older than 5 years) ($R=0.796$, $p<0.001$), we can only at best report that there is a very weak association in our healthy subgroup ($R=0.14$, $p=0.046$) [18]. Other reports on this topic from Herrick *et al.* and Ingegnoli *et al.* are more in line with our results [28, 31].

It is novel in the capillaroscopic research of jRMD to report on capillaroscopic dimension as the mean number of dilations per mm [18, 29, 32]. We observed a significantly higher number of dilations, and also a higher proportion of subjects with giants in jSSc, MCTD and JDM compared to HC, consistent with observations in adults [33-35]. In keeping with the findings of Herrick *et al.*, our subgroup analysis in HC revealed a trend for the number of dilations to rise with age [31].

So far, only one study in children reports on the capillary morphology by using the standardised “simple” definitions from the EULAR Study Group on Microcirculation in Rheumatic Diseases [5, 7, 18, 32, 34, 39]. Concerning the presence of microhemorrhages, we observed higher prevalence rates than previously reported in HC (39% compared to prevalence rates ranging from 10% till 20% in literature) [28, 32]. Keeping in mind that as it stands, no reliable distinction between pathological and non-pathological types of microhemorrhages can be made, especially in children who are more frequently exposed to (micro)traumatic events [32, 36].

Not unexpectedly, we observed “scleroderma patterns” in CTDs and “non-specific abnormalities” as the predominant pattern in cSLE [24, 25, 27, 28, 37-39]. Remarkably, in three out of five “unexpected” cases with a “scleroderma pattern”, trauma or the compulsive habit of nail biting was noted, which underlines the need of repeated assessments in these cases and careful follow-up.

The consecutive input from centres dispersed across Europe, the American continent and South-Asia is a major advantage of our study. Despite the dominance of the white ethnicity within the study population, we obtained images from a relatively larger proportion of JIAS-patients from the South-Asian subcontinent (40%) and from a relatively larger proportion of black patients amongst those suffering from cSLE, which reflect data from a real-life world study population. Both diseases are known to have a higher prevalence in these ethnicities [40, 41].

Another advantage of our study is the standardised acquisition of NVC images from different centres with similar magnification (200X) and the centralised reading method. By using the videocapillaroscope, which is considered the gold standard device to obtain reliable nailfold images, and by applying a standardised methodology, we believe that our results add value for the implementation of the NVC-examination in clinical practice [7]. While instructions on the NVC-technique and capturing method were provided to the operators only by a written study protocol, the high reported general evaluability (97.6% of the jRMD-subjects) attests the applicability of this technique [18, 34]. However, it needs to be kept in mind that, while thoroughly validated in adults, the used NVC- technique and methodology needs further validation in children and adolescents.

This study has some limitations, linked to its exploratory nature. Firstly, the subgroups of patients with jRMD remain relatively small and no pairwise comparison with age -and sex matched healthy controls was performed. Likewise, for the interpretation of the age-related NVC-characteristics in the healthy subset, it is important to note that only seven children were below five years old. Secondly, inherent to the study design, we did not obtain reliable clinical information from our healthy controls. Children were mostly recruited at schools, in the absence of their parents. An underlying diagnosis of RP or rheumatic disease might as such have been missed Thirdly, our analysis did not correct for centre contribution because we were not able to match all jRMD patients with HC from the same centre and because samples per subgroup were very small for some centres.

To conclude, this study pioneers in the standardised NVC-assessment of children and adolescents with jRMD, recruited across the world and had the aim of describing their NVC-assessment in a detailed and comprehensive manner. Our results support the regular use of NVC in children with PRP, CTD and even lSc. A further step of this international project is to follow children and adolescents prospectively in order to shed light on clinical associations with NVC-abnormalities in larger disease specific samples. The EULAR Study Group of Microcirculation in Rheumatic Diseases advocates the use of standardised NVC-assessments and terminology to improve the accuracy of NVC-studies and to facilitate comparisons in the future.

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SUPPLEMENTARY FILE 1: Centre contributions and EC approval numbers

Centre name (EC approval number)	HC N=204	PRP n=20	jRMD n=189
Ghent University Hospital, Ghent-Belgium (B670201627545)	62*	0	29
Brugmann University Hospital, Brussels-Belgium (B670201627545)	0	0	13
Amsterdam UMC, Amsterdam-The Netherlands (NL60885.018.17)	88*	7	33
University of Genoa and "Giannina Gaslini" Children Hospital, Genoa-Italy (392REG2017)	13	2	26
Gaetano Pini Hospital, Milan-Italy (752REG2017)	0	0	7
An der Schön Klinik Hamburg-Eilbek, Hamburg-Germany (PV5373)	3	10	35
Klinik für Allgemeine Pädiatrie und Neonatologie, Giessen-Germany (AZ 66/17)	10	0	14
Medical University of Lublin, Lublin-Poland (KE-0254/44/2017)	0	0	3
University Hospital La Paz, Madrid-Spain (PI-2635)	5	0	12
Mayo Clinic College of Medicine, Rochester-USA (18-003557)	21	0	3
University of Utah, Utah-USA (00118171)	1	1	2
Clinica Universitaria Bolivariana, Medellin-Colombia **	0	0	0
SRCC Children's Hospital, Mumbai-India (R-201901)	1	0	12

Table S1. Contributions per participating centre for healthy controls (HC), primary Raynaud's phenomenon (PRP), and juvenile diseases (jRMD) (n=413)

* Data obtained from schools

** Data transfer contract had not been finished at the moment of data analysis of this study.

SUPPLEMENTARY FILE 2: Extractable nuclear antigen specifications per subgroup

	PRP n=20	**cSLE n=20**	**JDM n=22**	**jSSc n=13**	**lSc n=21**	**MCTD n=18**	**JIA n=95**
ANA, n/total valid (%)	5/20 (25.0)	17/18 (94.4)	13/16 (81.2)	13/13 (100.0)	2/11 (18.2)	14/16 (87.5)	35/59 (89.8)
dsDNA, n (%)		6 (33.3)					
RNP, n (%)		8 (44.4)		1 (7.7)		11 (68.7)	
Sm, n (%)		4 (22.2)				3 (18.7)	
CenP, n (%)				1 (7.7)	1 (9.1)		1 (1.7)
PM/Scl, n (%)			1 (6.2)	3 (23.1)			
Scl70, n (%)				3 (23.1)			
Th/To, n (%)				2 (15.4)			
SSA and/or SSB, n (%)		6 (33.3)	1 (6.2)	1 (7.7)		4 (25.0)	
Ku, n (%)						1 (6.2)	
RibP, n (%)		2 (11.1)					
NXP2, n (%)			1 (4.5)				

Table S2. Specifications on the extractable nuclear antigen per disease subgroup (n=209)
ANA, anti-nuclear antibodies detected by indirect immunofluorescence-screening on Hep-2 cell substrates; CenP, anti-centromere protein antibody; cSLE, childhood onset systemic lupus erythematosus; JDM, juvenile dermatomyositis; JIA, juvenile idiopathic arthritis; jSSc, juvenile systemic sclerosis; lSc, localized scleroderma; MCTD, mixed connective tissue disease; NXP2, nuclear matrix protein antibodies; PM/Scl, anti-exome antibodies; PRP, primary Raynaud's phenomenon; RibP, anti-ribosomal P antibodies; RNP, ribonucleoprotein antibodies; Sm: anti-Smith antibodies.

SUPPLEMENTARY FILE 3: Evaluability and Matching

	HC n=204	**PRP** n=20	**cSLE** n=20	**JDM** n=22	**jSSc** n=13	**lSc** n=21	**MCTD** n=18	**JIA** n=95				
								OA n=22	**PA** n=23	**ERA** n=20	**PsA** n=15	**JIAS** n=15
Subjects with **<4/16 "good"** images, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (6.7)	3 (20.0)
Subjects with **4-8/16 "good"** images, n (%)	8 (3.4)	1 (5.0)	2 (10.0)	2 (9.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.5)	2 (8.7)	0 (0.0)	1 (6.7)	2 (13.3)
Subjects with **9-12/16 "good"** images, n (%)	19 (9.3)	0 (0.0)	2 (10.0)	0 (0.0)	3 (23.1)	0 (0.0)	1 (5.6)	6 (27.3)	3 (13.0)	5 (25.0)	2 (13.3)	2 (13.3)
Subjects with **13-16/16 "good"** images, n (%)	177 (86.8)	19 (95.0)	16 (80.0)	19 (86.4)	10 (76.9)	21 (100.0)	17 (94.4)	15 (68.2)	18 (78.3)	15 (75.0)	11 (78.6)	8 (53.3)

Table S3. Visibility of NVC-images per subgroup (n=413)
An image was considered as a "good" image when all parameters were evaluable. On the other hand, an image was scored as "bad" if any capillaroscopic parameter was not evaluable. A "bad" image was still included for analysis, but the concerning parameter was then encoded as a missing. Standardly, 16 images were taken at subject level. Subjects with less than four "good" images were excluded for further analysis (n=5).

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5

A nailfold capillary scleroderma pattern may be associated with disease damage in childhood-onset systemic lupus erythematosus: important lessons from longitudinal follow-up

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ABSTRACT

OBJECTIVES: To observe if capillary patterns in childhood-onset SLE (cSLE) change over time and find associations between a capillary scleroderma pattern with disease activity, damage or scleroderma-like features.

METHODS: Clinical and (yearly) capillaroscopy data from a longitudinal cohort of cSLE-patients (minimum of 4 SLICC criteria, onset < 18 years) were analyzed. Disease activity was measured by SLEDAI and disease damage by SDI. A scleroderma pattern was defined according to the 'Fast track algorithm' from the EULAR Study Group on Microcirculation in Rheumatic Diseases. An abnormal capillary pattern, not matching a scleroderma pattern, was defined as 'microangiopathy'.

RESULTS: Our cohort consisted of 53 cSLE patients with a median disease onset of 14 years (IQR 12.5-15.5 years), median SLEDAI score at diagnosis was 11 (IQR 8-16), median SLEDAI at follow up was 2 (IQR 1-6). A scleroderma pattern (ever) was seen in 18.9%, while only 13.2% of patients had a normal capillary pattern. Thirty-three patients had follow-up capillaroscopy of which 21.2% showed changes in type of capillary pattern over time. Type of capillary pattern was not associated with disease activity. Raynaud's phenomenon (ever) was equally distributed among patients with different capillaroscopy patterns (p=0.26). Anti-RNP antibodies (ever) were significantly more detected (Chi square, p=0.016) in the scleroderma pattern subgroup (n=7/10, 70%). Already 5 years after disease onset more than 50% of patients with a scleroderma pattern had SLE-related disease damage (HR 4.5, 95%CI 1.1-18.8, p=0.034), but they did not develop clinical features of systemic sclerosis at follow-up. Number of detected fingers with a scleroderma pattern was similar between cSLE, jSSc and jUCTD.

CONCLUSION: This longitudinal study shows that the majority of capillary patterns in cSLE are abnormal and they can change over time. Irrespective of disease activity, a capillary scleroderma pattern in cSLE may be associated with higher risk for SLE-related disease damage.

INTRODUCTION

Abnormalities in nailfold capillaries of systemic lupus erythematosus (SLE) patients, visualized by capillaroscopy, have been described in literature [1]. Two systematic literature reviews showed that these capillary abnormalities mainly consist of capillary hemorrhages and abnormal capillary shapes in adults with SLE but literature in childhood-onset SLE (cSLE) is scarce and mainly inconclusive [1, 2]. By qualitative analysis, capillary patterns in SLE are mainly described as 'non-specific changes' but a scleroderma pattern has also been described in SLE with percentages varying from 3 to 26% [3-9]. A scleroderma pattern in nailfold capillaries was first described in patients with systemic sclerosis (SSc) and is characterized by giant capillaries, sometimes in combination with large pathological capillary hemorrhages, loss of capillaries and abnormal capillary shapes with a deterioration of capillary architecture [10]. Studies have suggested that SLE-patients with a scleroderma pattern might be patients with (subclinical) overlapping features of other connective tissue diseases (CTD), such as SSc and dermatomyositis (DM) [8]. Additionally, the finding of a scleroderma pattern in SLE patients have been associated with the occurrence of Raynaud's phenomenon as well as with the presence of anti-ribonucleic protein (RNP) antibodies. Even more, these patients seem at risk for the development of pulmonary arterial hypertension or pulmonary fibrosis [3, 7, 8, 11]. In a cross-sectional study we previously showed that a scleroderma pattern in nailfold capillaries can be observed in patients with cSLE, characterized by typical SLE symptoms like lupus nephritis, malar rash, serositis, aphtous ulcers, leukopenia, thrombocytopenia, but without any clinical signs of SSc [9].

Vasculopathy is an important feature of SSc and this is a dynamic process in this disease changing over time. Changes of the nailfold capillary pattern, both deterioration, improvement as well as complete normalization have been described during follow-up of SSc-patients [12-16]. In one of these longitudinal studies, the appearance of digital ulcers correlated with the type of capillary patterns identified over time [15]. Only one study with longitudinal follow-up of nailfold capillaroscopy in SLE patients is available, describing changes in capillary pattern in cSLE as well as adult onset SLE-patients. This study suggested that a scleroderma pattern might be considered a red flag for the potential development of scleroderma spectrum disorders such as SSc and mixed/undifferentiated connective tissue disease [11]. More longitudinal follow-up data are obviously needed for better understanding of the meaning of a scleroderma capillary pattern in SLE.

The primary aims of this longitudinal prospective study are to observe if (ab)normal capillary patterns in individual cSLE patients change over time and if so, if these (changes in) capillary patterns associate with disease activity and disease damage. Additionally it is studied if a capillary scleroderma pattern in cSLE-patients indicates an increased risk for development of clinical symptoms for SSc over time. Secondary objective is to compare total finger counts with a scleroderma pattern in cSLE to patients diagnosed with juvenile systemic sclerosis (jSSc), juvenile dermatomyositis (JDM) and juvenile undifferentiated connective tissue disease (jUCTD) to give more value to this abnormal observation.

METHODS

Study design and patients

Between April 2016 and April 2021, patients with systemic auto-immune diseases who visited the (out)patient clinics of the Amsterdam UMC and Leiden UMC were included. Prospective capillaroscopy data were obtained in a cSLE cohort and three disease control cohorts (jSSc, JDM and jUCTD) visiting the (outpatient) clinics. Inclusion criteria for cSLE-patients were diagnosis according to the 2012 Systemic Lupus International Collaborating Clinics (SLICC) classification criteria [17] and age of disease onset < 18 years old. Patients were diagnosed with JDM and jSSc according to their respective EULAR/ACR criteria [18, 19]. Juvenile UCTD patients were defined as patients with Raynaud's phenomenon and anti-nuclear auto-immune antibodies, but without fulfilling the classification criteria for cSLE, JDM or jSSc. Capillaroscopy was part of routine clinical follow-up, demographic and clinical data were recorded from the patient charts. For cSLE patients that already diagnosed in the past (before capillaroscopy), retrospective clinical and demographical data were used from the time of diagnosis (autoimmune serology, SLEDAI at diagnosis and type of organ involvement).

All cSLE-patients visiting the (outpatient) clinic in Amsterdam UMC were asked to participate in this longitudinal cohort study, patients from Leiden UMC were asked to participate during two pre-planned visits using the same videocapillaroscope from Amsterdam UMC. Patients were excluded if it was impossible to collect images with good quality (due to nailfold skin thickness) or when a patient was too sick to undergo capillaroscopy examination. Demographic and clinical characteristics and disease activity were collected at the study visit. Age, gender, ethnic background, Raynaud symptoms (ever in time) and periungual trauma were noted. Disease onset was defined at the date of first SLE-symptom. Disease activity was measured using the Systemic Lupus Erythematosus Activity Index (SLEDAI)[20]; disease damage by the SLICC damage index (SDI) [21]. Disease activity was also graded by severity: inactive or mild for SLEDAI <3, moderate for SLEDAI between 3-6 and severe for SLEDAI >6.

This study was approved by the ethical committee from the Amsterdam University Medical Centers (Dutch trial register registration no. NL60885.018.17). All patients were coded with a unique study number. For follow-up, according to the Dutch Medical Research Involving Human Subjects Act, an informed consent was signed by children from 12 years of age, and/or both parents (if alive and authorized) for children below 16 years old.

Nailfold capillaroscopy technique and image analysis

Nailfold videocapillaroscopy (NVC) was performed with a x200 magnification lens from Optilia. Images were collected by two investigators (DS or SB, respectively six and three years of experience in capillaroscopy examination). Before start of examination, the patients stayed in a room of 20-22 ^{0}C for a minimum of 15-20 minutes. Patients were in sitting position with the hand on a table at the level of their heart during capillaroscopy examination. A drop of oil was applied to the fingers before examination. In total, eight fingers per cSLE-patient (excluding the thumbs) were examined. Per finger, four images were stored.

Qualitatively, three capillary patterns were described. First, a scleroderma pattern was defined as extremely lowered density (≤ 3 capillaries / mm) with abnormal shapes or the presence of giants by 'fast track algorithm' according to the 'EULAR Study Group on Microcirculation in Rheumatic Diseases standardized capillaroscopy evaluation chart' [10. 22]. Second, a capillary pattern was designated as 'microangiopathy' (also described as 'non-specific changes') if the observed capillary pattern showed abnormal capillary morphology (according to the EULAR study group criteria) [22, 23] and/or capillary hemorrhages, but did not match the criteria for a scleroderma pattern. Third, a normal capillary pattern was defined if a patient did not have any capillary abnormalities. If the type of capillary pattern in a patient changed over time, the worst pattern was used in analyses. In case of microangiopathy or scleroderma pattern, the number of fingers demonstrating this abnormal pattern was counted.

Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics type 26. Descriptive statistics were reported in terms of percentages, means and standard deviations or medians and inter-quartile ranges. For differences of variables between groups, Chi-square, Mann-Whitney U and Kruskal Wallis test analyses were used depending on (distribution, groups and number of categories of) outcome data. Logistic regression was used for occurrence of disease damage and scleroderma pattern as binary outcome data with odds ratio (OR) reported with 95% confidence interval (CI). Variables for univariate logistic regression were demographic (age and gender), auto-antibodies anti-RNP and anti-Sm, organ manifestations and SLEDAI at diagnosis. Significant variables from univariate analyses and anti-RNP antibodies, nephritis and neuropsychiatric organ involvement (known to give risk for respectively overlap disease or damage from literature [7, 24]) were chosen as co-variates for multivariate logistic regression analysis. Longitudinal analysis (with significant variable(s) from univariate analysis) for comparison between different capillary patterns and disease damage as endpoint was performed by Cox regression analysis. P-values <0.05 were considered as statistically significant.

RESULTS

Of 56 eligible patients, capillary images could be analyzed in 53 cSLE-patients at the first study visit (Figure 1). Thirty-three of these 53 patients (62.3%) had a minimum of one follow-up visit for NVC with a range of 8-60 months after first capillaroscopy. N=14 and n=8 patients respectively had two and three follow-up visits for NVC. The reasons for no follow-up NVC (n=20) were: no consent (n=2), lost to follow-up (n=4), transition to adult care (n=6), <1 year of diagnosis (n=4), no time/logistic reason (n=3) and no evaluable images (n=1) (Figure 1). Clinical follow-up data in our longitudinal cSLE-cohort ranged from 0.5-16 years after disease onset. The baseline characteristics are listed in table 1. The patient groups with or without follow-up NVC were comparable for all baseline characteristics.

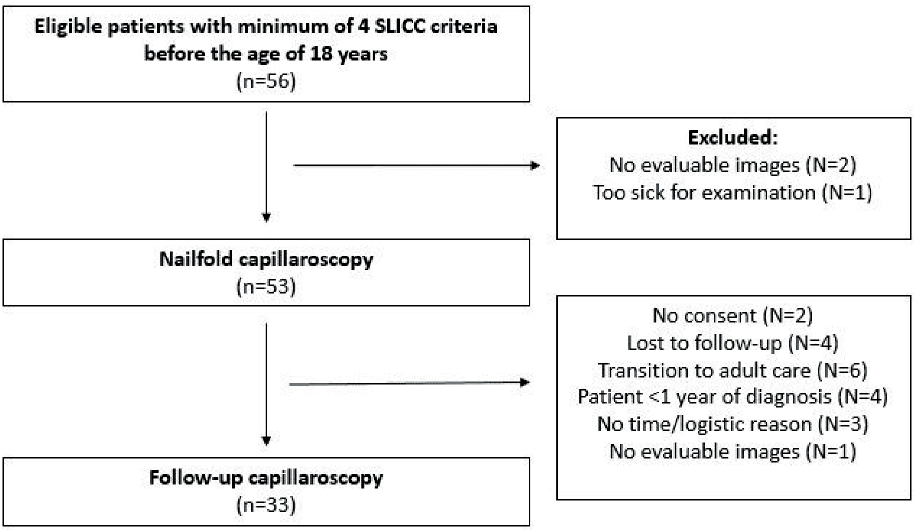

Figure 1. Flow chart longitudinal study cohort of cSLE-patients

Type of capillary patterns and change in time

At baseline, 13.2% (7/53) showed a capillary scleroderma pattern. In total in our cSLE cohort, 10/53 (18.9%) of all patients showed a nailfold scleroderma pattern (ever) at minimal one examination, 36/53 (67.9%) showed microangiopathy and 7/53 (13.2%) a normal capillary pattern. Range for NVC follow up time was 1-5 years. Figure 2 shows examples of nailfold capillary images with a scleroderma pattern in our cSLE-patients. Observed microangiopathy pattern consisted of abnormal capillary shapes and capillary hemorrhages (without the typical giant capillaries or extremely lowered density ≤ 3/mm as in a scleroderma pattern).

At longitudinal follow-up with NVC assessment, most patients (26/33, 78.8%) showed the same capillary pattern as baseline (see supplementary file 1). Of the 7 patients with a baseline capillary scleroderma pattern, in 5/7 patients NVC was repeated, which showed that 4/5 patients (80%) had a persistent scleroderma pattern and one patient (n=1/5, 20%) changed to a microangiopathy pattern (with severe active disease). Range of NVC follow-up in these patients with a scleroderma pattern was 14-50 months.

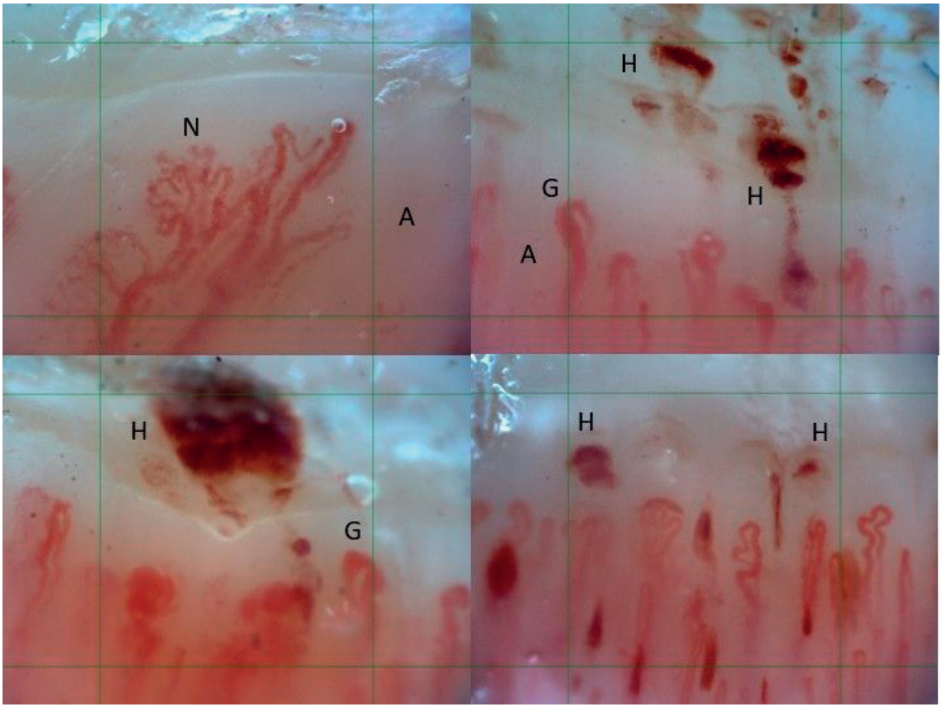

Figure 2. Capillary scleroderma patterns in patients with cSLE showing giant capillaries (G), hemorrhages (H) and neovascularization (= abnormal shapes) (N) with avascular areas (A).
Green grid: 1 mm.

Of the 39 patients with a baseline microangiopathy pattern, in 24/39 patients NVC was repeated. Images from one patient could not be interpreted because of indistinct visualization of capillaries (poor quality images). Eighteen of these patients (n=18/24, 75%) showed persistent microangiopathy and three patients (3/24, 12.5%) changed to a scleroderma pattern (two patients with inactive/mild and one with severe disease activity). In three other patients (3/24, 12.5%) microangiopathy changed to a normal pattern (two patients with inactive/mild disease and one with moderate disease activity). Range of NVC follow-up in these patients was 9-42 months.

Of the 7 patients with a normal capillary pattern, in 4/7 patients NVC was repeated. In all patients (n=4/4, 100%) this normal pattern persisted (two patients with inactive/mild disease and two with moderate disease activity). Range of NVC follow-up in these patients was 8-22 months.

Mean time between NVC in patients with a change in capillary pattern was 21.1 months (range 9-50 months). Mean time between NVC in patients without any change in pattern was comparable with 15.8 months (range 6-52 months) (see supplementary file 1). Almost all patients but one (with a worsening of capillary pattern to scleroderma pattern), with and without change in capillary pattern, were on treatment for SLE.

Two patients with a scleroderma pattern had digital lesions at presentation of their disease and thus at time of first capillaroscopy. At follow-up their digital lesions improved but the capillary scleroderma pattern persisted in both patients.

Capillary patterns and association disease activity/-damage

There was no significant difference in 'SLEDAI at diagnosis' between patients with different capillary patterns (sub-analysis of n=31 treatment-naive patients, Kruskal-Wallis, p=0.18). Overall, patients in follow-up had lower disease activity with median SLEDAI 2 (IQR 1.5-6) than patients at diagnosis with a median SLEDAI of 12 (IQR 8-17). Raynaud's phenomenon (ever) was equally distributed among patients with different capillaroscopy patterns (Chi square, p=0.26). Anti-RNP antibodies (all subtypes, ever in time) were significantly more detected (Chi square, p=0.016) in the scleroderma pattern subgroup than in the other groups, (in 42.8%, 22.2% and 70% of patients with a normal, microangiopathy and scleroderma pattern respectively). Of the anti-RNP positive patients with a scleroderma pattern, 85.7% (n=6/7) also showed positive anti-ds-DNA antibodies and 71.4% (n=5/7) also showed anti-Sm antibodies. The anti-RNP antibodies were directed against 70kD-protein in 7/18 patients, in the other patients the anti-RNP antibodies were directed against A- or C-protein. The more severe disease variables nephritis and neuropsychiatric involvement were equally distributed at diagnosis among different capillary patterns (resp. p=0.29 and p=0.44). By univariate regression analysis, discoid rash (OR 9.3, 95% CI 2.0-42.8, p=0.004) and anti-RNP antibodies (OR 6.8, 95% CI 1.5-30.9, p=0.013) were significantly associated with the occurrence of a capillary scleroderma pattern. By multivariate regression analysis this was only seen for discoid rash (OR 5.9, 95% CI 1.2-30.2, p=0.033) (see supplementary file 2).

Table 1. Demographical variables and clinical characteristics of all cSLE patients (total and per subgroup with/without follow-up capillaroscopy).

	Total, n=53	Capillaroscopy follow-up, n=33	No capillaroscopy follow-up, n=20	p-value°
Female, n (%)	47 (88.7)	29 (87.9)	18 (90)	0.81
Ethnicity, n (%)				
African/Afro-Caribbean	21 (39.6)	14 (42.4)	7 (35)	0.23
White	21 (36.6)	13 (39.4)	8 (40)	
North-African/Middle-Eastern	4 (7.5)	3 (9.1)	1 (5)	
Asian	4 (7.5)	3 (9.1)	1 (5)	
Mixed/other	3 (5.7)	0	3 (15)	
Age at first capillaroscopy in years, median (IQR)	17 (14-17)	16 (14-17.5)	17 (14.3-17)	0.14
Raynaud's phenomenon / acro-cyanotic symptoms, n (%)	17 (32.1)	10 (30.3)	7 (35)	0.72
Age at onset in years, median (IQR 25-75)	14 (12.5-15.5)	15 (13.5-15.5)	13.5 (11.3-15.8)	0.22
Prednisone naïve, n (%)	31 (58.5)			
ANA at diagnosis, n (%)	52 (98.1)			
ANA + anti-ds-DNA	37 (69.8)	23 (69.7)	14 (70)	0.99
ANA + anti-RNP	18 (34)	14 (42.4)	4 (20)	0.10
ANA + anti-Sm	18 (34)	11 (33.3)	7 (35)	0.90
Cutaneous involvement, n (%)	38 (71.7)	22 (66.7)	17 (85)	0.14
Nephritis, n (%)	17 (32.1)	11 (33.3)	6 (30)	0.80
Neuropsychiatric involvement, n (%)	8 (15.1)	5 (15.2)	3 (15)	0.99
Antiphospholipid antibodies, n (%)	6 (11.3)	3 (9.1)	3 (15)	0.33
SLEDAI at diagnosis, median (IQR)	11 (8-16)	12 (8-17)	10 (6.5-14.8)	0.38
SLEDAI at first capillaroscopy, median (IQR)	6 (3.5-12)			
SLEDAI at second capillaroscopy, median (IQR)		2 (1-6)		
Capillary pattern: normal / microangiopathy / scleroderma pattern, n (%)	7 / 36 / 10 (13.2 / 67.9 / 18.9)	4 / 21 / 8 (12.1 / 63.6 / 24.2)	3 / 15 / 2 (15 / 75 / 10)	0.44
Disease damage present, n (%)	10 (18.9)	6 (18.2)	4 (20)	0.87

° Chi square/Fisher exact analysis between the two subgroups

At final follow-up, disease damage (as measured by SDI), by univariate regression analysis, was significantly associated with detection of a capillary scleroderma pattern (ever) (OR 7.6 95% CI 1.6-35.9, p=0.01). In this univariate analysis neuropsychiatric involvement was also significantly associated with the risk of development of disease damage (OR 6.5 95%CI 1.3-33.2, p=0.024) as was discoid lupus (OR 5.1, 95% CI 1.2-22.6, p=0.003). By multivariate analysis, only neuropsychiatric involvement was a significant variable for occurrence of disease damage (OR 7.8, 95% CI 1.2-15.4, p=0.032) (see supplementary file 3). Cox regression analysis for occurrence of disease damage between patients with versus without a scleroderma pattern showed a significantly higher hazard for developing disease damage (Figure 3, Hazard Ratio (HR) 4.6 (95%CI 1.1-18.8, p=0.034). In this longitudinal regression model for disease damage, neuropsychiatric involvement was again a significant co-variable (HR 8.1, 95% CI 1.9-35.8, p=0.005), whereas discoid rash was not (p=0.158). Specific details on disease damage were end-stage renal disease (n=1), growth failure (n=1), extensive scarring in face (n=2), avascular skeletal necrosis (n=2), cognitive impairment/major psychosis (n=1), history of cerebrovascular accident (n=3), loss of digits (n=1), cardiac valve disease (n=1) and seizures requiring therapy for > 6 months (n=1). In figure 3 is seen that, between 4-5 years after disease onset, half of the cSLE-patients with a capillary scleroderma pattern had irreversible disease damage compared to <10% of cSLE-patients without a capillary scleroderma pattern.

Patients with a capillary scleroderma pattern did not show criteria to diagnose SSc (puffy hands, sclerodactyly or skin thickening, telangiectasia, interstitial lung disease or digital tip ulcers) or overlap disease during follow-up (table 2).

Capillary patterns in cSLE compared to disease controls

At time of first capillaroscopy, the median number of affected fingers was 5 (IQR 3-7) with microangiopathy and 4 (IQR 2-6) with a scleroderma pattern. At second capillaroscopy, the median number of affected fingers was 6.5 (IQR 2.3-8) with microangiopathy and with a scleroderma pattern 6 (IQR 4.5-7). Table 3 shows the number of fingers with a capillary scleroderma pattern in cSLE compared with patient cohorts of JDM (n=12), jUCTD (n=13) and jSSc (n=7). At first NVC and compared with cSLE, the number of fingers with a scleroderma pattern was significantly higher in patients with JDM, but this was not significantly different for patients with jSSc or jUCTD versus cSLE, nor was it different at follow-up.

Table 2. Clinical characteristics of cSLE-patients (n=10/53) with a capillary scleroderma pattern

SLE- characteristics	**Patient 1**	**Patient 2**	**Patient 3**	**Patient 4**	**Patient 5**	**Patient 6**	**Patient 7**	**Patient 8**	**Patient 9**	**Patient 10**
Butterfly rash	+	+	-	-	-	-	-	+	-	+
Photosensitive rash	+	+	+	+	+	+	-	+	+	+
Lupus nephritis	-	-	+	+	-	+	-	-	+	+
Autoimmune cytopenia	+	+	+	-	-	+	+	+	+	+
Positive coombs	+	+	+	+	+	+	-	-	+	+
Low C3/C4	+	+	+	+	-	+	-	-	+	+
Serositis	-	-	+	+	-	+	-	-	+	-
Damage	+	+	+	+	-	-	-	-	+	-
Type of auto-antibodies	ANA, Anti-ds-DNA, anti-Sm, anti-RNP, anti-Ro52, anti-SS-A	ANA, Anti-ds-DNA, anti-Sm, anti-RNP, anti-Ro52, anti-SS-A	ANA, Anti-ds-DNA, anti-Sm, anti-RNP, anti-SS-A	ANA, Anti-ds-DNA, anti-Sm, anti-RNP, anti-Ro52, anti-SS-A	ANA	ANA, Anti-ds-DNA, anti-C1q	ANA, Anti-ds-DNA, anti-Sm, anti-RNP	ANA	ANA, Anti-ds-DNA, anti-Sm, anti-RNP, anti-Ro52, anti-SS-A	ANA, Anti-ds-DNA, anti-Sm, anti-RNP, anti-Ro52, anti-SS-A

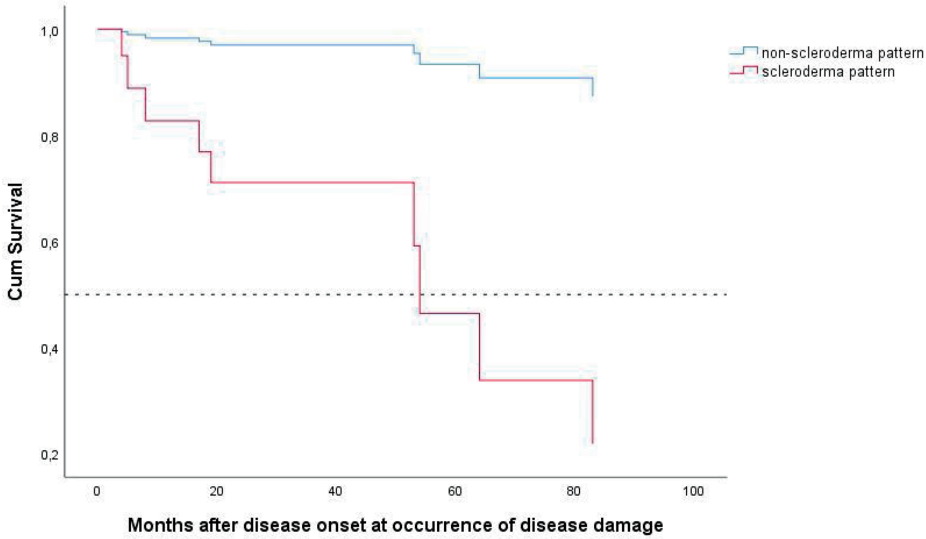

Figure 3. Cox regression analysis for occurrence of disease damage in cSLE patients with nailfold capillary non-scleroderma pattern (n=23) versus a capillary scleroderma pattern (n=10):
Hazard Ratio (HR) 4.6 (95% CI 1.1-18.8) for scleroderma pattern group, p=0.034

Table 3. Different patients groups with a capillary scleroderma pattern and follow-up data.

Patient group	JDM n=11	jUCTD n=13	jSSc n=7	cSLE n=53	p-value
Scleroderma pattern at first capillaroscopy, n (%)	7 (63.6)	9 (69.2)	7 (100)	7 (13.2)	**0.001/<0.001/<0.001**°
No fingers with scleroderma pattern, median (IQR)	8 (7-8)	3 (2.5-8)	8 (6-8)	4 (2-6)	**0.03** / 0.76 / 0.10[b]
FU patients, n (%)	7 (77.8)	3 (23.1)	5 (71.4)	33 (62.3)	
Scleroderma pattern at FU, n/total FU patients (%)	3/7 (42.9)	2/3 (66.7)	5/5 (100)	8/33 (24.2)	0.208 /0.181/**0.003**°
No fingers with scleroderma pattern in FU, median (IQR)	7 (7)	5 (5)	8 (5-8)	5.5 (2.5-7)	0.09 / 0.89 / 0.07[b]

FU = follow-up . Bold indicates statistically significant p values (<0.05). ° Chi square/Fisher exact analysis between the two subgroups. [b] Mann-Whitney U test between cSLE and resp. JDM/jUCTD/jSSc.

DISCUSSION

This is the first study reporting longitudinal follow-up data of nailfold capillaroscopy combined with clinical data in cSLE. We showed that nailfold capillary patterns in cSLE can change over time, but these changes were irrespective of disease activity. Strikingly, cSLE patients with a capillary scleroderma pattern had a higher risk for SLE-related disease damage, although SLEDAI (at baseline nor at follow-up) did not significantly differ between different capillary pattern groups. Subsequently, as we have shown that capillary patterns can worsen over time, it seems necessary to repeat capillaroscopy during clinical follow-up. Furthermore, we are also the first to show that in cSLE both microangiopathy and a scleroderma pattern, if detected, were observed in the majority of eight examined fingers. At baseline and follow-up, the total number of fingers with a scleroderma pattern, if present in cSLE, was comparable to patients with either jSSc/jUCTD (baseline) and JDM/jSSc/jUCTD (follow-up). This suggests that this finding is not coincidental in cSLE, and in juvenile patients might not only be a specific finding for jSSc.

Anti-RNP antibodies were significantly more detected in cSLE-patients with a capillary scleroderma pattern, but Raynaud's phenomenon was not, also not during follow-up. A capillary scleroderma pattern has been linked to overlap disease or mixed connective tissue disease [25-27]. In our study with up to 10 years of clinical follow up after diagnosis however, cSLE patients with a capillary scleroderma pattern did not evolve into a scleroderma overlap disease. Clinical follow-up of these patients included routine medical history, physical examination, laboratory biomarkers and pulmonary function tests (minimal every 2-3 years), cardiac ultrasound (on indication) and pulmonary CT scan for signs of fibrosis (on indication). The mean follow-up was 7 years after diagnosis, one patient was lost to follow-up. Looking at the available anti-RNP titers (in only one-third) in our patients, these titers were quite low and this might subsequently mean that this subgroup of cSLE-patients are not at risk for overlap disease. Interestingly, our data do suggest that cSLE patients with anti-RNP seem to be a more severe subgroup with more SLE-related disease damage, which was also stated by Dayal et al. [28]. On the other hand, a recent study in SSc showed that the presence of anti-RNP antibodies fits with a subgroup of SSc with a better prognostic outcome in terms of survival [29]. Thus, the detection of anti-RNP antibodies might have a different prognostic meaning in these different systemic autoimmune diseases or scleroderma spectrum disorders. Most patients with anti-RNP antibodies in the scleroderma pattern group also had multiple, more SLE-specific, auto-antibodies detected, besides other clinical SLICC criteria. This shows that these patients are different than UCTD-patients with only anti-RNP and Raynaud symptoms.

Our finding that a capillary scleroderma pattern is a significant risk factor for irreversible SLE-related disease damage in cSLE is new. Longitudinally, we found that half of the cSLE-patients with a nailfold capillary scleroderma pattern had already irreversible disease damage within only 5 years of diagnosis, meaning that this disease damage develops at a young age, probably around their twenties. We have also shown that this damage could not be predicted by SLEDAI at diagnosis nor by SLEDAI at follow-up, and most of these patients had low disease activity over time. It has been described that SLE-patients with neuropsychiatric involvement and/or nephritis reflect a severe subgroup [24]. In our study this was confirmed for neuropsychiatric involvement, but not for nephritis. Previously, in our cross-sectional study in cSLE, we did find a significant correlation between nephritis and the number of capillary hemorrhages. We have also described that the number of capillary hemorrhages significantly correlated with SLEDAI [9]. In this follow-up study, the capillary abnormalities remained visible although patients had low global disease activity or even inactive disease. In addition, patients with low disease activity and a scleroderma pattern at NVC, were significantly more at risk for developing disease damage, despite their low disease activity over time. This might imply an ongoing vasculopathy in SLE leading to this disease damage, irrespective of disease activity, as measured by SLEDAI. The disease damage in our cSLE-cohort was typical SLE-induced disease damage such as cerebrovascular incidents, seizures (>6 months of treatment), major psychosis, end-stage renal disease and avascular skeletal necrosis and extensive (facial) scarring. Only 7/53 patients never used prednisolone as treatment, all others used prednisone at some time in different dosages. As was shown in the specific details of disease damage, two patients suffered from skeletal necrosis and one from growth failure, probably related to chronic prednisolone use. In univariate regression analysis, prednisolone use (ever) was not associated with occurrence of disease damage, but cumulative steroid dose was not calculated in this study. All other patients had specific SLE-related disease damage, which was not related to medication. Also, most disease damage already occurred in the first years after diagnosis.

As in our cross-sectional study [9], microangiopathy with abnormal capillary shapes and high number of capillary hemorrhages, was again a predominant and longitudinal a persistent finding in this current longitudinal study. As we have shown as well, two types of hemorrhages can be reliably differentiated and reproduced by different raters [30]. The same two types of capillary hemorrhages were observed over time in our longitudinal cohort. Other studies have described the capillary abnormalities in (c)SLE as non-specific abnormalities [1, 31, 32]. We hypothesize that the predominant finding of ‘microangiopathy’ in (c)SLE might be capillary leakage and revascularization and might be due to endothelial dysregulation in SLE, and may not be so non-specific at all. Dysregulation of endothelial cells in SLE has been linked to an increased risk of cardiovascular disease in these patients [33-35], which is one of the most important prognostic factors for mortality in SLE, especially in cSLE patients [36]. Thus, this microangiopathy should be further analyzed in this severe and chronic disease and might be a possible new biomarker or a “lupus-pattern” reflecting (early) vasculopathy in SLE which warrants additional therapy to prevent future damage.

A limitation of our study is the relatively low number of patients that was included. Although almost all eligible patients from the outpatient clinic could be included, the prevalence of these systemic autoimmune diseases at pediatric age is rare. Another limitation of our study is that not all patients had follow-up visits with capillaroscopy due to several reasons. The typical age of patients at cSLE onset is in their teens which means that patients are relatively soon transferred to adult care after diagnosis, roughly around the age of 18 years. In the Amsterdam UMC, these patients were included in our longitudinal cohort but not all longitudinal capillaroscopy data could be completed. Patients with transition to other hospitals were lost to follow-up.

CONCLUSION

We conclude that a capillary scleroderma pattern in cSLE did not reflect a SLE-subgroup at risk for developing SSc-like symptoms but we suggest that a capillary scleroderma pattern in SLE may be associated with a higher risk of developing SLE-related disease damage.

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Supplementary table 1. Follow-up of capillary patterns in time (in months)

Subject	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Time 1-2	Time 2-3	Time 3-4	Time 4-5	Time 5-6	Time 6-7
1	N	N						12					
2	N	N						22					
3	N	N						12					
4	N	N						8					
5	MA	MA	MA	Poor quality images				20	5	18			
6	MA	MA	MA	MA				15	20	16			
7	MA	MA	MA					15	20				
8	MA	MA	**MA**	**Scl**				16	14	**20**			
9	**MA**	**Scl**	Scl	Scl	Scl			**13**	13	13	9		
10	MA	MA	MA					15	15				
11	MA	MA						16					
12	MA	MA	MA					18	29				
13	MA	MA						52					
14	MA	MA						25					
15	**MA**	**Scl**						**50**					
16	MA	MA	MA					12	13				
17	MA	MA						36					
18	MA	MA	MA					10	26				
19	MA	MA						16					
20	MA	MA	MA					15	19				
21	MA	MA	MA					13	17				
22	MA	MA						23					
23	**MA**	**N**						**15**					
24	**MA**	**N**						**9**					
25	MA	MA						13					
26	**MA**	**N**						21					
27	MA	Poor quality images						20					
28	MA	MA						19					
29	Scl	Scl	Scl	Scl	Scl	Scl	Scl	9	8	5	6	10	9
30	Scl	Scl	Scl	Scl				10	14	14			
31	Scl	Scl	Scl	Scl				16	15	18			
32	**Scl**	**MA**						**20**					
33	Scl	Scl						14					

N= Normal pattern, MA=MicroAngiopathy, Scl=Scleroderma pattern. Bold: change in capillary pattern

Supplementary table 2. Logistic regression analyses for occurrence of scleroderma pattern (ever)

Univariate analysis	OR (95% CI)	p-value
SLEDAI at diagnosis	1.05 (0.96-1.15)	0.277
Discoid lupus	**9.25 (2.0-42.77)**	**0.004**
Nephritis	0.47 (0.09-2.48)	0.372
Neuropsychiatric involvement	1.54 (0.26-9.08)	0.632
Anti-phospholipid antibodies	0.71 (0.15-3.37)	0.669
Anti-RNP antibodies	**6.79 (1.49-30.92)**	**0.013**
Anti-Sm antibodies	2.31 (0.57-9.36)	0.242
Multivariate analysis		
Discoid lupus	**5.89 (1.15-30.19)**	**0.033**
Anti-RNP antibodies	4.11 (0.79-21.25)	0.092

OR= odds ratio, CI=confidence interval. NA=not available. Bold=statistical significant

Supplementary table 3. Logistic regression analyses for occurrence of SLE disease damage (by SDI)

Univariate analysis	OR (95% CI)	p-value
Age at diagnosis	1.19 (0.89-1.61)	0.243
Gender	1.18 (0.12-11.42)	0.884
SLEDAI at diagnosis	1.09 (0.99-1.19)	0.063
Discoid lupus	**5.1 (1.17-22.61)**	**0.003**
Nephritis	0.47 (0.09-2.48)	0.372
Neuropsychiatric involvement	**6.5 (1.27-33.20)**	**0.024**
Anti-phospholipid antibodies	0.92 (0.46-1.85)	0.819
Anti-RNP antibodies	3.88 (0.93-16.19)	0.063
Anti-Sm antibodies	1.38 (0.34-5.70)	0.655
Prednisolon use (ever)	NA	0.999
Scleroderma pattern (ever)	**7.6 (1.61-35.85)**	**0.01**
Multivariate analysis		
Discoid lupus	2.24 (0.33-15.44)	0.411
Neuropsychiatric involvement	**7.75 (1.19-15.44)**	**0.032**
Nephritis	0.50 (0.07-3.66)	0.496
Anti-RNP antibodies	2.01 (0.32-12.63)	0.455
Scleroderma pattern (ever)	4.75 (0.73-31.11)	0.104

OR= odds ratio, CI=confidence interval. NA=not available. Bold=statistical significant

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Part II

New biomarkers in (systemic) connective tissue diseases

6

Gene signature fingerprints divide SLE patients in subgroups with similar biological disease profiles: a multicenter longitudinal study

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ABSTRACT

OBJECTIVES: Clinical phenotyping and predicting treatment responses in Systemic Lupus Erythematosus (SLE) patients is challenging. Extensive blood transcriptional profiling has identified various gene modules that are promising for stratification of SLE patients. We aimed to translate existing transcriptomic data into simpler gene signatures suitable for daily clinical practice.

METHODS: RT-PCR of multiple genes from the Interferon M1.2, Interferon M5.12, neutrophil (NPh) and plasma cell (PLC) modules followed by a principle component analysis, was used to identify indicator genes per gene signature. Gene signatures were measured in longitudinal samples from two childhood onset SLE cohorts (n=101 and n=34, respectively) and associated with clinical features. Disease activity was measured using SELENA-SLEDAI. Cluster analysis subdivided patients into three mutually exclusive fingerprint-groups termed 1) all-signatures-low, 2) only IFN high (M1.2 and/or M5.12) and 3) high NPh and/or PLC.

RESULTS: All gene signatures were significantly associated with disease activity in cross-sectionally collected samples. The PLC-signature showed the highest association with disease activity. Interestingly in longitudinally collected samples, the PLC-signature was associated with disease activity and showed a decrease over time. When patients were divided into fingerprints, the highest disease activity was observed in the high NPh and/or PLC group. The lowest disease activity was observed in the all-signatures-low group. The same distribution was reproduced in samples from an independent SLE cohort.

CONCLUSIONS: The identified gene signatures are associated with disease activity and suitable tools to stratify SLE patients into groups with similar activated immune pathways that may guide future treatment choices.

INTRODUCTION

Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by its heterogeneity at the clinical, cellular and molecular level [1]. This often poses a challenge for clinicians to reliably divide patients into homogeneous disease subgroups. Additionally, patients with distinct clinical disease phenotypes respond to the same medication and vice versa, underlining that solely using clinical phenotype to decide which treatment to start is not enough. Identification of tools to cluster patients in homogeneous groups with similar underlying aberrantly activated immune pathways to guide treatment blocking these pathways, is an important research topic. Transcriptional profiling resulted in the identification of so-called “gene signatures”. A gene signature is a group of simultaneously upregulated genes caused by a change in the cell’s biological processes. In SLE multiple gene signatures with correlations to unique clinical features have been identified. However, application into clinical practice is challenging, due to a lack of consensus regarding the genes representing a signature and the feasibility to implement transcriptional profiling on individual patients in the clinical setting.

The most well-known gene signature in SLE is the type I Interferon (IFN-I) gene signature, which is present in more than 50% of the patients and correlates to disease activity in several cross-sectional studies [2-6]. Transcriptomic data of SLE blood has revealed three different upregulated IFN-annotated modules, respectively called M1.2, M3.4 and M5.12 [7]. The M1.2 module is induced by IFN-I, while both the M3.4 and M5.12 modules are induced by a combination of IFN-I and type II IFN (IFN-II). When studied over time in SLE patients, each module displayed a different dynamic pattern, with the highest variation in the M5.12 module. These fluctuations indicated that the IFN signature could be used as biomarker for disease activity. However, the few studies that have investigated the parallel change of disease activity with IFN gene signatures over time, have shown a lack of association [8, 9]. This implicates the involvement of other immune pathways than the IFN route.

When focusing on pathways which have already been shown to correlate with SLE disease manifestations and/or disease activity, two other gene signatures stand out: the neutrophil (NPh) and plasma cell (PLC) signature [8-12]. Neutrophils and plasma cells are increased in SLE patients and play a role in disease pathogenesis [13, 14]. Neutrophils of SLE patients are more active, have lower phagocytic capacity and are prone to spontaneously release Neutrophil Extracellular Traps [15]. Plasma cells are the source of pathogenic auto-antibodies in SLE. The NPh signature was associated with lupus nephritis and vascular inflammation while the PLC signature correlated with disease activity [8-10, 16]. Moreover, in studies with extensive transcriptional profiling, these and other gene signatures were used to divide patients into subgroups with similar biological disease profiles [12, 17].

Here, we investigated whether we could translate complex transcriptomic data, reflecting multiple different immune pathways, into simple gene signatures suitable for introduction into clinical practice. Additionally, we studied their association with disease activity and clinical outcome using prospectively collected clinical data and blood samples over time from two childhood-onset SLE (cSLE) cohorts. cSLE is an excellent disease model to study, as cSLE represents the more severe clinical phenotype, has a higher genetic component and children with SLE lack the comorbidities common in adult-onset SLE and that may confound translational studies.

METHODS

Patient recruitment

Patients fulfilled the Systemic Lupus International Collaborating Clinics (SLICC) classification criteria or the 2019 EULAR/ACR [18, 19]. Blood specimens, demographics and clinical characteristics were prospectively collected. Disease activity was assessed by the Safety of Estrogens in Lupus National Assessment-Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI) at each visit [20]. Disease flares were indicated by an increase of >3 or >12 points from the previous visit for a mild/moderate or severe flare respectively. Disease domains were derived from the SELENA-SLEDAI items.

Additionally, 51 healthy controls (HC), without symptoms of underlying viral infections or the use of any medications, were included. Written informed consent was obtained from all participants in compliance with the Declaration of Helsinki. The study was approved by the medical ethics review committee of the Erasmus Medical Center, Rotterdam, the Netherlands (MEC-2019-0412).

Clustering strategy

A semi-manual and an automated clustering strategy was performed (supplementary figure S1). For the semi-manual clustering strategy, the combination of a positive or negative score per gene signature was used to identify 16 clusters. These clusters were subsequently divided into 3 so-called fingerprints consisting of patients with mutually exclusive combinations of positive and negative gene signatures. For the automated clustering strategy, an unsupervised hierarchical clustering method was used to identify clusters that were enriched in the cSLE cohort.

Supplementary methods

Details on blood collection and real-time PCR, gene selection and signature definitions, ultrasensitive IFN-α Simoa and statistics are described here.

RESULTS

Cohort description

Between March 2013 and January 2021, 101 Childhood-onset SLE (cSLE) patients with median disease duration of 0.5 (0-8.2) years at enrolment (Cohort-I, Table 1) were prospectively recruited at the outpatient clinic of three academic hospitals in the Netherlands and one in the Czech Republic. Fifty-one HC were included in the study. For 73/101 patients, blood samples from 2-4 longitudinal time points with a median follow up time of 344 (29-1542) days were available. As a replication cohort, 34 adults with cSLE with median disease duration of 15.8 (3.8-40) years were included (Cohort-II, Table 1).

Four dynamic gene signatures are present in SLE patients

Four gene signatures were assessed based on indicator genes in 51 HC and 101 cSLE patients (supplementary- Figure S1, table T1). All four gene signatures were significantly higher expressed in patients compared to HC (Figure 1A). High positive associations were present between the genes representing each gene signature, while poor associations were found between genes of different gene modules (Figure 1B). The M1.2 and M5.12 IFN signatures had the highest association, followed by the M5.12 and NPh signature, while poor associations were observed between the other signatures (Figure 1C).

In 73 cSLE patients, each gene signature was determined at a second time-point and in 42 cSLE patients at a third and/or fourth time point (Figure 1D). In 51 out of 73 patients (70%), at least one or more signatures changed from positive to negative or vice versa (Figure 1D). The M5.12 IFN signature showed the highest variability within individual patients (coefficient of variation [CoV] 0.66 ± 2.74), followed by the PLC- (CoV 0.54 ± 4.39), NPh- (CoV -0.35 ± 4.23) and M1.2 IFN (CoV 0.05 ± 1.14) signatures (Figure 1E). These findings imply that gene signatures are driven by different pathways and have a dynamic character over time, which makes them potential biomarkers for changes in disease activity.

Gene signatures are associated with disease activity

To investigate associations with disease characteristics we stratified patients into groups based on a low or high gene signature using the mean + 2xSD_{HC} per score as a threshold. A high M1.2 IFN, M5.12 IFN, NPh or PLC signature was more prevalent in patients with a higher SELENA-SLEDAI (supplementary figure S2A-D). The highest association was found for the PLC signature (p<0.0001, r=0.473) (Figure 2A).

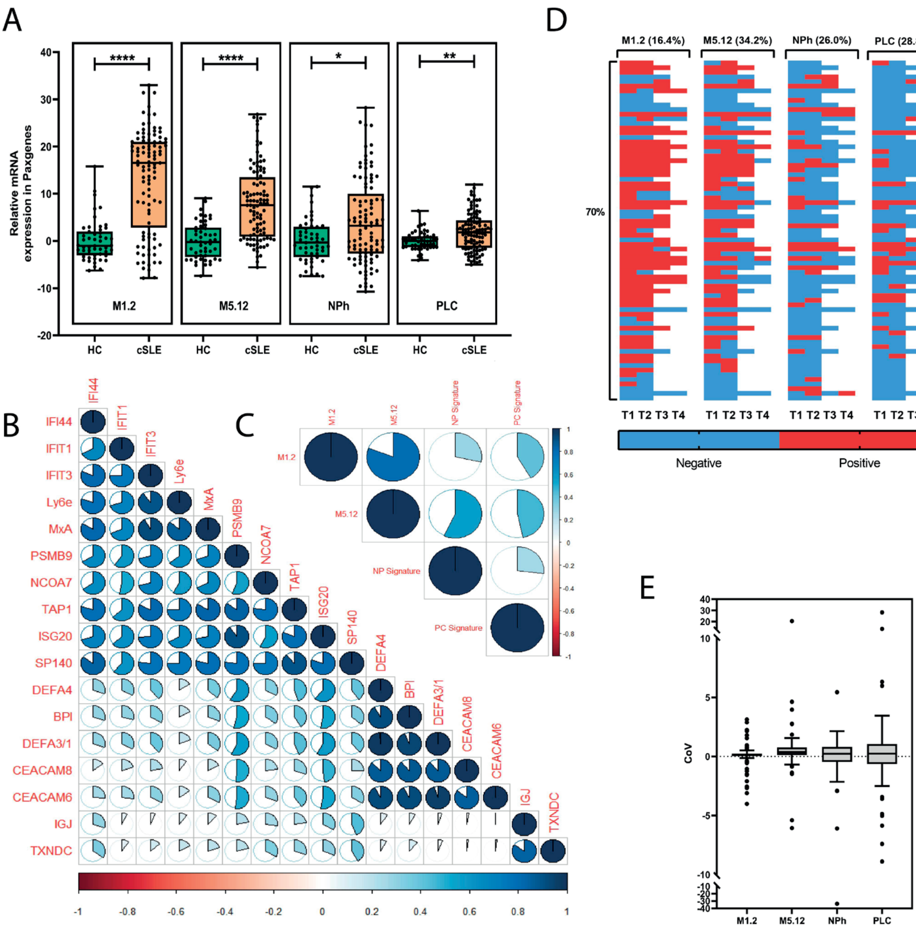


Figure 1. Four dynamic gene signatures are present in cSLE patients
A). Relative expression of four gene signature scores in HC (N=51) vs cSLE patients (N=101). B). Correlation matrix between gene signature associated genes based on relative expression. C). Correlation matrix between each individual gene signature. D). Heatmap indicating a positive or negative gene signature score over time in 73 cSLE patients. Each row represents the same patient. Horizontal percentages indicate the number of patients that showed a dynamic signature over time. Vertical percentage indicates the number of patients with at least one dynamic gene signature. E). Coefficient of variation (CoV) per gene signature indicating the intraindividual difference per gene signature. Each dot represents the CoV of one patient. Lines indicate the mean ± SD.
Mann-Whitney-U test was used to compare two groups; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. For correlations Spearman's rho was used. A full circle represents a rho of 1. HC, healthy control. T, time point. NPh, Neutrophil signature. PLC, Plasma cell signature.

Furthermore, we investigated the association of disease domains, derived from the SELENA-SLEDAI, with the different gene signatures (Figure 2B). Univariate analysis showed skin-, hematological-, and immunological domain involvement to be associated

Table 1. Patients and healthy control characteristics

				COHORT-I				COHORT-II	
		Category	Total cSLE cohort (n=101)	EMC cohort (n=49)	AUMC cohort (n=35)	TCH cohort (n=12)	RUMC cohort (n=5)	Adult cSLE cohort (n=34)	HC (n=51)
Demographics									
	Gender	*Female*	84 (83.2%)	41 (83.7%)	30 (85.7%)	8 (66.7%)	5 (100%)	32 (94.1%)	40 (78.4%)
		Male	17 (16.8%)	8 (16.3%)	5 (14.3%)	4 (33.3%)	0 (0%)	2 (5.9%)	11(21.6%)
	Ethnicity	*White*	43 (42.6%)	25 (51.0%)	15 (42.9%)	1 (8.3%)	2 (40%)	26 (76.5%)	45 (88.2%)
		Non-white	58 (57.4%)	24 (49.0%)	20 (57.1%)	11 (91.7%)	3 (60%)	8 (23.5%)	6 (11.8%)
	Age at enrolment (years)		15.6 (5.1-23)	15.2 (5.2-18.1)	16.7 (11.8-23)	14.4 (5.1-17.2)	16.1 (15.5-17.6)	32.4 (18.5-56.4)	29 (20-65)
	Disease duration at enrolment (years)		0.51 (0-8.2)	0.18 (0-6.8)	1.11 (0-8.2)	0.04 (0-1.8)	0.59 (0-4.8)	15.8 (3.8-40)	.
	SELENA-SLEDAI at enrolment		4 (0-27)	4 (0-18)	4 (0-27)	4 (0-15)	4 (0-10)	4 (0-14)	.
		≤4	53 (52.5%)	25 (51.0%)	18 (51.4%)	7 (58.3%)	3 (60%)	23 (67.6%)	.
		5 to 7	12 (11.9%)	9 (18.4%)	2 (5.7%)	0 (0%)	1 (20%)	8 (23.5%)	.
		≥8	36 (35.6%)	15 (30.6%)	15 (42.9%)	5 (41.7%)	1 (20%)	3 (8.9%)	.
Longitudinal samples			73 (72.2%)	43 (87.8%)	18 (51.4%)	9 (75%)	3 (60%)	0	0
	Number of visits (median)		2 (1-4)	2 (1-4)	2 (1-4)	2 (1-4)	2 (1-3)	.	.

Table 1. Patients and healthy control characteristics (*continued*)

				COHORT-I				COHORT-II	
		Category	**Total cSLE cohort (n=101)**	**EMC cohort (n=49)**	**AUMC cohort (n=35)**	**TCH cohort (n=12)**	**RUMC cohort (n=5)**	**Adult cSLE cohort (n=34)**	**HC (n=51)**
	Follow up time (days)		344 (29-1542)	267 (51-1542)	511 (98-1059)	91 (29-182)	120.5 (105-182)	.	.
	Flare	*Mild/moderate*	14 (19.2%)	7 (14.2%)	6 (17.1%)	1 (8.3%)	0 (0%)	.	.
		Severe	7 (9.6%)	1 (2.0%)	6 (17.1%)	0 (0%)	0 (0%)	.	.
Treatment at enrolment									
	None		27 (26.7%)	15 (30.6%)	8 (22.9%)	3 (25%)	0 (0%)	0 (0%)	51 (100%)
	Hydroxychloroquine		67 (66.3%)	33 (67.3%)	24 (68.6%)	6 (50%)	4 (80%)	31 (91.2%)	.
	Mycophenolate Mofetil		28 (27.7%)	16 (32.7%)	9 (25.7%)	0 (0%)	3 (60%)	14 (41.2%)	.
	MTX		3 (3.0%)	2 (4.1%)	0 (0%)	0 (0%)	1 (20%)	2 (5.9%)	.
	Azathioprine		8 (7.9%)	2 (4.1%)	5 (14.3%)	1 (8.3%)	0 (0%)	16 (47.1%)	.
	Cyclophosphamide		0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	8 (23.5%)	.
	Prednisone		29 (28.7%)	15 (30.6%)	8 (22.9%)	4 (33.3%)	2 (40%)	31 (91.2%)	.
	Rituximab		4 (4.0%)	0 (0%)	4 (11.4%)	0 (0%)	0 (0%)	1 (2.9%)	.
	Belumimab		1 (1.0%)	0 (0%)	1 (2.9%)	0 (0%)	0 (0%)	0 (0%)	.

Data are presented as median (range) or as number (% of total). Non-white ethnicity = Hindu, Suriname, Hispanic, Asian, African-American, Mixed. MTX = Methotrexate. EMC (Erasmus Medical Center); AUMC (Amsterdam University Medical Center); TCH (Czech Republic Palacky University Olomouc); RUMC (Radboud University Medical Center); cSLE = childhood onset SLE; HC = healthy controls.

with a high M1.2 IFN signature. No domain was associated with a high M5.12 IFN signature. Constitutional and musculoskeletal domain involvement showed an association with a high NPh signature. Additionally, all domains except for the skin, renal and CNS were associated with a high PLC signature. In the multivariate model, skin involvement was associated with a high M1.2 IFN signature, musculoskeletal domain associated with a high NPh and PLC signature, and the constitutional domain was associated with a high PLC signature (Figure 2B).

Next, we determined whether changes over time in disease activity are accompanied by changes in gene signatures. For this purpose, 20 treatment naïve (Tx_{naive}) children with at least one subsequent sample after start of treatment (median time between samples: 62.5 days) were chosen as the optimal patient group to investigate this. The decrease in disease activity over time was accompanied by a significant reduction of the PLC signature but was not mirrored by changes in M1.2 IFN, M5.12 IFN and NPh signatures (Figure 2C-D). Interestingly, when investigating the effect of medication, the additional use of Mycophenolate Mofetil (MMF) and/or Prednisone to Hydroxychloroquine treatment also led to a decrease in the PLC signature ($p=0.0005$), whereas the addition of Prednisone increased the NPh signature ($p=0.0195$) (Figure 2D). In addition, the IFNa2 levels, neutrophil counts and anti-dsDNA levels were measured in the same samples to study other factors than medication use, that may influence the signatures. Neutrophil counts and anti-dsDNA levels showed the same trend as the NPh and PLC signatures, while the IFNa2 levels in general decreased in contrast to the varying activation of the M1.2 and M5.12 IFN signature (supplementary figure S3A-C). Together, these data indicate that gene signatures are associated with disease activity and are influenced by medication use and cell compositions. However, the correlation coefficients (figure 2A) were low indicating that testing individual gene signatures will not be sufficient to identify homogeneous subgroups of patients.

Gene fingerprints identify SLE patients with similar disease activity

In our search to find homogenous subgroups of patients, we first used a semi-manual clustering strategy. Based on the four described gene signatures, patients were allocated into 16 unique clusters (Figure 3A). A cluster represents a combination of either a positive or negative gene signature score. To reduce data complexity the clusters were distributed over 3 mutually exclusive groups with matching underlying activated immune pathways forming a so-called fingerprint. Fingerprint-1 is described as “all-signature-low” which indicates patients with a low score in all four gene signatures. Fingerprint-1 consists of patients in cluster 16 (n=25; 24.8%). Fingerprint-2 represents patients with high IFNs, meaning a high M1.2 and/or high M5.12 score and consists of patients in clusters 1, 2 and 6 (n=34; 33.7%). Lastly, fingerprint-3 defines patients with high NPh and/or PLC signature independent of the IFN signatures and includes patients in clusters 3, 4, 5, 7 to 15 (n=42; 41.5%). Notably, the majority of these patients have a positive M1.2 and/or M512 gene signature (36/42) (Figure 3A-B).

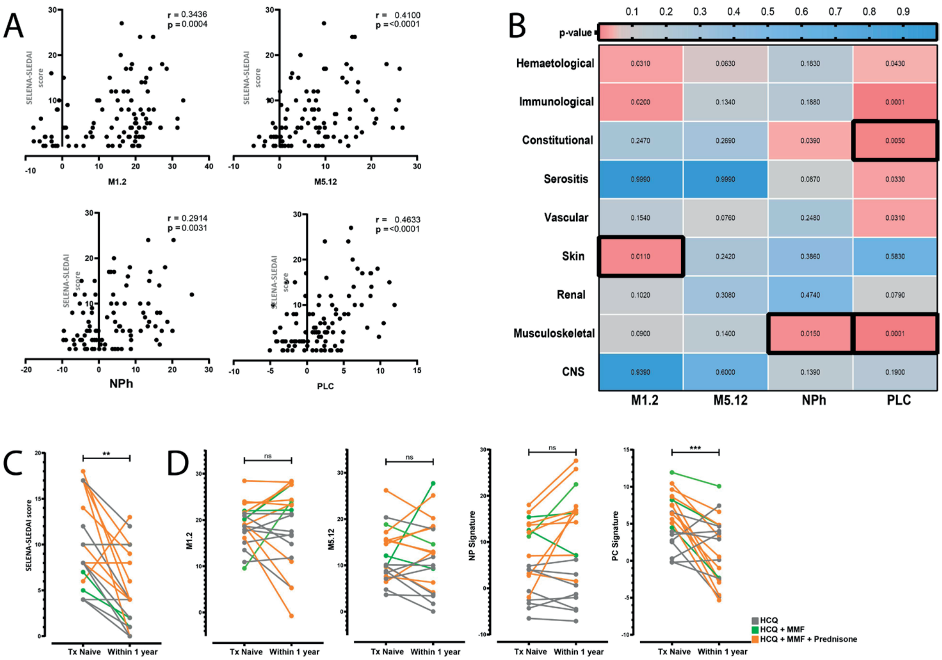


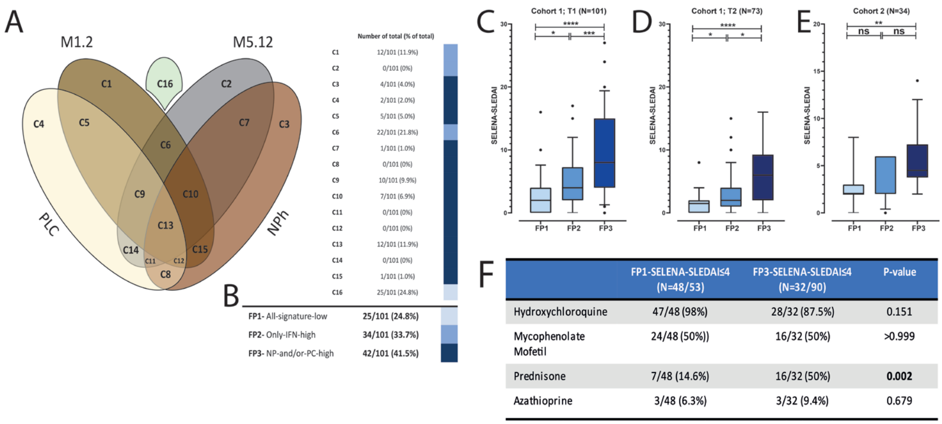
Figure 2. Gene signatures are associated with disease activity

A). Correlation between the SELENA-SLEDAI and gene signature scores. B). Heatmap indicating the correlation between a high signature and a specific disease domain derived from the SELENA-SLEDAI. Numbers indicate the p-value based on univariate analysis. Black lined boxes indicate domains that were significant in the multivariate model. C) Longitudinal SELENA-SLEDAI. D) gene signature scores from 20 Tx_{naive} cSLE patients at the first and second time point (median time between two samples = 62.5 days).
Mann-Whitney-U test was used to compare two groups; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. For correlations Spearman's rho was used. Fisher's exact test was used to compare categorical data. NPh, Neutrophil signature. CNS, central nervous system. PLC, Plasma cell signature. Tx_{naive}, treatment naïve. HCQ, Hydroxychloroquine. MMF, Mycophenolate Mofetil.

As a first step we cross-sectionally analyzed data from samples taken at entry in the study (Figure 3C). Disease activity was significantly different between the three fingerprint groups. Patients with fingerprint-1 (median SELENA-SLEDAI = 2) had the lowest disease activity, while patients with fingerprint-3 had the highest disease activity (median SELENA-SLEDAI = 8). Interestingly, patients with fingerprint-2 formed an intermediate group, indicating that these patients had immunological activation and clinical disease activity but less prominent than patients with fingerprint-3 (Figure 3C). In the fingerprint-3 group, the highest number of patients was treatment naïve ($Tx_{naive;}$ N=20/42) and recently diagnosed (median 10 days, supplementary figure S4A-B). Only 8 patients in this group had a disease duration of >1 year, of which 3 had a disease flare.

To filter out the component of high disease activity and lack of immunosuppressive medication in Tx_{naive} patients (n=27/101) at the first time point, we analyzed 73 samples taken at a subsequent second time point. This confirmed the observation from the first time point: patients within fingerprint-1 had the lowest disease activity, while patients with fingerprint-3 had the highest disease activity (Figure 3D).

To further address whether disease duration influenced our findings, the fingerprints were determined in a cohort of 34 adults with cSLE with a median disease duration of 15.8 years (cohort-II, Table 1). Distribution of patients within cohort-II based on fingerprints, showed an identical association with disease activity. This excludes disease duration from being a factor influencing the fingerprints (Figure 3E). Moreover, to investigate why a selection of patients with low disease activity (SELENA-SLEDAI ≤4) had fingerprint-3, we compared the use of medication between all patients with SELENA-SLEDAI ≤4 with fingerprint-1 and fingerprint-3 (Figure 3F). Patients in the fingerprint-3 group with SELENA-SLEDAI ≤4 were more often on prednisone (p<0.002). This indicates that these patients, despite having low disease activity, still have activation of the underlying immune pathways leading to high NPh and/or PLC gene signature expression.



	FP1-SELENA-SLEDAI≤4 (N=48/53)	FP3-SELENA-SLEDAI≤4 (N=32/90)	P-value
Hydroxychloroquine	47/48 (98%)	28/32 (87.5%)	0.151
Mycophenolate Mofetil	24/48 (50%))	16/32 (50%)	>0.999
Prednisone	7/48 (14.6%)	16/32 (50%)	**0.002**
Azathioprine	3/48 (6.3%)	3/32 (9.4%)	0.679

Figure 3. Gene fingerprints identify SLE patients with similar disease activity
A). Venn-diagram of gene clusters. Overlap between Venn's indicates a positive gene score of the involved gene signatures. B). Patient distribution over three fingerprint groups. Colored bars represent the clusters that are involved in each fingerprint. C). SELENA-SLEDAI distribution per fingerprint group in cSLE cohort-I; first time point (n=101). D). SELENA-SLEDAI distribution per fingerprint group in cSLE cohort-I; second time point (n=73). E). SELENA-SLEDAI distribution per fingerprint group in cSLE cohort-II; replication cohort (n=34). F) Medication use in patients with FP1 and FP3 with a SELENA-SLEDAI ≤4. Dots represent individual patients.
Mann-Whitney-U test was used to compare the two groups; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. NPh, Neutrophil signature. PLC, Plasma cell signature. FP, fingerprint. ns, not significant. T, time point.

Gene fingerprints and clinical phenotype

We performed univariate and multivariate logistic regression analyses to test for specific organ domains involved in patients in the fingerprint-groups, fingerprint-1 was associated with significantly less skin involvement in the multivariate model and fingerprint-3 was associated with involvement of the musculoskeletal, constitutional and immunological organ domains (supplementary table T2).

Auto-antibody profiling of the patients revealed that patients with fingerprint-3 had higher anti-dsDNA levels than patients with fingerprints-1 and 2, reflecting the higher disease activity found in patients with fingerprint-3 (supplementary figure S4C-D). Interestingly, patients with fingerprint-3 were also more often anti-dsDNA positive than the other patients. Anti-SSA antibodies were primarily present in patients with fingerprint-2 and 3, while anti-SM and anti-RNP did not differ between the fingerprint groups (supplementary figure S4D).

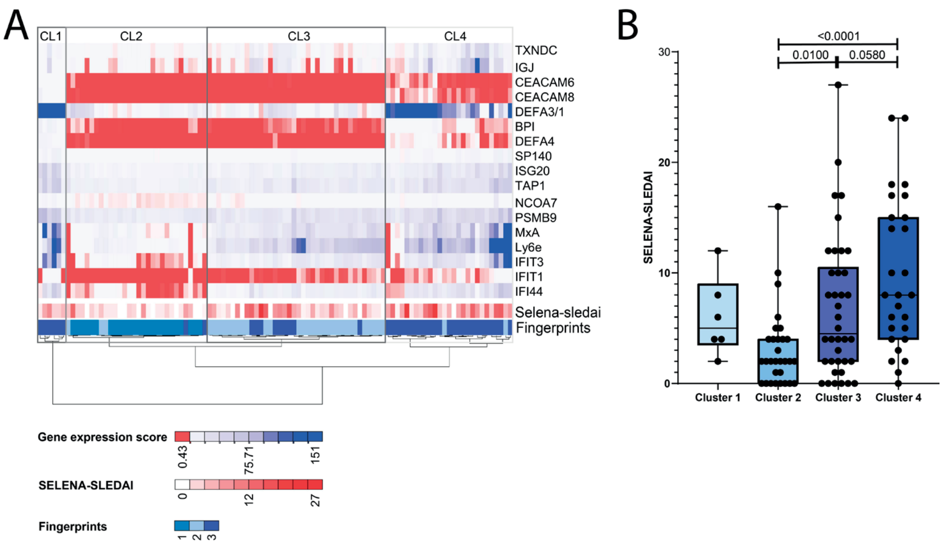


Figure 4. Hierarchical clustering parallels the identified gene fingerprints
A) Unsupervised hierarchical clustering using Ward's agglomerative method, passing the Euclidean distance between samples, identifying Cluster 1,2,3 and 4. Fingerprints 1,2,3 and SELENA-SLEDAI are depicted in the lowest two rows of the heatmap. Each column represents one patient. B). Association between SELENA-SLEDAI and clusters. Dots represent individual patients.
Mann-Whitney-U test was used to compare two groups (4B); *$p<0.05$; **$p<0.01$; ***$p<0.001$; ****$p<0.0001$. Red to white color indicates the magnitude of gene expression described as 2^-(dCT).

Hierarchical clustering identifies gene fingerprints

To investigate the robustness of the identified gene fingerprints, we additionally performed an automated clustering strategy to assess clusters that were enriched in our patient cohort. Unsupervised hierarchical clustering identified four major clusters that paralleled the identified gene fingerprints (figure 4A). Cluster 1 represented a small group of patients, all with fingerprint-3. Cluster 2 represented patients with fingerprint-1. Cluster 3 represented patients with primarily fingerprint-2, while cluster 4 represented patients with primarily fingerprint-3. As in the respective fingerprint-groups, patients with cluster 2 had the lowest disease activity, while patients with cluster 4 had the highest disease activity (figure 4B). Interestingly, within cluster 3, patients that had a fingerprint-3 had a higher disease activity. These data indicate that gene fingerprints are robust tools that match with an automated clustering method and correctly identify patients with similar disease activity.

DISCUSSION

We studied four gene signatures in SLE derived from previously described transcriptomic data to develop a method that can easily be applied in clinical practice. These gene signatures were associated with disease activity in general and with disease domains derived from the SELENA-SLEDAI. Upon subgrouping of patients into fingerprints we found a significant difference in disease activity between the fingerprintAui groups. Fingerprint-1 was associated with low disease activity, while fingerprint-3 represented the patients with high disease activity. We replicated these findings in samples collected over time in the same patient cohort as well as in cross sectional samples of a replication cohort. Hierarchical clustering identified similar gene fingerprints indicating the robustness of our strategy.

Transcriptional profiling is elaborate and costly, resulting in signatures that consist of large gene sets that are simultaneously upregulated [12]. Translation of these data into signatures of a restricted number of genes would facilitate introduction into clinical practice. Therefore, we used a principal components analysis (PCA)- approach to identify genes that explain more than 95% of the total variance in the gene groups. Between the indicator genes that describe a specific gene signature, we observed a high association while genes from different gene signatures lacked this association. These results are in line with previous studies, where indicator genes from each individual gene signature are driven by their own unique pathway [12, 21]. Previously, Chiche et al. described the intra-individual variation of the M1.2 and M5.12 IFN gene signatures obtained from transcriptomics in longitudinal samples from 29 SLE patients [7]. We were able to reproduce these findings for the M1.2 and M5.12 IFN gene signatures obtained via the PCA approach, in our longitudinal cohort of 73 patients. Interestingly, demographic and clinical differences between the two cohorts didn't affect these observations. We further demonstrated for the first time, that the NPh and PLC signatures, showed a dynamic character over time. Importantly, the presence of the described signatures in our cohort was in line with previous findings obtained by microarray analysis by Banchereau and colleagues [12]. This indicates that the signatures are a reliable approach that can substitute transcriptomic analysis.

Confirming previous data, disease activity was associated with each individual gene signature [5, 7, 10, 12]. In line with the findings of Banchereau et al, the PLC signature showed the best association with disease activity in a cross-sectional cohort [12]. Additionally, we now show in our longitudinal cohort of Tx naïve patients that the PLC signature aligns significantly with disease activity as well. Yet, the correlation coefficients are rather low. Disease activity is measured by scoring the involvement of various disease domains. Here we show that each gene signature is linked to specific disease domains. The M1.2 IFN signature was associated with the skin domain while the NPh and PLC signatures associated significantly with the musculoskeletal and constitutional domains. In contrast to previous findings the NPh signature was not associated with renal involvement [10, 12, 16]. As shown by Banchereau et al and Wither et al., the NPh signature is mostly increased during the active phase of lupus nephritis [10, 12]. The contrasting results between our study and previous published results might be due to the relative low number of patients that had active lupus nephritis at time of sample collection. Nevertheless, these data indicate that a low correlation of individual gene signatures with disease activity could be a consequence of the effect of these signatures on different disease domains.

Our longitudinal data indicate that there might be an association between prednisone use and a positive NPh signature. This observation is in line with previous data, showing that neutrophil numbers are increased in individuals using corticosteroids [12, 22, 23]. Our study is the first longitudinal study that confirms previous findings by Banchereau et al. showing that corticosteroids influence the NPh signature. Also, the finding that the PLC signature in longitudinal cSLE samples is sensitive to changes in disease activity and is affected by the use of prednisone and MMF is in line with previously described results [12]. Studies in the MRL/lpr mouse model for SLE showed that prednisone treatment was associated with a significant decrease of plasma cell numbers [24], that linked to a decrease in BLIMP-1, which regulates plasma cell formation [25]. Interestingly, BLIMP-1 correlated to increased plasma cell numbers and disease activity in SLE patients [25, 26]. Moreover, the neutrophil count and anti-dsDNA represented the NPh and PLC signatures, indicating that cell compositions are drivers of the gene signatures. Considering the IFNa2 levels, we show that the IFN gene signatures don't have the ability to parallel changes in disease activity while ultra-sensitive analysis of IFNa2 can. This is in line with previous findings [27]. This finding underscores that in contrast to the NPh and PLC signatures the IFN gene signatures are not influenced by the use of medication and potentially have a biological role in disease manifestation. Future longitudinal studies in SLE patients are needed to confirm our observations.

We identified 3 fingerprints by cluster analysis of four different gene signatures. These fingerprints were able to discriminate between patients who are in remission (fingerprint-1- “all signature low”) or have high disease activity (fingerprint-3- “high NPh and/or PLC”) in two different cohorts. This observation is in line with previous work showing that adult SLE patients with a low IFN-I signature had significantly lower disease activity compared to patients with a high IFN-I and high NPh signature [23]. Moreover, the demonstration that unsupervised hierarchical clustering analysis paralleled the identification of the gene fingerprint groups shows the robustness of this novel approach.

Our logistic regression model indicated that fingerprints were associated with different organ domains. The identified associations highly reflect the drivers of each fingerprint group. Fingerprint-1 is particularly driven by the M1.2 IFN gene signature, as this signature is associated with skin involvement. Fingerprint-2 seems to be driven by the M5.12 gene signature as this signature was not associated with any organ domain. Lastly, fingerprint-3 is driven by the NPh and PLC signatures, as these signatures are associated with the musculoskeletal and constitutional domains. Moreover, our results illustrate that autoantibody-profiles are different among patients within various fingerprint groups adding to the knowledge on the relation between autoantibody profiles, disease activity and disease phenotype [28, 29], yet also highlighting their gaps and underlining that mere autoantibody-profiles are not enough for subtyping SLE patients.

Previous data showed that the presence of an IFN signature is associated with an increased chance of disease flare in 5-years [30], supporting a role in disease pathogenesis. Also, higher baseline serum IFN-alpha levels measured by Simoa during SLE remission identified patients at risk for relapse [31]. Interestingly, in our cohorts the patients with only high IFN scores clustered together in fingerprint-2 and they had intermediate disease activity scores when compared to patients with fingerprint-1 and fingerprint-3. Further studies will have to show whether these patients may be specifically at risk to develop a disease flare. Recently, Northcott et al showed that high expression of IFN-I is associated with limited efficacy of glucocorticoids in SLE patients suggesting that IFN gene signatures can predict treatment efficacy and therefore are candidates for future individualized treatment choices [32].

This study has several strengths. This is a study measuring four different gene signatures in a longitudinal multicenter cohort of SLE patients with ± 25% being Tx_{naive}. Moreover, the reproducibility of our observations in an independent replication cohort and unsupervised clustering strategy with similar findings shows the robustness of this approach. Our study also has limitations. With the current cohort of 101 patients, we are underpowered to study specific disease phenotypes likes lupus nephritis and neuropsychiatric lupus. Furthermore, disease activity was assessed by the SELENA-SLEDAI. A disadvantage of this scoring system is that it does not consider improvement or worsening of disease items. Therefore, the SELENA-SLEDAI is less sensitive to changes in disease activity compared to other measurement scales [33]. Lastly, it is important to mention that gene signatures and fingerprints, especially the NPh and PLC signatures, may be influenced by medication use. Therefore, medication use should always be considered as a confounding factor before results on fingerprints are interpreted.

CONCLUSION

In conclusion, this study shows that the approach using PCA to identify indicator genes for gene groups, is a successful method to translate existing transcriptomic data into a tool that can be applied in clinical practice. We confirmed the activation of four gene signatures previously identified by transcriptomics and reproduced these data in an independent replication cohort. Moreover, combining the gene signatures into so-called fingerprints enabled us to stratify patients into subgroups with similar activated immune pathways that were associated with disease activity over time in our longitudinal cohort study. The heterogeneity of SLE is reflected in the variability of drug responsiveness between patients. This is expected to become more given the current focus on the development of new biologicals that specifically target specific molecules or immune pathways. We have identified a molecular tool, stratifying patients into groups with similar biological disease profiles that has the potential to guide individualized treatment choices and improve the trial-and-error treatment approach of the present time. Our findings should be confirmed in large longitudinal studies to elucidate the applicability of this tool for prediction of responses on treatments interfering with the aberrantly activated immune pathways.

ACKNOWLEDGEMENTS

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SUPPLEMENTARY METHODS

Blood collection and real-time PCR

Blood was collected in PAXgene RNA tubes and was stored in -80^{0}C until use for whole blood RNA purification. RNA isolation, cDNA preparation and real-time PCR were performed according to manufacturer's protocol. In short, total RNA was isolated from PAXgene tubes and reverse-transcribed to cDNA. For calculation of relative gene expressions, samples were normalized to expression of the household gene Abl. Relative expression values were determined from normalized CT values using the 2^-ΔCT method.

Gene selection and gene signature definitions

For each gene signature, genes were selected from the most frequently described genes from transcriptional studies published previously (supplementary table T1, figure S1). To identify correlated groups of genes and reduce data complexity, the expression of 10 IFN-I inducible genes from the M1.2 IFN module, 15 neutrophil (NPh)- and 7 plasma cell (PLC) associated genes were added to a principle component analysis (PCA). Kaiser-Meyer-Olkin measure of sampling adequacy were respectively 0.744, 0.796 and 0.792.

The gene scores for each signature were defined by the sum of the relative expression of 2-5 indicator genes. A log2 transformation was used as an intermediate step in the calculation process to generate both + (up) and – (downregulated) gene expression values. The gene signature formula is listed below. For the M1.2 IFN signature these were IFI44, IFIT1, IFIT3, Ly6e and MxA; for the NPh signature DEFA4, BPI, CEACAM6, CEAMCAM 8 and DEFA3/1; and for the PLC signature IGJ and TXNDC5. The gene score for the M5.12 IFN signature was defined by the relative expression of 5 indicator genes (PSMB9, ISG20, NCOA7, SP140 and TAP1) as previously described [21]. Mean and SD of each gene in HC were used to standardize expression levels. The gene scores per subject represent the sum of these standardized scores, calculated as previously described [4, 5]. Patients were divided in groups being positive and negative for a signature, using a threshold of mean + 2xSD_{HC}.

Gene signature formula

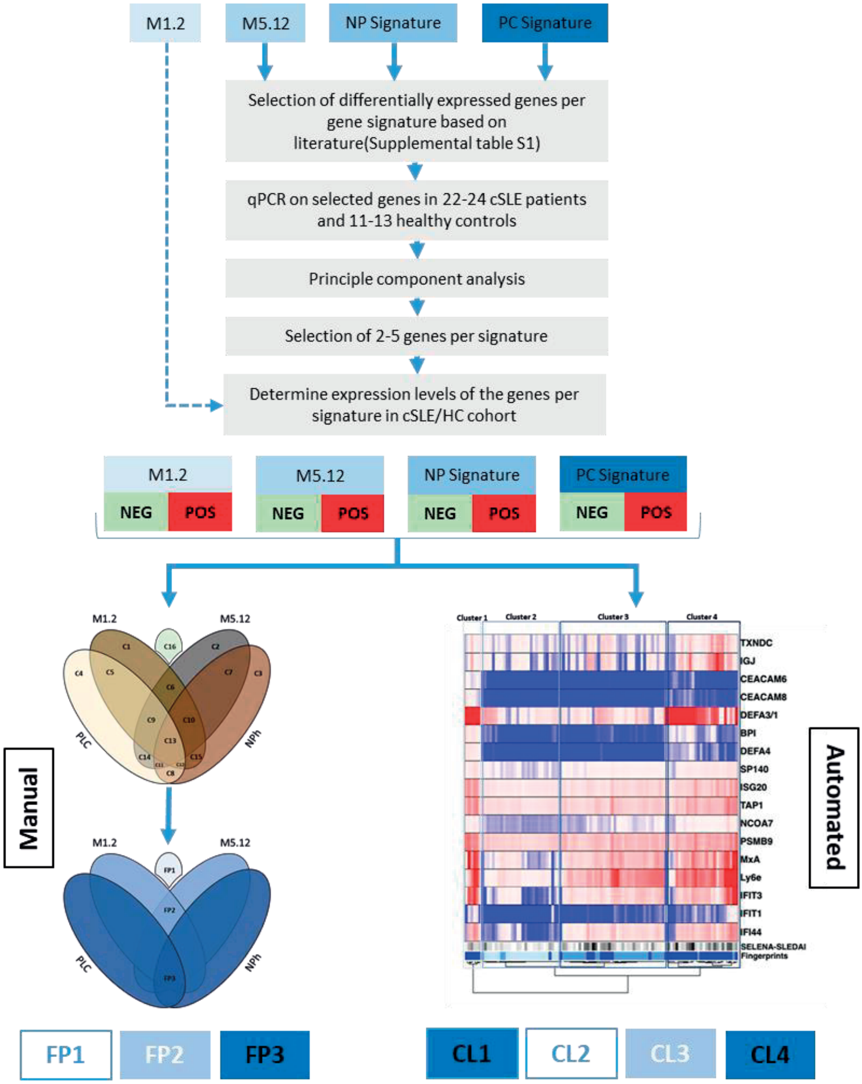
∑(Log2^-ΔCt(subjects)- mean Log2^-ΔCt(healthy controls)) / st.dev. Log2^-ΔCt(healthy controls).

Ultrasensitive IFN-α Simoa

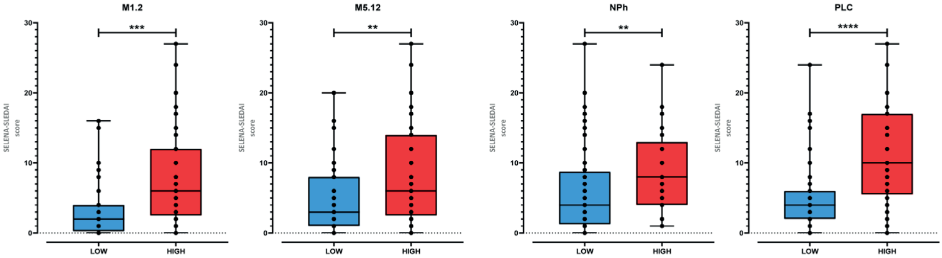
IFN-α2 was measured in duplicates from serum samples (diluted two-fold in sample diluent) using the Simoa IFN-α Advantage Kit (no. 100860, Quanterix, Billerica, MA, USA) following the instructions of the supplied manual. Sample processing and analysis were done using an HD-X analyser (software version 1.6.1905.300; Quanterix). The lower limit of detection was 5 fg/ml.

Statistics

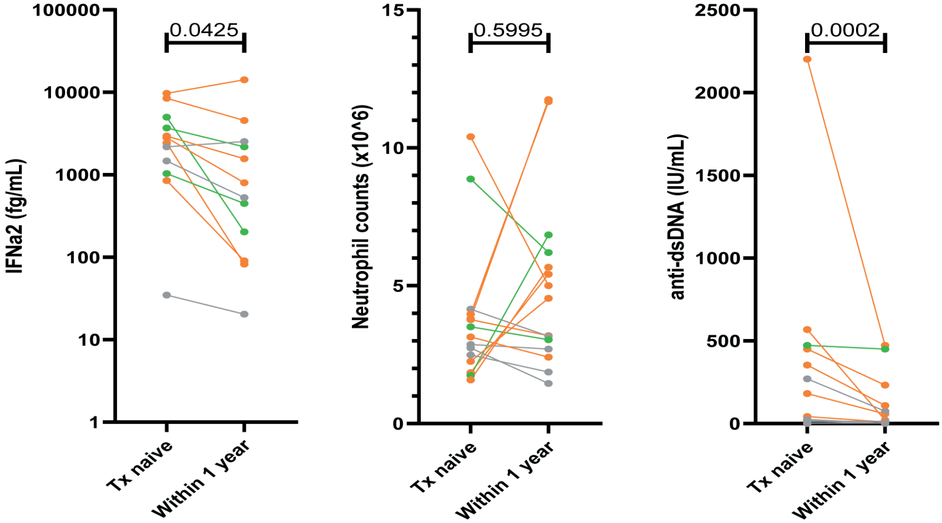
The non-parametric Mann-Whitney U (two groups) and Kruskal-Wallis (three groups) tests were used to analyze comparisons between medians. The paired *t* test was used to compare means of paired data. Chi squared test and Fisher's exact test were used to compare categorical data. Spearman's rho (rs) coefficient or Pearson correlation coefficient was calculated to assess correlation. Regression analysis was performed to relate organ domains to gene signatures or fingerprints. All domains with an individual effect of $p<0.1$ were used to build a multivariable model. Values of $p<0.05$ were considered statistically significant. Unsupervised hierarchical clustering using Ward's agglomerative method, passing Euclidean distance between samples was used to identify clusters enriched in cSLE patients. Graphpad Prism 8.0 (Graphpad Software, La Jolla, CA, USA) and R statistical software were used for graph design and statistical analysis. Due to the exploratory nature of our study, we refrained from performing multiple testing correction.



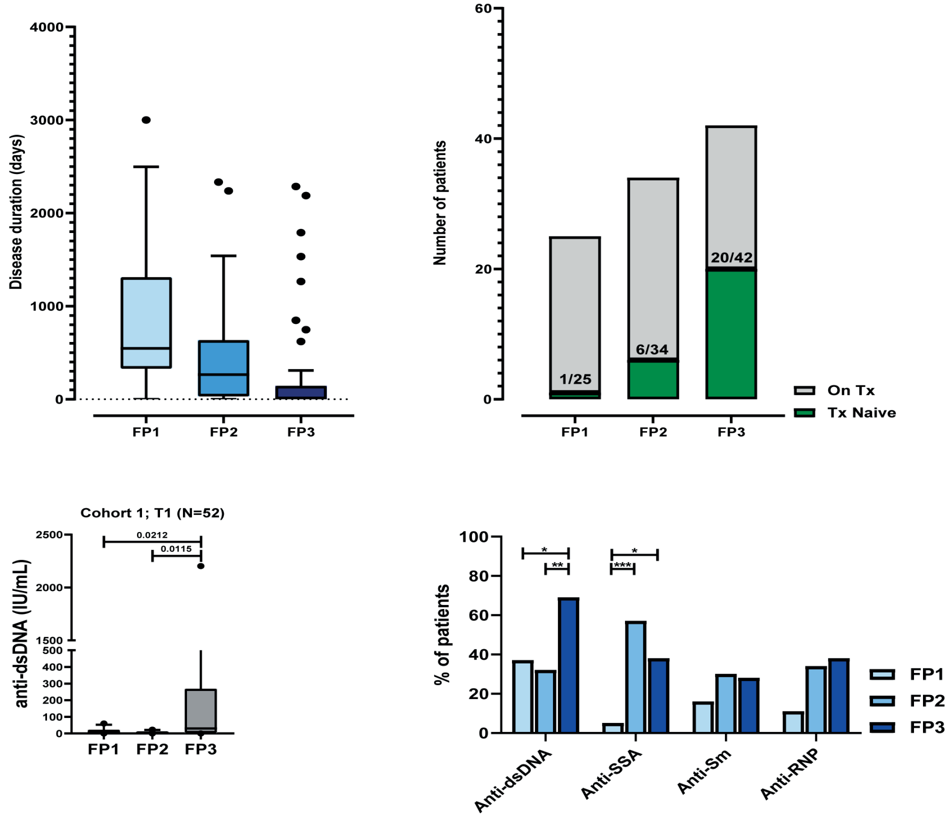
Supplementary figure S1. Flowchart illustrating the process of gene selection and clustering strategies
For the M1.2, NPh and PLC signature, genes were selected by choosing the most frequently described genes from transcriptional studies published previously. Gene expression was determined in 22-24 cSLE patients and 11-13 healthy controls by qPCR. Expression data was added into a principle component analysis. For each gene signature 2-5 indicator genes were selected. Expression of these indicator genes were further determined in the rest of the blood samples. Indicator genes of the M5.12 IFN signature were previously described by our group (21). A semi-manual and an automated clustering strategy were performed. The semi-manual approach identified 16 clusters based on the combination of a positive or negative score per gene signature. The gene signatures measured at the first study time point of each patient were used. These clusters were subsequently divided into 3 so-called fingerprints. As a starting point we chose a group of patients in which all gene signatures were low. We combined the two IFN gene signatures together due to their high inter-correlation (Figure 1A). We defined Fingerprint 2 by having either a positive M1.2 or a positive M5.12 gene signature but without having a positive NPh or positive PLC gene signature. Fingerprint 3 was defined by having a positive NPh and/or positive PLC gene signature, independent of the IFN gene scores. Notably, the fingerprint patient groups were mutually exclusive, each patient only fits in one fingerprint group. For the automated clustering strategy, an unsupervised hierarchical clustering method was used to identify clusters that were enriched in the cSLE cohort.
IFN: Interferon, NPh: Neutrophil, PLC: Plasma cell, FP: Fingerprint, CL: Cluster.



Supplementary figure S2. SELENA-SLEDAI score in patients with a low or high gene signature (N=101)
A). M1.2 IFN signature. B). M5.12 IFN signature. C). NPh signature. D). PLC signature. The mean + 2xSD$_{HC}$ was used as a threshold for dividing patients into the low or high group.
Mann-Whitney-U test was used to compare the two groups; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. IFN: Interferon, NPh: Neutrophil, PLC: Plasma cell.



Supplementary figure S3. IFNa2, Neutrophil count and anti-dsDNA level in 20 Tx naïve patients over time
A). IFNa2 (n=12). B). Neutrophil count (n=15). C). anti-dsDNA (n=16).
Mann-Whitney-U test was used to compare the two groups; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. IFNa2, Interferon alpha 2.



Supplementary figure S4. Disease duration, medication use and autoantibodies per fingerprint
A). Distribution of disease duration between fingerprints. B). Number of treatment naïve patients per fingerprint. Numbers represent treatment naïve patients per group total. C). Anti-dsDNA level distribution per fingerprint in a selection of cSLE cohort 1; first time point (N=52). Levels are based on a fluorescent enzyme immune assay, reference value >10 IU/mL. D). Percentage of patients with a positive auto-antibody per fingerprint in cSLE cohort 1 (N=86).
Mann-Whitney-U test was used to compare the two groups; chi-square test was used to compare categorical data
*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. FP, Fingerprint. Tx, Treatment.
On Tx indicates Hydroxychloroquine with or without Mycophenolate Mofetil and/or Prednisone.

Supplementary table T1. List of genes per signature

M1.2 IFN	M5.12 IFN	NPh	PLC
IFI44	**PSMB9**	**DEFA4**	**IGJ**
IFIT1	**ISG20**	**BPI**	**TXNDC5**
IFIT3	**NCOA7**	**DEFA3/DEFA1**	**CD38**
Ly6e	**SP140**	**CEACAM8**	**GLDC**
MxA	**TAP1**	**CEACAM6**	**TNFRSF17**
Serping		**MPO**	**IGHA1**
IFI27		**ELANE**	**IGKC/IGKV5-1**
IFI44L		**AZU1**	
XAF1		**MMP8**	
ISG15		**LTF**	
		CTSG	
		LCN2	
		MS4A3	
		OLFM4	
		RNS2	

M1.2	
Bodewes, 2018	IFI44 IFIT1 IFIT3 Ly6e MxA Serping IFI27 IFI44L XAF1 ISG15
M5.12	
Bodewes, 2018	PSMB9 ISG20 NCOA7 SP140 TAP1
Neutrophil associated genes	
Bennett, 2003	RNS2 RNAse 2 MMP-9 RNS3 HUSI-1 ELASTASE 2 LCN2 CG cathepsin G MPO NCF DEF3 DEFA4 DEFA1 FALL-39 BPI AZU EST GCA S100P CD24A CD66A CD66B TC1 CRISPR3 F2RPA
Villanueva, 2011	MMP8 RNASE3 ELANE LCN2 MPO CTSG DEFA4 BPI AZU1 CEACAM8 CEACAM1 LTF CLU
Banchereau M5.15, 2016	ARG1 AZU1 BPI CAMP CEACAM6 CEACAM8 COL17A1 CTSG DEFA1 DEFA3 DEFA4 EIF1AY ELA2 HLA-DRB1 HLA-DRB5 HP LOC653600 LTF MMP8 MPO MS4A3 OLR1 RETN TCN1
Carlucci, 2018	MPO ELANE PRTN3 CTSG AZU1 DEFA4
Wither, 2018	MPO DEFA4 DEFA3 DEFA1 MMP8 CEACAM6 CEACAM8 LTF MS4A3 OLFM4 CRISP3 LCN2 BPI
Petri, 2019	DEFA4 CEACAM8 CEACAM6 MMP8 LCN2 BPI OLFM4 LTF
Plasma cell associated genes	
Streicher, 2014	IGHA1 IGJ IGKC IGKV4-1 TNFRSF17
Banchereau M4.11, 2016	CAMK1G CD38 GLDC IGJ TNFRSF17 TXNDC5
Petri, 2019	IGJ TXNDC5

*Grey color illustrates the indicator genes per gene signature.

Supplementary table T2. Binary logistic regression analysis of organ domains associated with different fingerprints as outcome measure

	Univariate								
	FP1			FP2			FP3		
	B	P	Exp	B	P	Exp	B	P	Exp
CNS	20.104	0.999	0	20.556	0.999	0	-1.34	0.281	0.262
Musculoskeletal	1.775	**0.094**	5.902	0.911	0.18	2.1488	-1.721	**0.004**	0.179
Renal	0.963	0.15	2.619	0.462	0.384	1.587	-1.197	**0.015**	0.302
Skin	1.281	**0.02**	3.6	-0.401	0.347	0.67	-0.683	0.109	0.505
Vascular	20.232	0.999	0	0.774	0.345	2.169	-1.658	**0.022**	0.19
Serositis	20.232	0.999	0	20.602	0.999	0	-21.991	0.999	0
Constitutional	1.402	0.191	4.062	1.869	**0.08**	6.482	-1.984	**0.005**	0.138
Immunological	0.912	**0.061**	2.489	0.816	**0.061**	2.261	-1.91	**0.0001**	0.148
Haematological	1.114	**0.02**	3.048	-0.147	0.73	0.863	-0.78	**0.076**	0.458
	Multivariate								
CNS									
Musculoskeletal	1.495	0.173	4.461				-1.404	**0.043**	0.246
Renal							-1.038	0.075	0.354
Skin	1.118	**0.05**	3.06						
Vascular							-0.267	0.769	0.766
Serositis									
Constitutional							-2.062	**0.012**	0.127
Immunological	0.415	0.429	1.515	1.752	0.103	5.765	-1.449	**0.008**	0.235
Haematological	0.852	0.092	2.344	0.734	0.097	2.084	-0.217	0.689	0.805

A cut-off of $p<0.10$ was set to select the variables for the multivariate logistic regression. In the analysis a value of 1 indicated the involvement of a fingerprint while 0 indicated no involvement of a fingerprint, a negative value for *B* therefore means more association with a certain domain, while a positive *B* indicated less association.
CNS: Central Nervous System, FP: Fingerprint.

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7

Rarities in rare: illuminating the microvascular and dermal status in juvenile localized scleroderma. A case series

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ABSTRACT

OBJECTIVE: To assess the (structural and functional) characteristics of the microvascular and dermal status in juvenile localised scleroderma (jLoS), using novel non-invasive standardised research tools commonly used in adult systemic sclerosis (SSc).

METHODS: Ten consecutive patients with a confirmed jLoS diagnosis were studied cross-sectional in this two-centre case series. For each patient, the most prominent lesion (i.e. “target lesion”) was chosen for further examination of the centre, edge and contralateral unaffected site. High-frequency ultrasonography was used to determine dermal thickness, durometer for skin hardness, and laser speckle contrast analysis (LASCA) for a dynamical evaluation of the microcirculation. The structure of the microcirculation was evaluated at the nailfolds of the 2nd-5th finger bilaterally, using nailfold videocapillaroscopy (NVC).

RESULTS: 6 linear and 4 plaque subtype jLoS lesions were included. Dermal thickness was thinner at the centre of the “target lesions” vs. the edges (p<0.001) and control sites (p<0.001). Skin hardness was harder at the centre of the “target lesions” vs. the edges (p=0.012) and control sites (p=0.003). A higher perfusion was found in the centre of the "target lesion" (124.87±66.40 PU) vs. the edges (87.27±46.40 PU; p<0.001) and control sites (67.85±37.49; p<0.001). Of note, all patients had a “non-scleroderma” pattern on NVC.

CONCLUSION: This case series suggests the supportive value of both microcirculatory and dermal assessments of skin lesions using novel non-invasive research tools, adopted from adult SSc, for the identification, scoring and/or monitoring of jLoS.

INTRODUCTION

Scleroderma comprises a group of rare (incidence of ±1 per 100.000/year) fibrosing disorders with similar histopathological findings [1-8]. Scleroderma can be broadly divided into systemic sclerosis (SSc, prevalence ranging between 7 and 44 per 100.000 individuals) and localised scleroderma (LoS, also called morphea, estimated prevalence ±50 per 100.000 individuals). SSc is a multisystem connective tissue disease hallmarked by a triad of vasculopathy, autoimmunity and fibrosis of the skin and/or internal organs, LoS is pathological process of local and chronic inflammation mainly affecting part(s) of the skin and underlying tissues, which eventually leads to fibrosis and atrophic changes [1, 6-12]. In contrast to SSc, LoS is a very rare condition that usually presents in childhood (juvenile LoS [jLoS]) with a mean age of onset at 6-8 years [9, 12]. The estimated incidence of jLoS is estimated at 3.4 cases per million children per year [13, 14].

It is believed that SSc and (j)LoS share common pathophysiological pathways with an initial inflammatory phase accompanied by endothelial activation, followed by a fibrotic phase characterised by tissue collagenisation and appreciable skin thickness sometimes accompanied by atrophic changes [3, 7-9, 15-17]. The hypothesis of a common pathophysiological pathway is further supported by recent observations from our research group, as we found both in literature and in a pilot study that the coexistence of SSc and (j)LoS (2.4 to 7.4%) is higher than their individual prevalence in the healthy population [16].

Although it is not a lethal disease like SSc, (j)LoS can lead to severe physical damage with growth deformities, functional disabilities and cosmetic impairment, which may eventually lead to chronic psychological besides physical problems [18-20]. As there are no antifibrotic therapies yet that can cure the disease, early anti-inflammatory aggressive systemic treatment regimens are often required to halt disease progression and prevent poor outcomes [21-25].

In daily clinical practice the validated Localised Scleroderma Cutaneous Assessment Tool (LoSCAT) is being used combining assessment of disease activity using the modified Skin Severity Index (mLoSSI) and assessment of disease damage using the modified Skin Damage Index (mLoSDI) [26, 27]. Because the LoSCAT only provides a global impression of all affected (j)LoS localisations, there is a need for more validated (imaging) tools to objectively identify and monitor inflammation and tissue damage *per* (j)LoS lesion. Those tools are an unmet need in making treatment decisions, for instance when to increase, switch, taper or to stop treatment in young aged patients and should consequently predict and monitor treatment response. The lack of these tools not only hampers clinical daily treatment decisions, but also complicates the conduct of clinical trials. Several non-invasive imaging modalities, such as thermography, laser doppler flowmetry and (doppler) ultrasound have been proposed, but are hampered by user dependency and lack of standardisation [28]. Magnetic resonance imaging (MRI) and cone beam computed tomography (CBCT) are other imaging possibilities with the disadvantages of high costs and invasiveness for young children, not only during examination (MRI) but also because of negative radiation risks (CBCT) [29-37].

Unlike in SSc, where the evaluation of the microcirculation (using non-invasive standardised tools such as nailfold videocapillaroscopy [NVC] and laser speckle contrast analysis [LASCA]) has earned a pivotal role and the evaluation of skin fibrosis using high-frequency ultrasonography (HFUS) and durometry is currently an area of significant research and standardisation efforts, exhaustive reports using these tools in jLoS are non-existent [1, 6-9, 38-54]. Against this background, we felt it was time to descriptively assess the (structural and functional) characteristics of the microvascular and dermal status in a case series of jLoS patients, using non-invasive standardised research tools (i.e. NVC, LASCA, HFUS and durometer) that are commonly used in adult SSc.

MATERIALS AND METHODS

Ethical Vote

This study was approved by the local institutional review boards and local ethics committees (Amsterdam University Medical Centre [2017-172], Ghent University Hospital [EC/2019/1639], and conducted in accordance with the Declaration of Helsinki and Helsinki and its amendments. All parents or legal guardians (for patients < 16 years old) and competent patients (over > 12 years old) signed written informed consent before inclusion.

Study population

This two-centre, observational study was conducted in 2019 at the tertiary Amsterdam University Medical Centre (The Netherlands) and in 2021 at the tertiary Ghent University Hospital (Belgium). Consecutive juvenile patients (≤18 years) with a confirmed diagnosis of jLoS, regardless of disease duration, who visited the paediatric rheumatology/immunology and dermatology department for an outpatient visit were recruited [6, 17]. The diagnosis of jLoS was made clinically and, if necessary in doubtful cases, confirmed by histopathological examination (6, 17). In addition, jLoS was classified according to Kreuter's guideline as limited, generalised, linear, deep or mixed type [6]. Patients without demonstrable skin involvement at the time of recruitment, or with the presence of other systemic diseases (e.g. juvenile systemic sclerosis, rheumatoid arthritis, lupus erythematosus) were deemed ineligible for the study.

Data collection

Demographic, anamnestic, clinical and serological data were collected cross-sectionally from electronic medical records. For the purpose of this study, all patients were subjected to a detailed skin and microvascular examination by a team of experienced (paediatric) dermatologists and rheumatologists on the same day.

Skin examination

First, a clinical skin evaluation was performed, including a detailed description of the jLoS lesions in terms of their number, anatomical locations and dimensions and an assessment of clinical signs of disease activity and damage by completing the mLoSSI and the mLoSDI (which are combined in the LoSCAT) [26, 27]. More specifically, disease activity was measured by assessing 3 separate items (new lesion/lesion extension, erythema and skin thickness) at 18 cutaneous anatomical sites (head, neck, chest, abdomen, upper back, lower back, upper arms, forearms, hands/fingers, buttocks/thighs, legs and feet), with a predefined score of 0-3. The scores for each anatomical site were based on the most severe (i.e. highest) score for each item [27]. Disease damage was measured by the comparable mLoSDI, scoring 0-3 on three items (i.e. dermal atrophy, subcutaneous atrophy and dyspigmentation) in the same 18 cutaneous anatomical areas as the mLoSSI [4]. Identically to the mLoSSI, the most severe score obtained from each item was used to calculate the mLoSDI [26].

When multiple lesions were present, the most prominent lesion (i.e. “target lesion”) was chosen for further examination, and in case of large lesions, the most affected site was designated by an expert dermatologist (M.M.-H.). Following the clinical skin evaluation, a bi-instrumental examination of the centre, edge and contralateral unaffected site of the “target lesion” was performed by an experienced investigator (A.V.) using HFUS to assess skin thickness, and a durometer to determine skin hardness. In case of presence of jLoS lesions on the contralateral site, the perilesional unaffected skin was examined.

HFUS images were taken by using a commercially available ultrasound system with a linear probe operating at 18 MHz in B-mode (Logiq S8, GE Healthcare, Chalfont St. Giles, Buckinghamshire, UK). Briefly, the probe was placed perpendicular to the skin by hand, without applying pressure, using a layer of ultrasound gel acting as a coupling agent between the skin surface and the probe. Images were obtained of the centre, edge and contralateral/perilesional unaffected site of the “target lesion”. The dermal thickness (DT), expressed in mm, was subsequently determined by measuring the distance between the epidermis-dermis interface and the dermis-subcutis interface three times and calculating an average DT value for each area [55, 56].

Durometer measurements were performed using a hand-held electronic durometer (RX-DD Digital Durometer, type OO), which has a calibrated continuous scale from 0 to 100 standard durometer units (DU). The durometer was placed perpendicular to the skin and left at rest by gravity of the durometer's weight. As during the HFUS examination, durometer readings were taken in the centre, edge and contralateral/perilesional unaffected site of the "target lesion" to obtain a durometer value for each of these areas [57, 58].

Microcirculation

The microcirculation was evaluated both structurally and dynamically, respectively by using nailfold videocapillaroscopy (NVC) and laser speckle contrast analysis (LASCA).

First, the structure of the microcirculation was evaluated by examining the nailfolds of the 2nd-5th finger bilaterally with an **NVC** probe equipped with a 200x magnification lens. Two adjacent fields in the middle of the nailfold, extending over 1mm and corresponding to the distal row of capillaries, were captured per finger, resulting in 16 images per patient. The NVC images, with a 1 mm grid, were coded and read centrally at the Ghent University Hospital. Quantitative and qualitative assessments of these images were performed according to the consented capillaroscopic definitions of the EULAR Study Group on Microcirculation in Rheumatic Diseases [38, 47, 59, 60].

For the dynamic evaluation of the microcirculation, **LASCA** was performed under standardised conditions as previously described, using a commercially available LASCA instrument (Pericam PSI, Perimed, Jarfalla, Sweden). During the measurements, the areas of interest, being the centre, edge and contralateral/perilesional unaffected site of the "target lesion", were illuminated perpendicularly with a laser beam for 30 seconds, at a fixed distance (20±0.5 cm). Then, the blood perfusion (BP) was evaluated by drawing a standardised circular region of interest (ROI) with a fixed diameter of 1cm in the middle of the area of interest, using LASCA software (PIMSoft 15.1, Perimed AB, Jarfalla, Sweden). Hence, a BP value, expressed in arbitrary perfusion units (PU), was recorded for each of these areas [49-53, 61].

Statistical analysis

Descriptive statistics were used to summarise the data. For nominal categorical variables, absolute numbers with percentages are shown, for ordinal categorical and skewed continuous variables, medians with interquartile ranges (IQR) are shown, and for symmetric continuous variables, means with standard deviation (SD) are shown. To compare the means between the "target lesions" and control sites, paired sample T-tests were used. Pearson's correlations examined the relationship between the mLoSSI/mLoSDI and LASCA, HFUS and durometer measurements of the "target lesions". Significance was defined as $p<0.05$. Statistical analysis are performed with SPSS, version 27 (IBM SPSS Inc., USA).

RESULTS

Study population

Ten patients with a confirmed jLoS diagnosis were included at the tertiary Amsterdam University Medical Centre (*n*=9) and the tertiary Ghent University Hospital (*n*=1). Their demographic, clinical and laboratory characteristics are summarised in table 1. The mean age was 14.6 years, and 60% were female patients. When categorised according to the jLoS subtype, there were 6 patients with linear (5 extremities, 1 ECDS) and 4 with plaque subtype. A total of 22 lesions were found, which were located on the trunk (*n*=12; with 4 on the chest, 4 on the abdomen and 4 on the back), lower extremities (*n*=9; with 6 on the upper legs, 2 on the lower legs, and 1 on the feet), and face (*n*=1).

NVC evaluation

By quantitative analysis, the mean capillary density was 7.4 (±0.8) capillaries/linear mm. No giant capillaries were observed. The mean number of abnormal capillary shapes was 0.3 (±0.3) capillaries/linear mm and 5 (50%) patients showed microhaemorrhages. By qualitative analysis, all patients had a “non-scleroderma pattern”. Of them, 5/10 (50%) were classified as having a “normal” NVC pattern, and 5/10 (50%) as having “non-specific abnormalities” (table 1, see end of manuscript).

Clinical and instrumental measurements

Table 2 (see end of manuscript) lists per patient the mLoSSI and mLoSDI measurements, as well as all instrumental measurements of the centre, edge and contralateral/perilesional unaffected sites of each “target lesion”. When examining skin thickness and hardness, significant differences were observed in the centre of the “target lesions” compared to both the edge of the “target lesions” and control sites (tables 3 and 4). More specifically, the dermal thickness was thinner in the centre of the “target lesions” than at the edge of the “target lesions” ($p<0.001$, table 3) and the control sites ($p<0.001$, table 4). The centre of the “target lesions” was harder than the edge of the “target lesions” ($p=0.012$, table 3) and the control sites ($p=0.003$, table 4). Furthermore, a significant higher BP was observed in the centre of the "target lesions" (124.87±66.40 PU) than at the edge of the "target lesions" (87.27±46.40 PU, $p=0.001$, table 3) and the control sites (67.85±37.49 PU, $p<0.001$, table 4).

Table 1. Demographic, clinical and serological characteristics of jLoS patients.

n°	Age	Gender	Race	jLoS subtype	jLoS location	ANA	Age at diagnosis (years)	Disease duration (years)	Systemic treatment (ever)	NVC pattern	mLoSSI*	mLoSDI*
1	16	Male	African	Linear	**Upper leg left**	-	8	8	MTX, GCs	Normal	2	6
2	17	Female	Asian	Plaque	Thorax left, abdomen left (x2), **abdomen right**, back, lower leg left	+	10	7	MTX, GCs	Normal	2	3
3	14	Female	Caucasian	Plaque	**Thorax left**	-	5	9	MTX, tocilizumab	Non-specific	2	7
4	16	Male	Caucasian	Linear	**Foot left**	+	12	4	MTX, GCs	Non-specific	2	3
5	15	Male	Caucasian	Linear ECDS	**Face left**	+	12	3	MTX, GCs	Non-specific	3	5
6	18	Female	Mediterranean	Linear	**Upper leg left**	-	4	14	MTX	Normal	3	5
7	12	Female	African	Plaque	**Upper leg right**, abdomen right, back central	-	11	1	MTX, GCs	Non-specific	4	5
8	10	Female	Caucasian	Linear	**Upper leg left**	+	9	1	MTX, GCs	Non-specific	4	6
9	14	Male	Caucasian	Plaque	**Back right**	-	7	7	MTX, GCs	Normal	1	2
10	14	Female	Caucasian	Linear	**Thorax left**, upper leg left, upper leg right, thorax left, back middle	-	14	0	MTX, GCs	Normal	3	3

* Target lesion. *ANA: antinuclear antibodies; ECDS: en coup de sabre; jLoS: juvenile localised scleroderma; mLoSSI: modified localised scleroderma severity index; mLoSDI: modified localised scleroderma damage index; MTX: methotrexate; NVC: nailfold video capillaroscopy; GCs: glucocorticosteroids.*

Table 2. Detailed overview of measurements of the "target lesion" and the corresponding control site.

Patient n°	jLoS location	mLoSSI (0-9)	LASCA			mLoSDI (0-12)	HFUS			Durometer		
			Centre	Edge	Control site		Centre	Edge	Control site	Centre	Edge	Control site
1	**Upper leg left**	2	144.03	96.64	75.75	6	0.10	0.12	0.14	37.5	20.5	13.2
2	**Abdomen right**	2	159.56	91.45	74.41	3	0.08	0.10	0.14	12.0	8.0	7.4
3	**Thorax left**	2	144.17	122.14	98.40	7	0.08	0.09	0.12	25.6	16.6	7.7
4	**Foot left**	2	34.83	24.42	22.16	3	0.08	0.10	0.15	33.4	29.9	19.1
5	**Face left**	3	261.73	184.99	142.68	5	0.09	0.13	0.15	16.6	8.2	4.8
6	**Upper leg left**	3	77.23	44.52	28.61	5	0.10	0.12	0.13	44.4	39.8	5.7
7	**Upper leg right**	4	49.85	42.11	30.43	5	0.08	0.09	0.12	44.0	36.1	36.2
8	**Upper leg left**	4	97.82	65.59	43.14	6	0.08	0.09	0.11	53.8	28.8	21.9
9	**Back right**	1	109.47	96.06	81.35	2	0.07	0.08	0.12	16.2	19.6	15.4
10	**Thorax left**	3	170.01	104.82	81.56	3	0.10	0.12	0.15	28.8	25.8	22.8

jLoS: juvenile localised scleroderma; LASCA: laser speckle contrast analysis; mLoSSI: modified localised scleroderma severity index; mLoSDI: modified localised scleroderma damage index

Table 3. Comparison of measurements of centre of "target lesions" versus edge of "target lesions".

Variable	Centre of "target lesions"	Edge of "target lesions"	*p*-value
HFUS, mean ± SD (mm)	0.086 ± 0.01	0.104 ± 0.02	**< 0.001**
Durometer, mean ± SD (DU)	31.23 ± 13.89	23.33 ± 10.77	**0.012**
LASCA, mean ± SD (PU)	124.87 ± 66.40	87.27 ± 46.60	**0.001**

Table 4. Comparison of measurements of jLoS "target lesions" versus control sites.

Variable	Centre of "target lesions"	Control site	*p*-value
HFUS, mean ± SD (mm)	0.086 ± 0.01	0.133 ± 0.01	**< 0.001**
Durometer, mean ± SD (DU)	31.23 ± 13.89	15.42 ± 9.88	**0.003**
LASCA, mean ± SD (PU)	124.87 ± 66.40	67.85 ± 37.49	**< 0.001**

Bold text: statistical significant finding ($p < 0.05$).
DU: durometer units; HFUS: high-frequency ultrasonography; LASCA: laser speckle contrast analysis; mm: millimetre; PU: perfusion units; SD: standard deviation.

DISCUSSION

This is the first case series in a jLoS population describing the use of non-invasive research tools to evaluate both microcirculatory and dermal properties, as commonly used in adult SSc. Our study provides evidence for future investigation of these tools in the detection and monitoring of (j)LoS lesions.

Our results demonstrate that both skin thickness (i.e. "atrophy") and skin hardness (i.e. "fibrosis") of a (j)LoS lesion can be quantitatively measured separately by HFUS and durometer, respectively. Another striking finding is that the centre of a (j)LoS lesion has a higher perfusion than the edge of the same affected skin lesion (scored by LASCA). This higher perfusion within (j)LoS plaques has also been demonstrated by other study groups that used thermography, although their control site was healthy skin instead of the edge of the "target lesions" [62].

Quantitative outcome measures are important for monitoring disease activity in (j)LoS, and the combined measurements of LoSCAT add up several different type of scoring items. To date, infrared thermography, MRI and ultrasonography have also been used to detect disease activity in (j)LoS but those tools may be limited in case of severe skin atrophy [28]. More biomarkers are needed for the treating physician in the chronic treatment of (j)LoS because it is still difficult to know when to increase, modify, taper or stop systemic treatment. Methotrexate, steroids and mycophenolate mofetil are known to be effective in systemic treatment of (j)LoS but not all patients respond to these drugs and they are frequently not tolerated due to adverse side effects in their chronic use. Recently, biologic disease-modifying anti-rheumatic drugs like tocilizumab and abatacept, based on different molecular mechanisms, have been described as promising new treatment regimens for (j)LoS [63, 64]. This current pilot study explores and suggests the potential of novel, and non-invasive, research tools that appear to be easily applicable by using them in systemic treatment decisions in the daily clinical practice of (j)LoS. The separate quantitative outcome measurements obtained by HFUS (for dermal thickness), by durometer (for skin hardness) and by LASCA (for microcirculatory dynamics) make these tools particularly interesting as potential new disease biomarkers.

A limitation of this study is the relatively low number of patients, which can be explained by the high rarity of jLoS. Another limitation is the cross-sectional design since this was a first pilot study. Of note, LASCA is not very accessible to most hospitals, although HFUS and durometer are easier to acquire tools, making these interesting modalities for future longitudinal studies.

CONCLUSION

This case series suggests the supportive value of both microcirculatory and dermal assessment using novel non-invasive research tools, adopted from adult SSc, also for the identification, scoring and monitoring of (j)LoS lesions. Longitudinal studies should elaborate further the value of those non-invasive tools in this, potential severely invalidating, chronic skin disease.

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8

Consider the wrist: a retrospective study on pediatric connective tissue disease with MRI

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ABSTRACT

OBJECTIVE: The aim of this study is to describe the clinical characteristics and MRI findings of the wrist in a cohort of children suffering from connective tissue disease with musculoskeletal involvement.

METHODS: Ten patients with pediatric connective tissue disease [median age 14.7 years (IQR 12.7-16.6 years), 70% female] were identified from a large MRI database. Clinical findings during the disease course were retrospectively obtained from patient charts and findings at the time of MRI were prospectively registered in the MRI database. MRI wrist datasets were evaluated by three readers in consensus for synovitis, tenosynovitis, bone marrow changes, bone erosions and myositis.

RESULTS: Patients suffered from connective tissue disease with clinical overlap of subtypes systemic lupus erythematosus, Sjögren syndrome and dermatomyositis. Median onset of disease was at 12.3 years (IQR 7.8-14.8 years). Clinical arthritis activity was scored low (median visual analogue scale physician 19, IQR 7-31). Notwithstanding, extensive inflammatory abnormalities such as synovitis and tenosynovitis were found in the wrist of 7/10 patients. Osteochondral involvement was detected in 3/10 patients.

CONCLUSION: In a small cohort of children with connective tissue disease and musculoskeletal symptoms, severe inflammatory abnormalities of the involved wrist were present in the MRI, while clinical disease scores suggested mild disease activity. Therefore, clinicians should consider the wrist as vulnerable for joint damage and can add MRI as a helpful tool in the management of patients with pediatric connective tissue disease and musculoskeletal involvement.

INTRODUCTION

Connective tissue disease with potential musculoskeletal involvement in children comprises several disorders, such as systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD), systemic sclerosis (SSc) and juvenile polymyositis/dermatomyositis (PM/DM). Besides the variety of musculoskeletal symptoms, the above-mentioned disorders can manifest in many other organ systems [1]. For SLE, the recent international recommendation on treatment is based on the treat-to-target principle: since damage predicts subsequent damage and death, prevention of damage accrual should be a major therapeutic goal [2]. In general this also applies to the other mentioned connective tissue diseases.

In daily practice, the physician of children with connective tissue disease acquires information on the disease activity to make treatment decisions. Besides clinical and laboratory findings, imaging can be valuable in initiating and evaluating therapy and in the awareness and prevention of complications. MRI is the modality of choice for the imaging of inflammatory musculoskeletal changes due to its multiplanar capabilities and its capacity for visualizing both soft-tissue (e.g. synovium, tendons, muscle) and osteochondral structures [3].

In juvenile idiopathic arthritis, the wrist is known for its frequency of involvement, vulnerability to erosive damage and association to poor prognosis, and therefore warrants detailed examination in children with joint complaints [4-6].

Although several large cohort studies on the musculoskeletal symptoms of children with connective tissue disease have been published [7-10], no information on pediatric MRI of the joints in these diseases is available. For adult SLE [11, 12], MCTD [13] and SSc [14] a small number of studies has been published on the MRI appearance of the wrist. Based on these studies, wrist involvement in children with connective tissue disease is expected to show abnormalities indicating inflammatory arthritis as well as the common extra-articular involvement of skin, muscles and tendons visualized by MRI as soft tissue edema, indicating sclerosis, myositis and/or tenosynovitis [11-14].

The aim of this study is to describe the clinical characteristics and MRI findings of the wrist in a cohort of children suffering from connective tissue disease with musculoskeletal involvement.

METHODS

Pediatric patients with connective tissue disease that underwent MRI of the wrist in a tertiary pediatric rheumatology center between 2008 and 2018 were retrospectively included in this case series. MRI scans were performed at an open-bore 1.0T MRI scanner (Philips Panorama HFO) or at a 3.0T MRI scanner (Philips Achieva). No sedation was used. The clinically most affected wrist was imaged before and after intravenous (IV) contrast (Gadovist, Bayer Schering Pharma, Berlin, Germany, 1.0 mmol gadolinium/mL, dose 0.1 mmol/kg). Before IV contrast, coronal/axial T1- and T2-weighted images were acquired. After IV contrast, coronal and fat suppressed axial sequences were obtained. Approval by the regional ethics board and informed consent by patients and/or parents was waived for this study by the Medical Ethical Committee of the Amsterdam University Medical Center, location AMC (reference number W13_166, date August 6th 2013) as all examinations were in the context of regular patient care and data were completely anonymized.

Clinical parameters (clinical history, presentation/symptoms, laboratory values) were retrospectively acquired from the patient charts. MRI datasets were evaluated in a consensus reading session by a musculoskeletal radiologist (>25 years of experience), a pediatric rheumatologist (8 years of experience) and a resident in pediatrics with 5 years of experience in pediatric musculoskeletal radiology. The MRI's of the wrist were evaluated for presence and extent of synovial inflammation and tenosynovitis by means of validated scoring systems [15, 16]. The synovial inflammation score was based on a comprehensive evaluation of inflammatory features such as degree and extent of synovial enhancement and the presence of effusion [15]. Synovial inflammation was assessed on six locations: distal-radioulnar joint, radiocarpal joint, intercarpal joint, first carpometacarpal joint, carpometacarpal joints 2-5 and the pisotriquetral recess. A 0-2 (none, mild or moderate/severe inflammation) scale was used for every location, leading to a maximum total score of 12. Tenosynovitis was also scored on a 0-2 scale, with 0 reflecting no enhancement nor thickening of the synovial sheath, 1 reflecting enhancement and mild thickening of the synovial sheath, and 2 reflecting enhancement and moderate to significant thickening of the synovial sheath [16]. Tenosynovitis was evaluated over the entire trajectory of the tendon and separately in each of the six extensor compartments whereas the flexor tendons were scored as one group. Maximum total tenosynovitis score was 14. Other expected ancillary findings such as bone marrow edema, bone erosions and myositis were also evaluated and documented.

Descriptive statistics such as median, interquartile range (IQR) and frequencies were used to describe patient characteristics and clinical findings. SPSS software version 24

(IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp) was used for the evaluation of all data.

RESULTS

Clinical findings

Ten patients (7 female) with connective tissue disease and MRI of the wrist were identified. This concerns approximately 13% of the total population of pediatric patients with mixed connective tissue disease in the given time period (n=80). Median age at onset was 12.3 years with an IQR of 7.8 to 14.8 years. Median time from disease onset to MRI was 1.4 years with an IQR of 0.4 to 6.6 years. At time of MRI, median age was 14.7 years (IQR 12.7-16.6). Clinical arthritis activity as assessed on a Likert scale (0-5) was mild (score 3) in 6 of the patients (67%); none of the patients were considered to have 'severe' clinical arthritis activity on this Likert scale. Furthermore, clinical arthritis activity as assessed on a visual analogue scale (0-100) showed a median of 19 (IQR 7-31). At time of MRI, 4 patients used no medication and the other 6 patients used different types of medication, consisting of non-steroidal anti-inflammatory drugs, methotrexate, prednisone, Plaquenil and Cellcept. Clinical symptoms and treatment strategies during the disease course are shown in Table 1.

Imaging findings

MRI of the wrist showed extensive inflammatory findings in 7/10 patients. Severe synovial inflammation was seen in 7/10 patients, with total scores ranging between 7 and 12. Severe tenosynovitis as reflected by a score of 10 and higher was found in 6/10 patients. In those patients with severe tenosynovitis, flexor tendons were involved in 5/6 patients. An example is given in Figure 1.

The three patients with minimal inflammatory findings had total synovial inflammation scores of 2- 4 and total tenosynovitis scores of 0-3 respectively (which is less than 33% of the maximum scores in both items). Myositis, reflected by high signal intensity on the axial T2-weighted sequence as well as on the fat-suppressed T1-weighted sequence after administration of IV contrast, was found in 3/10 patients (33%). When present, myositis was always found in the volar thumb muscles (Figure 2)).

Furthermore, 1 patient showed extensive infiltration of the subcutaneous fat, clinically correlating active sclerosis of the skin (Figure 3). Osteochondral abnormalities, such as bone marrow edema and bone erosions, were found in 3/10 patients (Figure 4).

Table 1. Characteristics of 10 pediatric patients with connective tissue disease

	Ethnicity	Auto-antibody profile	Other symptoms (besides arthritis)	Treatment ever used
1	African / Afro-Caribbean	ANA, anti-RNP, anti-centromere, anti-Scl-70, anti-SS-A, anti-ds-DNA	Myositis, Raynaud, sclerodactyly, digital ulcerations, interstitial pulmonary involvement, nephritis	Hydroxychloroquine, MTX, MMF, prednisolone, RTX, etanercept, allogenic SCT
2	African/ Afro-Caribbean	ANA, anti-RNP, anti-Sm, anti-Ro52, anti-ds-DNA, RF	Myositis, Raynaud, pleuritis, pericarditis, haemolytic anaemia, nephritis, aphtous ulcers	Hydroxychloroquine, MTX, MMF, RTX, prednisolone, cyclophosphamide, belimumab
3	Asian	ANA, anti-RNP, anti-Sm, anti-Ro52, anti-ds-DNA, RF	Lymphadenopathy, haemolytic anaemia, parotitis, nephritis	Hydroxychloroquine, MTX, prednisolone, RTX
4	Caucasian	ANA, anti-SS-A, anti-SS-B, anti-Ro52, RF	Sicca, parotitis	Hydroxychloroquine
5	Caucasian	ANA, anti-RNP, anti-ds-DNA	Autoimmune mediated encephalopathy, leukopenia, aphtous ulcers	Hydroxychloroquine, MTX, MMF, RTX, prednisolone, IVIG, plasmapheresis
6	African / Afro-Caribbean	ANA, anti-RNP, anti-Sm, anti-ds-DNA	Leukopenia, thrombocytopenia, haemolytic anaemia	Hydroxychloroquine, MTX
7	North African / Middle east	ANA, anti-centromere A / anti-PM-Scl100	Dermatomyositis	MTX, IVIG, prednisolone, RTX
8	Caucasian	ANA, anti-RNP	Raynaud	MTX
9	Caucasian	ANA, anti-RNP	Raynaud	Hydroxychloroquine, MTX
10	Caucasian	ANA	Cutaneous LE, leukopenia, aphtous ulcers	MTX, plaquenil

anti-ds-DNA= anti-double stranded DNA antibodies, anti-RNP= anti-RiboNuclear Protein, anti-Sm= anti-Smith antibodies, RF = IgM rheumatoid factor; MTX=methotrexate, MMF=mycophenolate mofetil (cellcept), RTX=rituximab, IVIG=intravenous immunoglobulins, SCT = stem cell transplantation

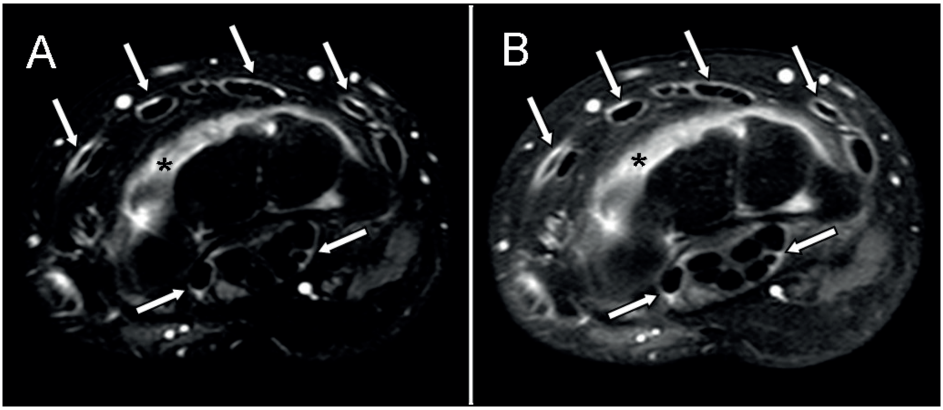

Figure 1. Multifocal inflammatory findings in the wrist from a 17 year old girl with mixed connective tissue disease.
The midcarpal region shows synovial inflammation (asterisk) and tenosynovitis (arrows) in the flexor and extensor tendons on axial T2-weighted images (A) and T1-weighted images with fat saturation after IV contrast (B).

Correlation clinical and imaging findings

In all patients, clinical arthritis activity scores were relatively low (i.e. VAS <50 (0-100) or Likert ≤4 (0-5)). On the other hand, in the majority of patients the total MRI scores for synovitis and tenosynovitis were clearly increased (i.e. total synovitis score >6 (0-12) and total tenosynovitis score >7 (0-14)). No correlation statistics were performed because of the small cohort.

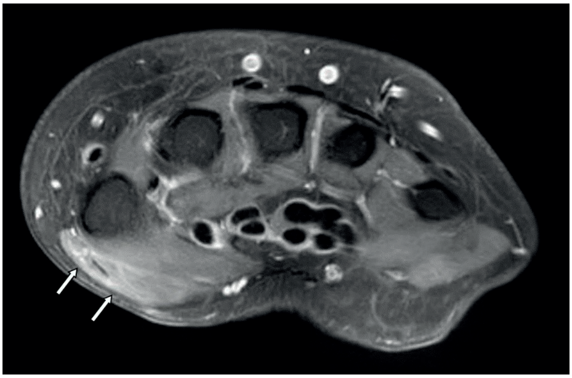

Figure 2. Osteochondral abnormalities in a 13 year old girl with mixed connective tissue disease.
Coronal T1- (A) and T2-weighted images (B) with numerous foci of low (A) and high (B) signal intensity within the bone marrow, in keeping with bone marrow edema and/or bone erosions indicating extensive osteochondral damage.

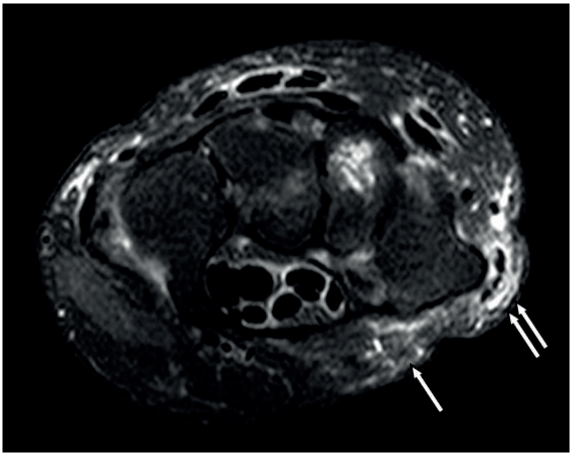

Figure 3. Soft tissue edema in mixed connective tissue disease in a 13 year old girl.
Axial T2-weighted image (A) visualizing infiltration of the subcutaneous fat (high signal intensity), possibly indicative of active sclerosis. Also bone marrow edema in the trapezoid and tenosynovitis (enhancement on T1 after contrast administration, B) in both the flexor- and extensor-compartments are present

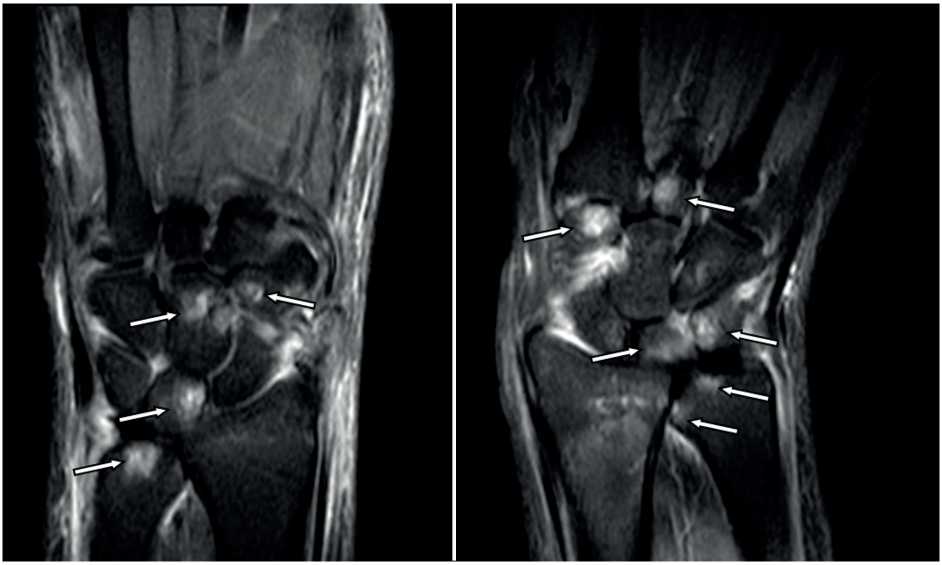

Figure 4. Myositis in an 8 year old boy with juvenile dermatomyositis.
Axial fat-saturated T1-weighted MR image of the wrist after administration of IV contrast, visualizing high signal intensity (enhancement) in the thenar muscles (arrows), indicative of local myositis.

DISCUSSION

To the best of our knowledge, this cohort of pediatric patients with connective tissue disease with musculoskeletal involvement is unique in describing the imaging findings of the wrist. Extensive inflammatory and osteochondral abnormalities on MRI of the wrist were observed in these patients and included involvement of flexor tendons, myositis and infiltration of subcutaneous fat indicative of active sclerosis. Interestingly, the perceived/scored clinical arthritis activity of the wrist by the patients and their clinicians (VAS) was strikingly low, compared to the extent of the abnormalities detected with MRI. Based on these findings, the wrist deserves thorough evaluation in daily practice as a possible vulnerable joint in children with connective tissue disease. The pattern of inflammation and damage on the MRI of the wrist as observed in patients with connective tissue disease is much more extensive compared to other arthritic diseases of childhood, such as juvenile idiopathic arthritis or reactive arthritis [4]. Although a direct comparison has not been performed, findings of pediatric connective tissue disease upon imaging of the wrist show higher synovial inflammation scores, the presence of extensive tenosynovitis and preference for the flexor tendons compared to JIA patients [4]. The findings including the extensive tenosynovitis and extracapsular inflammation have also been described upon MRI of the wrist in adults with MCTD [13]. Additionally, high signal intensity on T2-weighted images in muscle and subcutaneous tissue, suggestive for myositis and subcutaneous edema, have been described before in a recent study on pediatric mixed connective tissue disease [17].

The presence of bone erosions and bone marrow edema is commonly observed in adults with systemic autoimmune disease [11, 12]. However, Mosca et al. showed that bony depressions were equally frequent in the healthy control group and hence cast doubt on the clinical significance of these findings [11]. MRI studies in healthy children have shown that bony depressions in the wrist are very commonly present and may mimic erosive pathology [18, 19]. Nevertheless, erosions as found in our cohort can be considered 'pathologic', due to accompanying bone marrow edema and/or synovial inflammation.

The correlation between clinical and imaging findings could not be assessed due to the small cohort size. In general though, the clinical arthritis activity was scored low compared to the high MRI inflammation scores. Based on this finding, one could cautiously state that clinical arthritis activity underestimates the inflammation as assessed with MRI in pediatric connective tissue disease – a finding that needs confirmation in larger cohorts.

A limitation of the current study was the relatively low number of patients, resulting from the fact that connective tissue disease in children is a rare entity and MRI is not routinely performed in these affected children. Furthermore, the variety of different diagnoses within the connective disease decreased to the homogeneity of the cohort. On the other hand, homogeneity was achieved with the involvement and imaging of one specific joint, i.e. the wrist.

CONCLUSION

Children suffering from connective tissue disease with musculoskeletal symptoms show severe abnormalities on MRI of the involved wrist. Frequent findings include extensive (teno)synovitis, osteochondral damage, myositis and infiltration of the subcutaneous fat. Clinical arthritis activity was strikingly low compared to the extent of the MRI abnormalities. Considering the wrist as vulnerable for erosive disease, integration of MRI in the management of patients with pediatric connective tissue disease and musculoskeletal involvement deserves consideration to better interpret disease activity and subsequently damage.

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Part III

Discussion, summary and appendix

9

Discussion

NAILFOLD CAPILLAROSCOPY IN cSLE

One of the main objectives of this thesis was describing the abnormalities of the nailfold capillaries in cSLE patients and correlate these to patient- and disease characteristics. Nailfold capillaroscopy is an attractive imaging tool for daily clinical practice. It is non-invasive and gives direct image results during the nailfold investigation of the patient in (outpatient) clinic. Most devices for capillaroscopy are quite easy to use in daily care with self-teaching practice, but for interpretation of images some training is needed (1). These trainings are provided at (international) conferences in our field. Reliability of new definitions for abnormal capillary morphology have been tested in a pilot study of participants in a one-day EULAR capillaroscopy course. The reliability for abnormal capillary morphology was excellent, also in attendees with low experience in this field, proving capillaroscopy to be suitable for direct applicability in practice after a short training course (2).

In adults with SLE it had already been observed that tortuous capillaries, abnormal capillary morphology and capillary hemorrhages were significantly more prevalent in SLE patients as compared to healthy controls (3). After showing, in **chapter 2**, that the literature on capillaroscopy in cSLE was scarce, we have examined our own cohort of cSLE patients cross-sectionally. In **chapter 3** is shown that the nailfold capillaries in these patients have many abnormalities when compared to matched healthy controls. These capillary abnormalities were also seen in cSLE patients *without* Raynaud's phenomenon or acro-cyanotic complaints. In the above mentioned review on adults with SLE, capillary abnormalities were also seen with (2 studies) but also without (7 studies) a correlation with Raynaud's phenomenon (3). This is an interesting finding because capillary abnormalities and Raynaud's phenomenon in SSc are always closely linked. It raises the question if the pathophysiology of these capillary abnormalities is quite different in these two systemic autoimmune diseases. It is already known that both diseases have long-term vascular complications, although quite different in type: SLE patients suffer from cerebral stroke, myocardial ischemia or thrombosis while pulmonary hypertension is a well-known complication in SSc.

We have been the first to describe the nailfold capillary abnormalities in cSLE in so much detail. The specific capillary abnormalities that we observed in cSLE patients were giant capillaries (apical diameter > 50 micrometer), abnormal capillary morphology and capillary hemorrhages. Capillary hemorrhages were seen in up to 88% of cSLE patients and the number of capillary hemorrhages was significantly correlated with disease activity at diagnosis as well as with disease activity at the moment of capillaroscopy (by SLEDAI). The high percentage of cSLE patients that shows capillary hemorrhages is striking, as well as the high mean number of capillary hemorrhages per patient (at least one hemorrhage per mm, with a standard examination of eight mm in eight fingers per patient).

In SSc, the capillary hemorrhages are well known as part of the so-called ‘active stage’ capillary scleroderma pattern (4, 5). It has also been shown in SSc that the severity of this capillary scleroderma pattern (defined by early-, active- and late stage) is associated with the severity of organ involvement (6, 7). SSc is known to be a vascular disease with dysregulation of the endothelium (8). The endothelium in SLE is also dysregulated by intravascular pro-inflammatory cytokines due to auto-inflammation. This leads to oxidative stress, endothelial apoptosis and coagulopathy (9). As in SSc, the images from (video)capillaroscopy are visualizing (micro)vasculopathy in SLE. In **chapter 4**, some of the abnormal capillary findings in cSLE were confirmed in a multicenter cohort, although the number of investigated cSLE patients of this cohort was lower than in **chapter 3** and there was partial overlap of participating patients from **chapter 3 and 4**. In the international cohort of cSLE in **chapter 4**, it was confirmed that a large majority of 80% of cSLE patients showed capillary hemorrhages. cSLE patients also showed a significant lower capillary density when compared to healthy controls, although skin color was not taken into account. It has been shown that skin color can be of influence in capillary density (10, 11).

Chapter 3 and 4 show unique nailfold capillaroscopy data in cSLE because they describe, for the first time, observations of the abnormal capillary morphology by the new definition of EULAR study group on Microcirculation in Rheumatic Diseases from 2018 (2). In **chapter 3** we also described the *combination* of abnormal capillary morphology (shapes) and capillary hemorrhages, which were often seen in the same capillaries in our cSLE patient cohort. The significance of this finding was indicated by the percentage of observed patients (n=32/41, 78%) and number of fingers (78% in ≥3 fingers) affected, in which these abnormalities were combinable observed. The qualitative description of this finding was called ‘microangiopathy’ by us in **chapter 3**. In **chapter 4**, 75% of cSLE patients also showed the comparable ‘non-specific pattern’ versus 35% in healthy age-matched controls. In this international study, cSLE patients were the only disease subgroup with this finding of a ‘non-specific’ pattern. More recent studies show very similar results of SLE patients (children and adults) with abnormal ‘non-specific’ capillary patterns (12-14).

Another striking finding in **chapter 3** was the description of a new subtype of capillary hemorrhage, which we called ‘pericapillary extravasation’. To explore the reproducibility of this observation, inter- and intra-observer agreement had to be determined. As a first step, we have already shown that there is a good agreement to detect different subtypes of capillary hemorrhages (15). At this moment, the meaning of this new 'pericapillary extravasation' is not yet exactly clear. We hypothesize that it might be capillary leakage that can be visually detected by the good quality imaging from videocapillaroscopy with 200x magnification. SLE is a vascular disease with a high degree of vascular inflammation and endothelial damage. This endothelial damage may be reflected by these different subtypes of nailfold capillary hemorrhages that we observe in (c)SLE.

As mentioned in the introduction of this thesis, SLE patients are known to be at risk for cardiovascular disease which can lead to a much lower life expectancy. In the past, the cardiovascular health of SLE patients has been studied by measuring carotid intima-media thickness (CIMT) and presence of carotid plaques in SLE patients, which were significantly different compared to controls (16). A recent study shows that more sensitive imaging measurements such as 'total plaque area' and increased 'echogenicity of the plaque' is significantly higher in patients taking corticosteroids (CS) (17). It is known that risk factors with significant influence on CIMT are traditional cardiovascular risk factors (age, HDL and triglyceride) and lupus related risk factors (disease duration, ESR, SLEDAI and use of CS) (16, 17). Endothelial dysregulation seems to play an important factor in the risk for this premature atherosclerosis (9). In a systematic review and meta-analysis we found that several dysregulated EC markers seem associated with disease activity scores but, more interestingly, other EC markers show dysregulated levels without a clear association to disease activity scores (*Bergkamp et al., under review*). This finding suggests dysregulation of the endothelium during low disease activity states or maybe even in disease remission. The identified EC markers that we have found in this systematic literature review are involved in many endothelial functions: EC activation, EC apoptosis, disturbed angiogenesis, defective vascular tone control, immune dysregulation and coagulopathy. Hypothetically this could mean that, even in a low disease activity state, the endothelium of SLE patients might still be at risk for premature atherosclerosis and EC markers might reflect this pre-atherosclerotic state before CIMT measurements can detect signs of a plaque.

Future needs and perspectives in capillaroscopy and endothelial cell markers

One of the steps to be studied in this field is the sensitivity of different devices for nailfold capillaroscopy and the number of fingers that needs to be investigated for optimal results. Sensitivity should especially be investigated in various devices to see if they also detect (smaller types of) capillary hemorrhages. It is important to know if these hemorrhages are also detectable with a handheld dermatoscope, or if they can be missed with lower magnifications than those in the videocapillaroscopy (with 200-300x magnification). It would also be informative if less, and if so which, fingers can be investigated with the same sensitivity and quality of observations. If less fingers can be examined with good sensitivity of abnormal capillaries, this would be less time-consuming. For instance, capillary examination performed by dermatoscope in only 1-2 fingers takes only a few minutes. Hypothetically, digits 3 and 4 of the non-dominant hand, with less risk for traumatic abnormalities, might possibly be enough to detect capillary changes in systemic autoimmune diseases.

In chapter 4, a lower capillary density was found in cSLE patients. In future studies a correlation of lower capillary density in darker skin should be further examined and confirmed because this observation has been described in the past (10). This is important to know because people with different ethnic backgrounds have different incidence rates for SLE, as was discussed in the introduction of this thesis. In black patients a lower density might not be a pathological finding and this might be of importance in the interpretation of images from capillaroscopy in patients with different ethnic backgrounds.

The repeating observations of microangiopathy and non-specific pattern in cSLE lead to the hypothesis that a capillary ‘lupus pattern’ might exist as an entity. More (multicenter) studies with a detailed quantitative description of this abnormal capillary pattern in SLE (children and adults) should investigate this hypothesis further, according to international consensus definitions from the EULAR Study Group on Microcirculation in Rheumatic Diseases. Future longitudinal studies also have to show if the number of capillary hemorrhages/mm reflect treatment efficacy and microvascular health on the long-term. By combining EC marker analyses with capillaroscopy data it can also be investigated if the nailfold capillaries are a mirror of what happens to the protective internal lining of the blood vessel itself.

The reviewed studies in our systematic literature review on EC markers are mainly cross-sectional studies in adult SLE populations. To further unravel the pathophysiology of (cardio)vascular complications in SLE we are in progress to study most of these EC markers in our longitudinal cohort, with samples from pre-treatment and follow-up over time, during high and low disease activity and/or remission. Because the pediatric SLE population, in contrast to adults, does not have many other confounding risk factors for atherosclerosis, our study in cSLE patients will show results that are mostly determined by the sole effect of vascular inflammation in SLE, especially because pretreatment samples will be studied. Next steps would be to compare these results with measurements from blood samples from adult patients with a childhood-onset and a long disease duration, again preferably obtained during high and low disease activity states. In the future we have to focus on prevention of (subclinical) atherosclerosis in the subgroup of SLE patients at risk. The risk factors involved have to be defined in further detail for integration into standard clinical screening protocols regarding early detecting of premature atherosclerosis in SLE.

SLE-RELATED DAMAGE: (NEW) PREDICTIVE RISK FACTORS

In **chapter 5** was shown that a capillary scleroderma pattern has prognostic meaning in cSLE patients, as it predicts a higher risk for disease damage. cSLE patients with a capillary scleroderma pattern were at significant more risk to develop SLE-specific organ damage, which is irreversible. This finding was epochal because it was always thought that SLE patients with a capillary scleroderma pattern were patients at risk to develop overlapping symptoms with SSc. Nevertheless, in our cohort, these cSLE patients did not show any symptoms of overlap disease and the type of damage was purely lupus-related. Although the range of follow-up in these patients was up to 16 years after disease onset, our cohort consisted of relatively small patient numbers. More long-term follow-up studies with larger patient numbers should evaluate the risk for change into overlap disease of these (c)SLE patients with a capillary scleroderma pattern. If those patients are *not* at risk for overlap disease, this would mean that a capillary scleroderma pattern might not be a specific finding for SSc only.

The higher risk for (early) damage in cSLE versus aSLE is mostly reflected in ocular (cataract, retinal change or optic atrophy) and musculoskeletal damage (muscle atrophy, erosive arthritis, osteoporosis with fracture, avascular necrosis, osteomyelitis or ruptured tendon) (18). These data show that damage does not only result from inflammation but also from high and/or chronic (cumulative) CS treatment in SLE. Looking at the type of disease damage (by validated SDI scoring) in our cohort in **chapter 5**, this damage was partly *corticosteroid*-related (avascular skeletal necrosis and growth failure, n=3/53 patients) and partly *lupus*-related damage (skin scarring, end-stage renal disease, cerebrovascular accident, epilepsy, loss of digits, cardiac valve disease and cognitive impairment/major psychosis, n=10/53 patients). Two patients (n=2/10) showed both types of damage, lupus- and CS-related. Disease damage occurred already within five years after diagnosis and such early onset of damage has been shown in other studies as well (19, 20). One of these long-term studies showed that more than half of cSLE patients have damage after 10 years, with more increasing numbers in the years thereafter (19). Especially the presence of nephritis is a risk factor for mortality in young patients (21). This latter cohort consisted of 93% white patients. This might give an underestimation of damage because in general, black (female) patients have a higher incidence of SLE and are also at risk for more severe disease activity and -damage, as described in the introduction. Looking at very recent (unpublished) data from an international multicenter project (n=1096 cSLE patients) from the Pediatric Rheumatology European Society (PReS) Lupus working party, one-third of cSLE patients already have damage after 6 years of disease duration. Mean age at diagnosis in this study was 12 years, meaning that many of these patients already suffer from damage in the adolescent phase of their life. More importantly, damage was more likely with an early disease onset (< 10 years of age) but not correlated with SLEDAI at diagnosis or SLEDAI at last visit (*PReS Lupus Working party unpublished data*). We showed this same finding in our data from **chapter 4**. So although we currently are doing better in terms of lowering mortality rates in SLE, this means that we should still focus more on early detection of the diagnosis (in order to prevent lupus-specific damage) and on better/earlier use of CS- sparing regimes (to prevent CS-related damage).

In the new treat-to-target (T2T) approach the lowest effective dose of CS is now state-of-the-art and explicitly recommended in the treatment of (c)SLE (22)). T2T strategy has recently been proposed for adults with SLE but is now also of interest for treatment of cSLE (23, 24). This strategy defines specific targets that should be reached and aimed for while treating patients with SLE. One of the suggested treatment targets in SLE is the Lupus Low Disease Activity State (LLDAS). LLDAS is defined as low disease activity score (SLEDAI ≤4) with zero scores for renal, central nervous system, serositis, vasculitis and constitutional components, no increase in any SLEDAI component since the previous visit, physician global assessment ≤1, and prednisone dose ≤7.5 mg/day. LLDAS is a target which has been shown to significantly lower the risk for disease damage in adults with SLE (25-27). Recent studies show that LLDAS is also an achievable target in cSLE, even in the first year after diagnosis (28-30). This is a very important finding because disease damage in cSLE often occurs in the first years after diagnosis, and is often CS-related (20, 31). Another treatment target in T2T is disease remission for which different definitions are still being fine-tuned, for example remission "on" or "off treatment" (32). At this moment, an international cSLE T2T Lupus working party is establishing consensus definitions for different remission states, specifically for the cSLE patients.

Future needs and perspectives in SLE-related damage: prevention

Future studies have to show if T2T, by new treatment approaches, results in lower damage scores in cSLE on the long-term. The focus needs to be on lowering CS, but at the same time CS-sparing regimens need to be started early, preferably *as early as possible* after diagnosis. This means a high focus on the compliance of other (daily) oral medication (hydroxychloroquine, mycophenolate mofetil (MMF) and azathioprine (AZA)). Early (or earlier) starting of biologicals such as rituximab and belimumab (if available) should be considered when compliance of daily pills is a problem for the individual patient.

By defining subgroups with low and higher risk for damage in (c)SLE patients we can intervene early with different treatment protocols for different subgroups. A suggestion for stratifying SLE patients, by risk for cumulative CS use, is described in the next section on transcriptomics and personalized treatment.

The patients with high risk for long-term use of corticosteroids might profit from an early start with specific B cell depleting therapy early after diagnosis. A good example from current daily clinical practice is the difficult choice which patients should be treated with rituximab (RTX) and which patients should be treated with belimumab as first-choice of anti-B cell therapy. Both biologicals have shown efficacy in lowering disease activity and are therefore CS-sparing. Some RTX trials showed moderate efficacy, resulting in the opinion that RTX might not be the first choice in lupus nephritis, although this interpretation might not be correct (33). New insights in development of anti-drug-antibodies (ADA) against RTX might explain why some SLE patients fail in B cell depletion after RTX and this might also be the reason for different efficacy rates between SLE patients (34). It is important to find risk factors in patients that are prone to develop RTX-ADA in order to make the best treatment choices, especially because new treatment strategies also involve a combination of those two treatments with induction of RTX and maintenance therapy with belimumab. If RTX-ADA or anaphylactic reactions during infusion do not occur, treatment with RTX is an elegant therapy with 2 infusions every six months.

For SLE, CS are almost always needed for some time after diagnosis but it is important to pro-actively decrease the cumulative CS dose. For this reason, cumulative CS dose should be studied more often as co-variable or as outcome in longitudinal cohort studies in (c)SLE patients. Besides that, targeting anti-B cell therapy should be investigated as “top-down” strategy versus the currently used and slower “step-up” strategies, especially in high risk patients that are at risk for a high amount of cumulative CS use, as suggested in figure 1.

TRANSCRIPTOMICS IN cSLE: THE ROAD TO PERSONALIZED TREATMENT?

The heterogeneity of SLE patients makes it difficult to design standard treatment protocols for SLE. In the past decade, SHARE treatment recommendations were published to give guidance to (specialized) physicians in treatment choices for cSLE patients (35, 36). In clinical practice, as measured by SLEDAI and SDI, black SLE patients have more severe disease than patients with other ethnic backgrounds (37). This higher severity of disease was reflected by analysis of a large cohort of cSLE patients by increased plasmablast sig-

AT DIAGNOSIS

➔ **HIGH RISK GROUP** for long-term use of CS:
- MPNS + *direct* start of targeting anti-B-cell therapy (RTX/BEL) +/- MMF OR CYC

➔ **INTERMEDIATE RISK GROUP** for long-term use of CS:
- MPNS / oral CS + AZA/MMF depending on type of organ involvement

➔ **LOW- RISK GROUP** for long-term use of CS:
- (oral lower dose CS bridging) + AZA/MMF (depending on type of organ involvement)

All patients should receive hydroxychloroquine

EVALUATION AFTER 3-6 MONTHS:

➔ **HIGH RISK GROUP**:
- If effective: stay on anti-B cell therapy with/without MMF for minimum of 2 years and target to stop/taper to lowest dose of CS
- still active CS-dependent disease: switch to RTX/BEL or CYC
- caveat RTX-ADA if no B cell depletion, anaphylaxis or active disease

➔ **INTERMEDIATE RISK GROUP:**
- If effective: stay on AZA/MMF for minimum of 2 years and target to stop/taper to lowest dose of CS
- active disease and/or incompliance: reconsider anti-B cell therapy if CS-dependent

➔ **LOW- RISK GROUP**:
- if off CS, monitor disease activity and compliance for AZA/MMF
- if still CS dependent or incompliance: go to intermediate/high risk group

Figure 1. Example of personalized treatment regimens for SLE subgroups in the first year after diagnosis, stratified by risk for cumulative CS-usage
CS= corticosteroid(s), MPNS= methylprednisolon pulse, RTX= rituximab, BEL=belimumab, AZA= azathioprine, MMF= mycophenolate mofetil, ADA= anti-drug antibodies

natures in African-American patients, which correlated with higher SLEDAI (38). Those plasmablasts will form plasma cells and are the origin of the development of pathological auto-antibodies leading to systemic auto-inflammation. The ethnic background also influences IFN signatures (39). Recently, it was observed that SLE-patients can show different interferon signatures, differentiated in 'IFN high' and 'IFN low' subgroups but also in different modules by combining interferon type I and II expression profiles (38, 40-42). The results from **chapter 6** will help to stratify patients to make more personalized treatment choices. In our study, it was shown that by *combining* interferon signatures with neutrophil (NPh) and plasma cell (PLC) signatures, three different SLE-fingerprints can be seen in cSLE. These fingerprints showed that different types of IFN gene signatures were associated with different types of organ involvement and type of auto-antibodies (high titer of anti-ds-DNA and presence of anti-SSA, but not for presence of anti-Sm or -RNP). Our so-called 'fingerprint 3' with the highest disease activity and high NPh and/or PLC gene signatures consisted of patients who were more frequent on treatment with

CS. This could mean that this group is at the highest risk for disease damage. We need longitudinal studies to study these gene signatures in more detail over time, in search for associations with specific type of treatment and risk for damage, as also suggested by Northcott (41).

Current treatment choices are made depending on the severity of symptoms or clinical phenotype of the patient and they are re-evaluated on a regular basis, usually every 3-6 months,. Unfortunately many treatments (such as MMF, RTX or cyclophosphamide) often take up to 4-8 weeks to show some treatment effect. If after this period treatment efficacy is not enough, changes in treatment regimen have to be considered and this means delay *and* also more cumulative exposure to CS. Consequently, this leads to higher risk for CS-toxicity and higher risk for damage (due to longer period of higher disease activity *and* to longer CS use). There is need for more guidance leading to precision medicine to identify *which* patients need *which* therapy. This is not only necessary for patients with severe disease but also for patients with lower risk for high disease activity/–damage or flares. This latter group might not need (high dosage) CS, or only for a short period of time. There is need for more (predictive) disease biomarkers to identify patients that can taper faster to lower the risk for steroid-related damage.

Interferon signatures and 'fingerprints' as described in **chapter 6** will be helpful in stratifying patients into subgroups: patients with milder phenotype and patients with (potential) severe phenotypes that might need to start early with the currently available specific B-cell therapy or other novel therapy options (also see figure 1). However, it is necessary to measure IFN signatures directly after diagnosis in treatment-naïve patients because over time they do not always seem to correlate with disease activity. Northcott et al. showed that (initial) IFN high patients were more likely to be of East Asian ethnicity, younger at disease onset and more frequently showed anti-RNP, anti-Ro and anti-La auto-antibodies. IFN high patients also had significantly more active disease, lower attainment of LLDAS and higher rates of flare. In this study was concluded that IFN status, at diagnosis, had prognostic significance in the management of SLE (41). As was also shown in **chapter 6**, pre-treatment analysis for fingerprints in transcriptomics indeed seems to be helpful in identifying patients with higher disease activity but also seems to identify low-risk patients that might be able to taper steroids faster. By using this T2T strategy combined with transcriptomics, the risk for steroid-related damage may be reduced. The focus on lowering steroids as soon as possible is a reachable goal in developed countries like the Netherlands. A similar example is the shift in treatment of systemic juvenile idiopathic arthritis (sJIA) for which we start with anakinra (anti-IL-1) treatment up front, directly after diagnosis. As a consequence we see in our country that most of these sJIA patients do not have to use any CS at all during their disease course.

It is difficult to put this CS sparing strategy into financial numbers but in this example by starting with a more expensive medicine, it leads to faster remission rates and shorter treatment periods. This not only lowers the burden of chronic CS use but might also be cheaper in the long-term. Such insights are important to study further and can be implemented to other rheumatic diseases with high CS use, such as SLE.

Future needs and perspectives in transcriptomics

It will be informative to combine all of the hopeful (and novel) SLE disease biomarkers, that are described in this thesis. We are planning to analyse if (the number of) nailfold capillary hemorrhages, the presence of a capillary scleroderma pattern and/or dysregulated EC markers are correlated to the different IFN signatures. We need to know if specific transcriptomic 'fingerprints' or other biomarkers can predict the risk for damage better than SLEDAI, preferably at the moment of diagnosis. Correlating IFN-signatures with specific patterns in B cell subsets, especially in pre-treatment samples, is another interesting option to profile these (c)SLE patients. This could also lead to insights in further stratification to different SLE phenotypes with different risk profiles, and concomitant treatment strategies. Once optimal profiling phenotypes in cSLE are established, choosing personalized treatment regimens for different (c)SLE phenotypes is the next step forward. In the future this can also be implemented in drug trials. These trials are often set up by dividing patients with/without nephritis but it might also be interesting to look at responses between patients with different types of gene signature profiles.

OVERLAP DISEASE IN SYSTEMIC AUTOIMMUNITY

It is important to know in MCTD patients with systemic disease and overlapping symptoms which patients will develop to SLE- or SSc-like disease, especially in children that will probably suffer from this systemic autoimmunity for decades to come. Musculoskeletal involvement seems more prevalent in jMCTD than in adults with MCTD (43). Some studies describe arthritis in MCTD as evolvement into rheumatoid arthritis (RA) because of the combination of arthritis with a positive rheumatoid factor (RF) or anti-CCP antibodies. These specific, arthritis-linked, autoantibodies are probably not reflecting a different disease, such as RA, but may reflect disease severity and risk for bone erosions. The MRI abnormalities in **chapter 8** confirmed this severity of inflammatory and osteochondral abnormalities in a small cohort of CTD patients (3/10 RF-positive). Only some of these patients were jMCTD patients, others fulfilled the criteria for SLE and Sjögren's disease. Most important finding was that the clinical arthritis activity scores with visual analogue scale (VAS), by experienced clinicians were relatively low. Because of this discrepancy, caution should be given to a possible underestimation of the severity of musculoskeletal involvement of children with CTD, but follow-up studies in these patients are needed. Instead of an indication for RA, the relevance of RF in these MCTD patients may be related to more severe musculoskeletal disease.

The presence of a capillary scleroderma pattern does not seem to be specific for patients with SSc. This pattern has been observed in different CTD's including SLE and (J)DM (44). A capillary scleroderma pattern in (J)DM has also been described in many studies (45). A 'scleroderma-like DM-pattern' was even proposed by some, with the observation of specific giant-ramified capillaries, almost exclusively seen in the DM patients (46). In our capillaroscopy studies in **chapter 4 and 5 it** is shown that the majority of MCTD/UCTD, but also JDM patients, show a capillary scleroderma pattern. In those chapters it was also shown that a scleroderma pattern can be seen in up to 15% of cSLE patients, without any overlapping symptoms with SSc. Further investigation of the specific details of the capillary abnormalities in SLE- and (J)DM-patients with a scleroderma pattern will have to elucidate if this is (or is not) different from the capillary scleroderma pattern in (j)SSc. In Sjögren's disease, capillary abnormalities are not that common or only mild (47). In SLE patients the capillaries show more pathological findings as has been described in **chapter 2-5**. Schematically a spectrum of mild to severe vasculopathy, as seen by nailfold capillaroscopy, can be visualized as in figure 2. For jMCTD/UCTD, with overlapping clinical symptoms, the capillary pattern seems to be more severe with a high percentage of scleroderma patterns as was also shown in our patient cohorts from **chapter 4 and 5** (69 and 61%, respectively), although the cohort numbers were small (n=13 and n=18, respectively).

If the type of capillary pattern does not stratify MCTD patients, EC markers or IFN signatures may indicate whether an MCTD patient is likely to have (or will evolve into) SSc-like or more SLE-like disease. In SSc, endothelial dysregulation is prominent in many endothelial functions (8). In a recent study on EC markers in jMCTD, dysregulated levels of sICAM, IL-6 and vWF were observed in patients after a median disease duration of 17 years, compared to controls. By regression analysis with adjustment for cardiovascular risk factors, only sICAM-1 remained significant as sign for endothelial dysregulation from underlying auto-inflammation (48). In JDM, endothelial markers, such as endoglin and ICAM-1 seemed suitable for measurement of severe vasculopathy for identifying patients with capillary low end-row loop (ERL) score (<4 capillaries/mm). Nevertheless, combined with other biomarkers such as galectin-9 and CXCL-10 and clinical data, ICAM-1 and VEGF did not aid in identification of subgroups for disease severity (49). However, it is questionable if the variable ERL is enough to reflect the severity of vasculopathy in

JDM. The pathological capillaries in JDM also include many giants and hemorrhages in the active phase of this disease and a lower density can also be seen during lower disease activity states, without giants and hemorrhages. As mentioned earlier, it is important to take the ethnic background in the interpretation of capillary density into account. The complexity of endothelial dysregulation in these different CTDs is large and future studies have to show if EC markers and/or nailfold capillary changes will be helpful in differentiating MCTD from other CTD's.

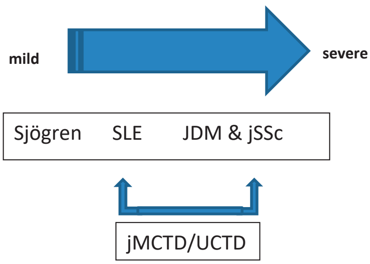

Figure 2. Spectrum of nailfold capillary abnormalities in different systemic autoimmune diseases
SLE=systemic lupus erythematosus, JDM=juvenile dermatomyositis, jSSc=juvenile systemic sclerosis, jMCTD=juvenile mixed connective tissue disease, UCTD=undifferentiated connective tissue disease

Future needs and perspectives in overlap disease of systemic autoimmunity

Some of the MCTD/UCTD patients might have this disease as a separate entity but they often show overlap symptoms and in time evolve into SSc or SLE. It is not yet known how to predict the clinical course of these patients. During follow-up of MCTD/UCTD patients, clinicians need to know which patients have to be screened for SSc-like symptoms (with pulmonary function tests and CT scans of the thorax) and which for more SLE-like symptoms (such as regular urine analysis). These organs (lungs and kidney) can be inflamed without the patient experiencing symptoms, especially in the early stages of organ involvement. We need large longitudinal databases, with a multicenter set-up due to the rarity of these diseases, in the pediatric *and* adult population. Longitudinal follow-up of clinical variables is essential but we also need to know what happens to the capillary abnormalities. Further detailed specification of the capillary pathological patterns is necessary, also in a longitudinal setting. Again, the key in understanding the pathophysiology of these overlapping diseases might be to combine clinical variables with EC markers, transcriptomics and capillaroscopy data to define better subgroups with prognostic meaning.

LOCALIZED SCLERODERMA

In **chapter 7** pediatric patients with an auto-inflammatory skin disease, defined as LS, have been described. LS is a very rare disease and is often not recognized, or diagnosed with a delay of many years after onset. Ongoing disease activity is difficult to objectify because there are different types of smoldering symptoms that reflect disease activity and can lead to invalidating damage. The surface area of the affected skin often shows slowly increasing hyper- or hypopigmentation, skin atrophy, slight redness of edges and progressive skin hardness. LS can slowly smolder for years, with ongoing disease activity leading to (preventable) skin damage but LS can also diminish spontaneously and stay inactive. It is important to realize that after many years of disease remission, LS can flare up again. Another problem besides recognition and progression of the disease is the fact that many physicians are not aware of the systemic treatment opportunities for LS. Many dermatologists have little experience with these systemic immunosuppressant's in children. Because of its low prevalence, we believe that all pediatric patients with (severe) LS should be treated in academic hospitals in multidisciplinary expertise teams with pediatric rheumatologists and dermatologists for the best quality of care.

The Localized Scleroderma Assessment Tool (LoSCAT) has been validated for assessment and follow-up by measuring disease activity (modified Localized Skin Severity Index (mLoSSI)) and –damage (modified Localized Skin Damage Index (mLoSDSI)), but it has some limitations as described in the introduction of this thesis with variability in inter-observer scoring (50). General aspects of the skin in children are different from adults allowing for adaptations of the pediatric skin thickness scoring in LS and jSSc (51). Lastly, only the most severe part of the lesion is being scored in LoSCAT which is most often the center of the lesion. Although surface area is also a scoring item, there is still a risk when using LOSCAT that slowly deteriorating skin (smoldering disease) is not well detected or documented.

In **chapter 7** we have shown that the skin durometer, HFUS and LASCA are promising non-invasive imaging techniques that can measure skin abnormalities in LS. Of these, the durometer is likely to be the most preferred in terms of costs and handiness. The durometer was able to produce measurements of skin hardness, not only with significant differences between affected and unaffected skin, but also between edge and center of the affected lesion. If this technique is used in follow-up of patients it is very important that the measurements will be performed at the exact same skin sites as before. This should be documented carefully in patient charts with photographic images. The durometer as imaging technique in LS (and/or in (j)SSc) needs to be further validated but it might be a disease activity biomarker with higher sensitivity and inter-observer rates than LoSCAT. High-frequency ultrasonography (HFUS) and laser speckle contrast analysis (LASCA) also showed potential to be used as imaging techniques in LS, but they have more practical limitations because of training, expertise and costs.

Some physicians that treat patients with LS are not always fully aware of the available systemic treatments for this disease, other than topical anti-inflammatory crèmes which are often not effective enough. Methotrexate (MTX) can be used as a systemic therapy, with or without prednisolone bridging. These two medicines frequently show side effects and MTX is often not well tolerated for long-term treatment, which is necessary in LS. Another systemic treatment option is MMF, which is mostly well-tolerated by patients. Nevertheless, for MMF compliance is often a problem.

Future needs and perspectives in treatment of localized scleroderma

In conclusion, there is an urgent need for new systemic treatment options in LS. Biologicals, such as tocilizumab and abatacept, have been described to be promising in treatment of LS but are still not officially approved. This gives an extra important reason for more longitudinal and multicenter studies in LS because these (new) treatments have only been described in case reports or case series, mainly because of the rarity of this disease (52-56). In future studies, new imaging techniques as described in **chapter 7** might be helpful in better monitoring (new) treatment effects, and especially the durometer seems an imaging technique that can be used in the context of multicenter trials.

CONCLUSIONS

This thesis aimed to provide direction on novel disease biomarkers from, mainly non-invasive, imaging tools in different (systemic) autoimmune diseases in children, with a specific emphasis on cSLE:

- By detection of nailfold capillaroscopy, capillary hemorrhages (in presence, type and per mm) are correlated with disease activity in (c)SLE.
- We hypothesize that a specific capillary 'lupus pattern', consisting of abnormal capillary morphology combined with hemorrhages, seems to exist and this observation needs to be studied further.
- A capillary scleroderma pattern is present in up to 15% of SLE patients and is associated with lupus-related damage and *not* with SSc-like overlap disease.
- Profiling of cSLE patients with 'fingerprints' from IFN-signatures and NPh/PLC modelling is a promising measurement for stratification at diagnosis (in a pre-treatment stage) for the implementation of personalized and CS-sparing treatment strategies.

- The severity of musculoskeletal inflammation in pediatric CTD, as visualized by MRI, seems to be underestimated by clinical arthritis activity as assessed on a Likert scale (0-5).
- The durometer can give quantitative measurements of inflamed skin in LS: it should be validated to measure disease activity in this rare localized connective tissue disease.

As mentioned in the introduction, diagnosis and survival rates for (c)SLE have been greatly improved in the last decades. Nevertheless, patients with SLE still suffer daily from this chronic lifelong disease with high risk for organ damage. A high number of daily pills, fatigue, lower quality of life and limited social participation are examples of the daily burden for a SLE patient which is a very difficult perspective for the future of a teenager that receives this diagnosis at a young age. Still, even though many insights and improvements were made in last decades, more disease biomarkers are needed to predict, and subsequently to prevent, disease damage and improve quality of life of these patients. Translational- and international collaborative research is the indispensable key to success. Most importantly, those (novel) disease biomarkers should be easy to implement in daily clinical care of pediatric patients with (rare) rheumatic diseases. In the end, those children with a pediatric onset of rheumatic disease are growing up to be adults. We need to further improve and adapt clinical care of these patients constantly, because these (pediatric) patients have to live their whole life with the effects of a chronic inflammatory disease.

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Summary

In the introduction of this thesis (chapter 1), background information is given about different autoimmune and so-called ‘connective tissue diseases’ (CTD) that are discussed in this thesis, such as Systemic Lupus Erythematosus (SLE), Mixed Connective Tissue Disease (MCTD) and Localized Scleroderma (LS). Although much progress has been made in the early diagnosis and treatment of these diseases in the last decades, still more biomarkers are needed that stratify patients by different (prognostic) disease severity grades. This stratification will lead to improve personalized treatment choices which will lower the risk for irreversible damage and improve their quality of life while living with a chronic disease.

PART I. NAILFOLD CAPILLAROSCOPY IN CSLE

This thesis consists of two parts. The first part is about nailfold capillaroscopy. The smallest blood vessels of the body (capillaries) can be visualized with this imaging device. Capillaroscopy is feasible and painless. Observations from capillaroscopy studies in children with CTD are being described with an emphasis on the severe autoimmune disease SLE. SLE with a childhood-onset is called cSLE.

In **chapter 2** a systematic literature review was provided on nailfold capillary abnormalities in cSLE. At the moment of literature search, in 2019, six articles were retained, of which two case-control studies and four case series. For capillary density, no difference was found between cSLE and healthy controls (one study). Differences in capillary diameter, capillary morphology, hemorrhages and semi-quantitative score were inconclusive or non-interpretable. A scleroderma pattern was reported in a minority of cSLE patients in three out of four case series. We concluded that literature on nailfold capillary findings in cSLE is scarce and inconclusive. To evaluate capillary characteristics in cSLE, prospective and longitudinal studies are needed with uniform definitions for capillary characteristics.

In **chapter 3** we described the capillary abnormalities from a cross-sectional study in cSLE (n=41), compared to capillary observations from matched healthy controls (n=41). The secondary objective was to correlate the observed capillary abnormalities with demographical variables and with disease-specific variables in cSLE patients. cSLE-patients showed significantly more ‘giants’ ($p=0.032$), ‘abnormal capillary shapes’ ($p=0.003$), ‘large capillary hemorrhages’ ($p<0.001$) and ‘pericapillary extravasations’ ($p<0.001$). By qualitative analysis, the pattern ‘microangiopathy’ was detected in 68.3% (28/41) and a ‘scleroderma pattern’ in 17.1% (7/41) of the cSLE-patients (but without scleroderma symptoms). ‘Microangiopathy’ consisted of the combination of capillary hemorrhages and abnormal capillary shapes. The difference of percentage positive anti-RNP antibodies in the group with or without a scleroderma pattern was not significantly different (p=0.089). The number of 'abnormal capillary shapes per mm' was correlated with treatment-naivety. The number of 'large pathological hemorrhages per mm' was correlated with SLEDAI score and presence of nephritis. Compared to healthy controls, 'pericapillary extravasations' were found in significantly higher numbers per mm (p<0.001) as well as in percentage of patients (p<0.001) and that was never described before in such detail. These observations confirmed for the first time that giants, abnormal capillary morphology and capillary hemorrhages are also observed in cSLE, as was already known for adults with SLE. A high frequency and total amount of "pericapillary extravasations" was also seen in our cSLE patients, possibly revealing a new subtype of capillary hemorrhage that might reflect endothelial damage in these pediatric patients

Chapter 4 shows a world-wide multicenter study with comparison of capillary findings between pediatric patients with different rheumatic diseases compared to healthy controls. Patients with rheumatic diseases showed many significant differences compared to healthy controls; capillary density was lower in jSSc, JDM, MCTD en cSLE and in these diseases significantly more hemorrhages were observed as well. A scleroderma pattern was seen in jSSc, MCTD and JDM but also in 15% of cSLE. In cSLE, an abnormal 'non-specific' capillary pattern was also found. In healthy children, capillary density only showed a weak correlation with age (R=0.14, p=0.046), in contrary with findings from earlier studies.

Chapter 5 describes the longitudinal data from our cSLE-cohort (n=53) with a median disease onset of 14 years (IQR 12.5-15.5 years), median SLEDAI score at diagnosis was 11 (IQR 8-16) and median SLEDAI at follow up was 2 (IQR 1-6). A scleroderma pattern (ever) was seen in 18.9% and micro-angiopathy in 67.9%, while only 13.2% of patients had a normal capillary pattern. Thirty-three patients had follow-up capillaroscopy of which 21.2% showed changes in type of capillary pattern over time. Type of capillary pattern was not associated with disease activity. Raynaud's phenomenon (ever) was equally distributed among patients with different capillaroscopy patterns (p=0.26). Anti-RNP antibodies (ever) were significantly more detected (Chi square, p=0.016) in the scleroderma pattern subgroup (n=7/10, 70%). Already 5 years after disease onset more than 50% of patients with a scleroderma pattern had SLE-related disease damage (HR 4.5, 95%CI 1.1-18.8, p=0.034), but they did not develop clinical features of systemic sclerosis at follow-up. Number of detected fingers with a scleroderma pattern was similar between cSLE, jSSc and jUCTD. This longitudinal study shows that the majority of capillary patterns in cSLE are abnormal and they can change over time. Irrespective of disease activity, a capillary scleroderma pattern in cSLE may be associated with higher risk for SLE-related disease damage.

PART II. NEW BIOMARKERS IN (SYSTEMIC) CONNECTIVE TISSUE DISEASES

The second part of this describes novel methods to might be used in treatment of cSLE patients and related CTD's. This concerned new techniques in blood analyses but also the 'older' technique MRI for imaging of the wrist. Also, results from new imaging techniques (durometer, high-frequency ultrasound and laser speckle contrast analysis) are described in children with the autoimmune disease localized scleroderma (LS) to quantitatively measure the inflammation in the skin.

Chapter 6 aimed to translate existing transcriptomic data into simpler gene signatures suitable for daily clinical practice in cSLE patients. RT-PCR of multiple genes from the Interferon M1.2, Interferon M5.12, neutrophil (NPh) and plasma cell (PLC) modules followed by a principle component analysis, was used to identify indicator genes per gene signature. Gene signatures were measured in longitudinal samples from two childhood onset SLE cohorts (n=101 and n=34, respectively) and associated with clinical features. Cluster analysis subdivided patients into three mutually exclusive fingerprint-groups termed 1) all-signatures-low, 2) only IFN high (M1.2 and/or M5.12) and 3) high NPh and/or PLC. All gene signatures were significantly associated with disease activity in cross-sectional collected samples. The PLC-signature showed the highest association with disease activity. Interestingly, in longitudinally collected samples, the PLC-signature was associated with disease activity and showed a decrease over time. When patients were divided into fingerprints, the highest disease activity was observed in the high NPh and/or PLC group. The lowest disease activity was observed in the all-signatures-low group. The same distribution was reproduced in samples from an independent SLE cohort. The identified gene signatures were associated with disease activity and are suitable tools to stratify SLE patients into groups with similar activated immune pathways that may guide future treatment choices.

In **chapter 7** a pilot study in LS patients. It describes imaging data from the most prominent lesion (i.e. "target lesion") with examination of the centre, edge and contralateral unaffected site from ten patients with LS. High-frequency ultrasonography was used to determine dermal thickness, durometer for skin hardness, and laser speckle contrast analysis (LASCA) for a dynamical evaluation of the microcirculation. Dermal thickness was thinner at the centre of the "target lesions" vs. the edges ($p<0.001$) and control sites ($p<0.001$). Skin hardness was harder at the centre of the “target lesions” vs. the edges ($p=0.012$) and control sites ($p=0.003$). A higher perfusion was found in the centre of the "target lesion" (124.87±66.40 PU) vs. the edges (87.27±46.40 PU; $p<0.001$) and control sites (67.85±37.49; $p<0.001$). This case series suggests the supportive value of both microcirculatory and dermal assessments of skin lesions using novel non-invasive research tools, adopted from adult SSc, for the identification, scoring and/or monitoring of LS.

Chapter 8 describes ten patients with pediatric connective tissue disease (with clinical (overlap of) subtypes systemic lupus erythematosus, Sjögren syndrome and dermatomyositis) with a median age of 14.7 years (IQR 12.7-16.6 years) and 70% female. MRI wrist datasets were evaluated by three readers in consensus for synovitis, tenosynovitis, bone marrow changes, bone erosions and myositis. Clinical arthritis activity was scored low (median visual analogue scale physician 19, IQR 7-31). Notwithstanding, extensive inflammatory abnormalities (synovitis and tenosynovitis) were found in the wrist of 7/10 patients. Osteochondral involvement was detected in 3/10 patients. It was concluded that severe inflammatory abnormalities in the wrist could be present while clinical disease scores suggested relatively mild disease activity. These are important findings because the wrist is known to be vulnerable for joint damage.

PART III. DISCUSSION, SUMMARY AND APPENDIX

In **chapter 9** all results from this thesis are discussed in a broader perspective. This leads to a recommendations on the current and future needs in patient care and research topics, especially for cSLE, jMCTD and LS.

NEDERLANDSE SAMENVATTING

In de inleiding van dit proefschrift (**hoofdstuk 1**), wordt achtergrondinformatie gegeven over de verschillende autoimmuun- en zogenaamde ‘connective tissue’ ziekten (CTD’s) zoals Systemische Lupus Erythematosus (SLE), Mixed Connective Tissue Disease (MCTD) en geLokaliseerde Sclerodermie (LS). Hoewel er in de afgelopen jaren veel progressie is geweest in vroegtijdige diagnose en behandelingen van deze ziektebeelden, zijn er nog steeds nieuwe biomarkers nodig die patiënten beter verdelen in (prognostische) groepen met verschillende ernst van de ziekte. Deze verdeling in subgroepen zal leiden tot verbeterde gepersonaliseerde keuze in behandeling, en dit zal leiden tot lager risico op onherstelbare schade en de kwaliteit van leven verbeteren ondanks het leven met een chronische ziekte.

Deel 1. Nagelriem capillaroscopie in kinderen met SLE

Dit proefschrift bestaat uit twee delen. Het eerste deel gaat over nagelriem capillaroscopie. Met dit apparaat kunnen de kleinste bloedvaatjes (capillairen) van het lichaam bij de nagelriem in beeld worden gebracht. Dit onderzoek is makkelijk uitvoerbaar en pijnloos. De bevindingen van capillaroscopie onderzoek worden beschreven bij kinderen met CTD, met de nadruk op de ernstige auto-immuunziekte SLE. SLE op de kinderleeftijd wordt childhood-onset SLE (cSLE) genoemd.

In **hoofdstuk 2** werd een ‘systematisch literatuuroverzicht' gegeven van alle gepubliceerde literatuur over capillaire afwijkingen bij cSLE. Op het moment van dit literatuuroverzicht in 2019 werden er zes artikelen gevonden waarvan twee casus-controle studies en vier casuïstiek series. In capillaire dichtheid werd geen verschil gevonden tussen cSLE en gezonde controles (1 studie). Verschillen in capillaire diameter, morfologie, bloedingen en semi-kwantitatieve score waren niet-conclusief of niet te interpreteren. Een sclerodermiepatroon werd gerapporteerd in een minderheid van de cSLE patiënten in drie van vier casuïstiek series. We concludeerden dat de literatuur over capillaire bevindingen van de nagelriem in cSLE beperkt is en niet-conclusief. Om capillaire karakteristieken in cSLE te evalueren zijn er prospectieve en longitudinale studies nodig met gebruik van uniforme definities voor capillaire afwijkingen.

In **hoofdstuk 3** beschrijven we de capillaire afwijkingen van een cross-sectionele studie in cSLE patiënten (n=41), in vergelijking met capillairen van vergelijkbare gezonde kinderen (n=41). Het tweede doel van deze studie was om de abnormale capillaire afwijkingen te correleren met demografische variabelen en met ziekte-specifieke factoren in cSLE patiënten. cSLE patiënten lieten significant meer ‘reuzencapillairen’ ($p=0.032$), ‘abnormale capillaire vormen’ ($p=0.003$), ‘grote capillaire bloedingen’ ($p<0.001$) en ‘pericapillaire extravasaties’ (p<0.001) zien. Met behulp van kwalitatieve analyse werd het patroon van ‘microangiopathy’ gedetecteerd in 68.3% (28/41) en een 'sclerodermie patroon' in 17.1% (7/41) van de cSLE-patiënten (zonder sclerodermie symptomen). 'Microangiopathy' bestond uit de combinatie van capillaire bloedingen en abnormale capillaire vormen. Er was geen verschil tussen percentage positieve anti-RNP antistoffen in de groep met of zonder een sclerodermiepatroon (p=0.089). Het aantal'abnormale capillaire vormen per mm' was gecorreleerd met treatment-naïviteit. The aantal 'grote pathologische bloedingen per mm' was gecorreleerd met SLEDAI score en aanwezigheid van nefritis. Vergeleken met gezonde controles werden 'pericapillaire extravasaties' in significant hogere aantallen per mm (p<0.001) gezien en ook in hoger percentage van patiënten (p<0.001) en dat was nog nooit zo in detail beschreven. Deze observaties bevestigen dus voor het eerst dat reuzencapillairen, abnormale capillaire morfologie en capillaire bloedingen ook worden geobserveerd in cSLE, net als bij volwassenen met SLE. Een hoge frequentie en aantal van ‘pericapillaire extravasaties' werd gezien in onze cSLE patiënten, dit lijkt een nieuw subtype van capillaire bloeding te zijn die mogelijk de endotheel-schade in deze patiënten reflecteert.

Hoofdstuk 4 beschrijft een internationale studie waarbij de capillaire afwijkingen bij kinderen met verschillende auto-immuunziekten werden beschreven, in vergelijking met gezonde controles. Patiënten met reumatische ziektebeelden lieten significant meer afwijkingen zien in vergelijking met gezonde controles; capillaire dichtheid was lager in jSSc, JDM, MCTD en cSLE en in deze ziektebeelden werden ook significant meer capillaire bloedingen gezien. Een sclerodermie patroon werd gezien in jSSc, MCTD en JDM maar ook in 15% van cSLE patiënten. In cSLE werd ook een afwijkend 'non-specifiek’ patroon gevonden. In gezonde kinderen liet capillaire dichtheid slechts een zwakke correlatie zien met leeftijd (R=0.14, p=0.046), in tegenstelling tot bevindingen uit eerdere studies.

In **hoofdstuk 5** werden de resultaten beschreven van ons longitudinaal cSLE cohort (n=53) met een mediane leeftijd van begin ziekte van 14 jaar (IQR 12.5-15.5 jaar), mediane SLEDAI score bij diagnose van 11 (IQR 8-16) en een mediane SLEDAI at follow-up van 2 (IQR 1-6). Een sclerodermie patroon (geobserveerd ooit tijdens follow-up) werd gezien in 18.9% van de patiënten en 'micro-angiopathie' bij 67.9%, terwijl slechts 13.2% van patiënten een normaal capillair patroon liet zien. N=33 patiënten kregen follow-up capillaroscopie waarvan 21.2% veranderingen liet zien in soort capillaire patroon over de tijd heen. Het type capillaire patroon was niet geassocieerd met ziekteactiviteit. Het optreden van Raynaud fenomeen (ooit) was gelijkmatig verdeeld over de patiënten met verschillende capillaire patronen (p=0.26). Anti-RNP antistoffen (ooit) werden significant meer gezien (Chi square, p=0.016) in de subgroep met sclerodermie patroon (n=7/10).

Meer dan 50% van de patiënten met een sclerodermie patroon had al SLE-gerelateerde schade door de ziekte opgelopen binnen 5 jaar na start van de klachten (HR 4.5, 95%CI 1.1-18.8, p=0.034), maar zij vertoonden geen klinische kenmerken van de ziekte SSc bij follow-up. Het aantal vingers met een sclerodermie patroon was hetzelfde bij patiënten met cSLE, jSSc en jUCTD. Deze longitudinale studie laat zien dat de meeste capillaire patronen in cSLE afwijkend zijn en ook kunnen veranderen over de tijd. Daarnaast lijkt, onafhankelijk van ziekteactiviteit, een sclerodermie patroon geassocieerd met een hoger risico op SLE-gerelateerde schade aan de organen.

Deel II. Nieuwe biomarkers in (systemische) ‘connective tissue’ ziektebeelden

Het tweede deel van dit proefschrift gaat over nieuwe onderzoeksmethoden die behulpzaam zouden kunnen zijn in de behandeling van kinderen met SLE en vergelijkbare “connective tissue” ziekten. Dit betreft nieuwe technieken om het bloed te analyseren maar ook over ‘oudere’ beeldvormende technieken zoals MRI van het polsgewricht. Daarnaast worden nieuwe beeldvormende technieken (durometer, hoogfrequentie echo en laser speckle contrast analyse) beschreven in kinderen met de auto-immuunziekte gelokaliseerde sclerodermie (LS) om op een kwantitatieve manier de inflammatie in de huid te meten.

Hoofdstuk 6 had als doel om bestaande ‘transcriptomic data’ te vertalen naar simpelere genetische signaturen die bruikbaar zijn in de dagelijkse praktijk bij cSLE patiënten. Meerdere genexpressies van de Interferon M1.2, Interferon M5.12, Neutrofielen (NPh) en Plasma Cel (PLC) modules, gevolgd door een 'principale componenten analyse', werden gebruikt om indicator genen per genetische signatuur te identificeren. Genetische signaturen werden gemeten in longitudinale samples van twee cSLE cohorten (n=101 en n=34) en geassocieerd met klinische verschijnselen. Cluster analyse verdeelde patiënten in drie exclusieve ‘fingerprints’ groepen genaamd 1) alle-signaturen-laag, 2) alleen IFN hoog (M1.2 en/of M5.12) en 3) hoog NPh en/of PLC. Alle genetische signaturen waren significant geassocieerd met ziekteactiviteit in de cross-sectionele samples. PLC-signatuur had de hoogste associatie met ziekteactiviteit. Een opvallende bevinding, in de longitudinale monsters, was dat de PLC-signatuur geassocieerd was met ziekteactiviteit en een lagere waarden liet zien over de tijd. Als patiënten werden verdeeld met 'fingerprints’ dan werd de hoogste ziekteactiviteit geobserveerd in de hoog NPh en/of PLC groep. De laagste ziekteactiviteit werd geobserveerd in de alle-signaturen-laag groep. Dezelfde verdelingen in groepen werden gereproduceerd in een onafhankelijk cSLE cohort. De geïdentificeerde gen signaturen waren geassocieerd met ziekteactiviteit en zijn passende tools om SLE patiënten te verdelen in groepen met dezelfde type immuun-activatie die toekomstige behandelingskeuzes meer richting kunnen geven.

In **hoofdstuk 7** wordt een pilot studie beschreven bij patiënten met gelokaliseerde sclerodermie (LS). Hierin worden de data beschreven van beeldvormingstechnieken bij de meest prominente huidlaesies ("target laesie") met onderzoek van het centrum en rand van de huidlaesie en van de contralaterale niet-aangedane kant van tien patiënten met LS. Hoogfrequentie echo werd gebruikt om de huiddikte vast te stellen, een durometer voor het meten van de hardheid van de huid en Laser Speckle Contrast Analyse (LASCA) voor dynamische evaluatie van de kleine bloedvaatjes (microcirculatie). De huiddikte was significant minder in het centrum van de 'target laesies' ten opzichte van de randen ($p<0.001$) en de niet-aangedane kant ($p<0.012$). Een hogere bloedperfusie werd gevonden in het centrum van de "target lesion" (124.87±66.40 PU) ten opzichte van de randen (87.27±46.40 PU; $p<0.001$) en de controle kant (67.85±37.49; $p<0.001$). Deze casuïstiek serie laat zien dat, overgenomen vanuit volwassen SSc, zowel metingen in microcirculatie als huiddikte/-hardheid van de aangedane huid waarde lijken te hebben als nieuwe non-invasieve instrumenten, in het vaststellen, scoren en/of monitoring van LS.

Hoofdstuk 8 beschrijft tien patiënten met een pediatrische "connective tissue" ziekte (met klinische overlap van de subtypes SLE, SSc, Sjögren syndroom en dermatomyositis) met een mediane leeftijd van 14.7 jaar (IQR 12.7-16.6 jaar) bestaand uit 70% vrouw. MRI beelden van de pols werden geëvalueerd door drie experts om consensus te krijgen voor het scoren van synovitis, tenosynovitis, beenmergveranderingen, boterosies en myositis. De klinische ziekteactiviteit werd als relatief laag gescoord (mediane visuele analoge school voor arts 19 (0-100), IQR 7-31). Desalniettemin werden er uitgebreide inflammatoire afwijkingen gevonden (synovitis en tenosynovitis) in de pols bij 7/10 patiënten. Kraakbeenbetrokkenheid werd gezien in 3/10 patiënten. Geconcludeerd werd dat er ernstige inflammatoire afwijkingen in de pols aanwezig kunnen zijn terwijl de klinische ziektescores als relatief mild werd beoordeeld. Dit zijn belangrijke bevindingen omdat specifiek de pols als gewricht kwetsbaar is voor gewrichtsschade.

Deel III. Discussie, samenvatting en appendix

In **hoofdstuk 9** worden alle resultaten van dit proefschrift bediscussieerd en in een breder perspectief geplaatst. Dit leidt tot aanbevelingen voor huidige en toekomstige behoeften in patiëntenzorg en voor onderwerpen van onderzoek, in het bijzonder voor cSLE, jMCTD en LS.

A

LIST OF ABBREVIATIONS

ACR: American College of Rheumatology
ADA: anti-drug antibodies
Anti-ds-DNA: anti-double-stranded DNA antibodies
Anti-RNP: anti-Ribonucleoprotein antibody
Anti-Sm: anti-Smith antibodies
aSLE: adult-onset Systemic Lupus Erythematosus
BEL: belimumab
BILAG: British Isles Lupus Assessment Group
CIMT: Carotid-Intima-Media-Thickness
CS: corticosteroids
cSLE: childhood-onset Systemic Lupus Erythematosus
CYC: cyclofosfamide
CTD: Connective Tissue Disease
EC: Endothelial Cell
EULAR: European League Against Rheumatism
HR: hazard ratio
HRQoL: health related quality of life
IFN: interferon
IQR: interquartile range
JDM: Juvenile Dermatomyositis
jMCTD: juvenile Mixed Connective Tissue Disease
jSSc: juvenile Systemic Sclerosis
LASCA: Laser Speckle Contrast Analysis (LASCA)
LLDAS: Lupus Low Disease Activity State
LoSCAT: Localized Scleroderma Assessment Tool
LS: Localized scleroderma
(M)CTD: (Mixed) Connective Tissue Disease
MMF: mycophenolate mofetil
MTX: methotrexate
NFC: nailfold capillaroscopy
NIH: National Institutes of Health
NPh: neutrophil
NVC: nailfold videocapillaroscopy
OR: odds ratio
PBP: peripheral blood perfusion
PLC: plasma cell
PReS: Pediatric Rheumatology European Society

RP: Raynaud's phenomenon
RTX: rituximab
SDI: SLICC Damage Index
SG MCRD: Study Group on Microcirculation in Rheumatic Diseases
SLE: Systemic Lupus Erythematosus
SLEDAI: Systemic Lupus Erythematosus Activity Index
SLICC: Systemic Lupus International Collaborating Clinics
SSc: Systemic Sclerosis
T2T: Treat to Target
UCTD: Undifferentiated Connective Tissue Disease

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Chapter 3: DS, JMvdB, VS and TWK initiated this study. DS, SB, AN, LBvdAA, GJdB, RtC, AEH, MvO, TWK and JMvdB recruited patients and healthy controls. DS, SB and JMvdB were involved in data analysis and writing of the manuscript. All authors were involved in editing and critical revision of the manuscript.

Chapter 4: KM, MC, DSM, IF, ALH, AS and VS had **s**ubstantial contributions to the ideation of the study, substantial contributions to the design of the study. KM, DSM, IF, MCL, YM, VB, RC, JDH, TF, JHZ, FI, AK, DK, HL, AM, MAMN, MMJ, UML, LNN, RO, CP, MR, RD, AR, SR, CU and JMvdB had substantial contribution to the acquisition of data. KM, DSM, MCL, EDS and VS had substantial contribution in analysis and interpretation of data. KM and VS were involved in drafting of the article. All authors were involved in editing and critical revision of the manuscript.

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Chapter 6: SK and MAV contributed to the study conception and design. MJW, CGvHM, ST and MAV contributed to the experimental work. MJW, DSM, CGvHM, SJvT, NG, EJHS, EPAHH, PCEHM, .DMCB, DD, MV, JMvdB, KB, SK and MAV contributed to the acquisition of data. MJW, DSM, SJvT, SK and MAV contributed to the analysis and interpretation of the data. MJW, SK and MAV contributed to the writing of the draft manuscript. MJW, DSM, CGvHM, SJvT, NG, EJHS, EPAHH, PCEHM, DMCB, DD, MV, JMvdB, KB, SK and MAV contributed to the rewriting of the manuscript.

Chapter 7: AvH, DSM, JMvdB and VS initiated this study. DSM, SdS, MAMH, ANSR, JMvdB and TWK recruited patients. AvH, DSM, SdS, SCB, MCL, ANSR, MAMH, JMvdB and VS were involved in acquisition of data. AvH, DSM, AI, MC and VS were involved in data analysis, interpretation of data and writing of manuscript. All authors were involved in editing and critical revision of the manuscript.

Chapter 8: CN, DSM and MM initiated this study. CN, JMvdB, ANSR, DSM, KN and MM were involved in acquisition of data. CN, KN, DSM and MM were involved in data analysis and writing of the manuscript. CN, KN, and DS. All authors were involved in editing and critical revision of the manuscript.

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PORTFOLIO

1. PhD training	Year	Workload
General courses		
Computing in R	2021	2.5
Basis legislation in Science (BROK)	2020 / 2016	2.0
Scientific writing course	2018	1.5
Practical Biostatistics	2016	1.5
Seminars, workshops and master classes		
JIR Winter School Management of CTD (virtual)	2022	1.5
JIR Winter School SLE	2018	1.5
Juvenile Systemic Sclerosis Symposium, Hamburg Germany	2016	1.0
Systemic JIA Preceptorship Genova, Germany	2015	1.5
Juvenile Systemic Sclerosis Symposium, Hamburg Germany	2014	1.0
Basis Immunology course Genova, Italy	2013	1.5
Presentations		
Lupus-Cora – oral presentation (virtual)	2021	0.5
EULAR – poster (virtual)	2021	0.5
PReS – oral presentation (virtual)	2021	0.5
PReS – Lightening talk (virtual)	2020	0.5
PReS – poster (virtual)	2020	0.5
EULAR study group on Microcirculation	2019	0.5
NVKR assembly scientific meeting	2019	0.5
JIR SLE Winter School – oral presentation	2018	0.5
JIR SLE Winter School – poster	2018	0.5
KAISZ patient organisation – oral presentation	2018	0.5
Amsterdam Kindersymposium – oral presentation	2017	0.5
Young Investigator Meeting (YIM) PReS – 2 posters	2017	0.5
PReS – poster	2017	0.5
Amsterdam Kindersymposium – oral presentation	2015	0.5
Young Investigator Meeting (YIM) PReS – poster	2015	0.5
International conferences		
Lupus-Cora (virtual)	2021	1.5
PReS (virtual)	2021	1.5

EULAR (virtual)	2021	1.5
PReS (virtual)	2020	1.5
EULAR Madrid, Spain	2019	1.5
Childhood Arthritis & Beyond Meeting Lake Louise, Canada	2019	1.5
PReS Lisbon, Portugal	2018	1.5
Therapeutic Drug Monitoring, Amsterdam NL	2018	1.5
EULAR Amsterdam, NL	2018	1.5
PReS/YIM Athens, Greece	2017	1.5
EULAR Madrid, Spain	2017	1.5
PReS Genova, Italy	2016	1.5
Autoimmunity Leipzig, Germany	2016	1.5
EULAR/YIM Rome, Italy	2015	1.5

Other

Writing METC-approved study protocol	2015-2016	4.0
Retro-/prospective data collection	2016-present	4.0

2. Teaching

Lecturing

Verpleegkundigen nascholing SLE	2022	0.5
AIOS cursorisch onderwijs	2021 / 2018	4.0
Studentencollege met patiënt	2019	0.5
Refereeravond SLE	2017	2.0

Tutoring, mentoring

Bachelor student (Ishika)	2022	1.0
Research internship (Rosanne, Sandy, Ilja)	2017/2019/2020	6.0
PhD student (Amara, Sandy)	2018-2022	8.0

ECTS PhD period 2015-2022	**Total**	**73**

3. Parameters of esteem

Grants and Funds	Year
Zeldzame Ziekten Fonds	2021
Stichting Steun Emma	2016

Expertise centers	
Amsterdam UMC expertise center for juvenile idiopathic arthritis	2016 - present
Amsterdam UMC expertise center for juvenile SLE	2021- present
Amsterdam UMC expertise center for localized scleroderma	2021 - present

Networks and associations	
cSLE T2T International Task Force - member	2021-present
VSOP – national website for expertise network in cSLE	2021-2022
UCAN-CAN-DU study group	2019-present
PReS SLE working party - member	2018-present
EULAR Study Group Microcirculation in Rheumatic Diseases - member	2016-present
NVKR – member	2011-present
NVK – member	2004-present

DANKWOORD

Het overkoepelende thema is van dit proefschrift voor mij is **vrouwen**. De ziekten die zijn beschreven en die ik dagelijks behandel treffen voor het merendeel meisjes en jonge vrouwen. Er was een tijd dat dit juist reden was dat hier minder onderzoek naar werd gedaan, en op veel plekken op aarde is de toegang tot zorg voor zieke meisjes en vrouwen helaas nog steeds niet vanzelfsprekend. Ik heb in de huidige tijd de kans gekregen om mij te ontwikkelen tot kinderarts-reumatoloog en zo iets te kunnen bijdragen aan verbetering. Ik besef me heel goed dat ik dit in vorige generaties niet had kunnen doen als vrouw. Ik ben enorm dankbaar dat ik mijn ambities heb kunnen waarmaken en besef me goed dat ik dit grotendeels heb te danken aan waar mijn wieg stond.

Dit proefschrift is opgedragen ter nagedachtenis aan Zenani en Minou. Ze worden allebei verschrikkelijk gemist. Zenani en Minou waren allebei prachtige jonge tieners die door mij, en door zoveel andere collega's, nooit zullen worden vergeten. Ze namen zelf geen deel aan mijn onderzoek maar zijn wel de reden waarom dit onderzoek nodig is. Ik wil hen extra eren en daarom komen ze ook terug in lay-out door het hele boekje heen. Ik hoop dat ik iets heb kunnen bijdragen aan verbetering van de toekomstige zorg in systemische auto-immuunziekten, zodat niemand meer zal hoeven doormaken wat zij, en hun dierbaren, hebben moeten doorstaan.

Ik wil alle patiënten en hun ouders bedanken voor hun vertrouwen en deelname aan de studies. Dit proefschrift is *voor* jullie geschreven. Het is zo ontzettend moeilijk om te moeten leven met een chronische ziekte en alles wat daarbij komt kijken, zeker als kind of adolescent. Ik vind het belangrijk dat er oog is voor de mens achter de patiënt en ik hoop dat jullie dit ook zo voelen met regelmatig een lach maar soms ook een traan in de spreekkamer. Ik zal me altijd voor jullie blijven inzetten.

Stichting Emma, familie van Maasdam en het Zeldzame Ziekten Fonds; bedankt voor jullie vertrouwen in mijn onderzoek. Het was zonder jullie financiële steun niet gelukt. Ik ben zo dankbaar dat ik de kans kreeg in 2016 om een pilot-onderzoek te doen en nu jaren later ligt dit proefschrift hier en zijn er vele nieuwe lopende projecten, plannen en ideeën. Bedankt voor jullie steun!

Merlijn: toen ik begon aan mijn fellowship in 2011 zei ik dat ik geen wetenschapper was. Jij zei dat ik binnen 5 jaar gepromoveerd zou zijn. Nu ligt er toch een boekje, niet binnen die 5 jaar, maar die promotie komt er en dat had ik echt nooit gedacht. Ik ben er heel erg trots op en jij hebt me er doorheen gesleept. Af en toe een duwtje, dan weer een luisterend oor en veel advies maar vooral de gunfactor van tijd. Dankzij die maandag research-dag is het mij gelukt. Je bent echt een maatje, inclusief soms wat gekibbel, maar bovenal iemand die mij door en door kent en begrijpt. Bedankt voor deze kans en route op mijn carrière, en wie weet is dit pas het begin. Ik heb nu zoveel nieuwe ideeën voor nieuwe onderzoeksprojecten, alleen helaas een gebrek aan tijd en vooral aan fondsen. Maar deze onderzoekslijn krijgt vorm en is in een stijgende lijn, we gaan ervoor. En daarna ga ik toch echt helpen om de plastic soep in zee op te ruimen.

Vanessa: de eerste keer dat ik je zag spreken was bij een workshop capillaroscopie tijdens een congres in San Diego in 2013. Ik had toen de eerste abnormale observaties bij capillaroscopie geanalyseerd in mijn SLE-patiënten en nam me voor contact te zoeken voor samenwerking met het idee: handig, want Gent is toch een beetje "om de hoek". Na 1 email was het contact snel gelegd en bezocht ik je in Gent, met mijn toenmalige onderzoeksgegevens. Alles kon direct de prullenbak in, dat was jouw advies: even slikken, maar je had wel gelijk. Als je het doet, dan moet het goed zijn. Hoe dat dan moest, dat heb jij mij geleerd. België bleek soms toch een afstand maar het lukte om samen projecten op te zetten en inmiddels is onze samenwerking niet meer uit ons leven weg te denken. Zonder jou was dit proefschrift niet gelukt, bedankt voor alles.

Taco: bij mijn sollicitatie was ik eerlijk en zei dat ik weinig onderzoek ambieerde. Dat was spannend en voelde een beetje naïef want ik was direct daarna bang om niet te worden aangenomen. Het tegendeel was waar. Je had juist iemand nodig voor patiëntenzorg en ik kon volop aan de slag. Toen kwamen opeens de onderzoeksvragen vanzelf. Vervolgens ligt er hier nu een proefschrift dat echt helemaal door mijzelf is opgebouwd en uitgevoerd en dat is zoveel waard. Je legde geen druk op mij in de snelheid van uitvoering en daar ben ik je dankbaar voor. Ik kon het op mijn eigen tempo doen, en op mijn eigen manier, ook al was dat soms anders dan hoe jij het zag. Bedankt voor steun en bovenal voor al die leerzame klinische lessen, je bent een geweldige dokter en een wandelende encyclopedie.

Rebecca: je stond aan de wieg van de ontwikkeling van kinderreumatologie in Nederland, en ook aan de wieg van mijn carrière in dit vakgebied. Aan het eind van mijn opleiding tot kinderarts kon ik een paar maanden met je meekijken en gaf je me een kans om het zwangerschapsverlof van Petra op de vangen. Het doel was verbeterde samenwerking tussen LUMC en AMC, en dat is ook precies gelukt. Inmiddels werken we nauw samen binnen KRANS en hebben we zelfs een gezamenlijke opleiding, wekelijkse patiëntenbesprekingen maar bovenal zeer korte lijntjes. Precies hoe jij het voor je zag en misschien nog wel meer dan dat. Bedankt voor al je hulp en steun. Je zei tegen me: vindt je eigen niche. Kijk nou, dat is gelukt!

Mario: mijn eerste ontmoeting met jou was toen ik ad hoc binnenliep in een radiologiebespreking om een formulier te laten ondertekenen voor mijn aanstelling in het AMC. Je reageerde hierop heel vriendelijk met een grap waardoor ik me niet meer opgelaten maar direct welkom voelde. Dat bleek een voorbode voor de goede sfeer en samenwerking van de jaren daarna. Altijd tijd nemen voor een spoedbeoordeling en de oprechte interesse in het verhaal van de patiënt achter de MRI-plaatjes. Ook al was de JIA-onderzoekslijn geheel anders dan die van de SLE, het is toch gelukt om die twee samen te brengen in dit proefschrift en jou in mijn promotiecommissie te krijgen. Bedankt voor je oprechte blijheid en interesse.

Sander: onze samenwerking begon tijdens mijn verplichte stage in volwassen reumatologie. Wat vond ik het moeilijk om weer volwassen patiënten te zien na al die jaren kindergeneeskunde. Doodsbang was ik om wat te missen. Jij was altijd een baken van rust, begrip en steun. Precies die kenmerken zie ik nu terug zie als je mijn arts-onderzoeker Sandy spreekt als mentor. Dat is voor jou vanzelfsprekend maar voor een ander zo waardevol in de academische hectiek. Ik ben dankbaar voor de samenwerking die de komende jaren zal groeien door onze gezamenlijke plannen. Want samen is beter; together everyone achieves more.

Ronald: het is een grote eer dat je in mijn commissie zit. Bedankt voor je uitnodiging in de ARC SLE research meetings, je oprechte interesse in waar ik mee bezig ben en navraag hoe het met mij gaat tijdens begeleiding van een levensbedreigend zieke patiënt.Het was even schrikken een paar maanden geleden. Gezondheid is het allerbelangrijkste en dat beseffen we als dokters elke dag. Bedankt voor je voorbeeld dat je hoogleraar kan zijn en nog steeds de mens achter de patiënt ziet. Dat inspireert mij enorm en is precies hoe geneeskunde ooit is bedoeld door Hippocrates.

Annet: dank je wel voor je enthousiasme om in mijn promotiecommissie deel te nemen, het is een eer. Je bent een expert in systemische auto-immuunziekten bij kinderen in Nederland, maar ook je internationale rol en betekenis is groot. Ik vind het spannend wat je van mijn proefschrift zal vinden. We hebben geprobeerd om de samenwerking op te zetten, de bureaucratische muren van UMCU bleken echter voor mij ondoordringbaar en vanwege tijdsgebrek gaf ik het op. Via een andere route met het landelijke CHILL-project hoop ik het alsnog te realiseren om capillaroscopie te kunnen uitvoeren bij de SLE patiënten in Utrecht. Geduld is een schone zaak, bedankt voor je steun.

Alexandre: jaren geleden vroeg ik je om mee te kijken bij een jonge patiënte waarbij ik twijfelde over een overlap-beeld. Dat was nog in de pre-fusie fase van AUMC. Je kwam speciaal hiervoor naar locatie AMC en dat waardeerde ik heel erg. De voor mij grappigste herinnering is toen je zei dat mijn proefschrift bijna af was: die inleiding en discussie schrijf je in een weekend, die leest toch niemand. Je had helemaal gelijk, dat klopt. Behalve de commissie en daar zit je nu in. Ik hoop dat je het met interesse hebt gelezen en ik weet zeker dat we een mooie gedachten wisseling zullen hebben met al jouw ervaring in dit vakgebied. Ik kijk uit naar een goede samenwerking de komende jaren op locatie AMC. Ter info: er komen vele transitie patiënten aan.

Amara: je meer dan welkome komst (in een zeer moeilijke tijd) en helaas ook weer vertrek in onze vakgroep ging gepaard met tranen. Toen we definitief dat nieuws hoorden vielen we elkaar huilend in de armen. Een dierbaar tijdperk was voorbij. We waren jarenlang een team waarin we elkaar maar hoefden aan te kijken of we wisten wat de ander dacht. Vanzelfsprekend hadden we ook direct een band doordat onze kinderen precies dezelfde leeftijd hebben en het ploeteren was in die tropenjaren. Ik wil je dolgraag helpen om dit gevoel, van een geprint proefschrift dat voor je ligt, ook te ervaren. Je weet dat ik er altijd voor je ben, let's go for it! En in de tussentijd gaan we weer genieten van terrasjes tijdens congressen nu het eindelijk weer kan.

Sandy: wat ben ik blij dat je reageerde op het berichtje op StudentBoard jaren geleden, nota bene voor het onderzoek van Amara. Je bent nu niet meer weg te denken uit mijn SLE onderzoek. Ik zeg het te weinig maar je wordt zo enorm gewaardeerd en ik ben zo trots op je ontwikkeling. Je bleef vertrouwen hebben in ons als team, ook zelfs met een paar maanden zonder salaris. Je vertrouwen werd beloond, uiteindelijk kwamen er gelukkig echt (sporadische) fondsen binnen om je salaris te betalen. Ik heb midden in jouw promotietraject een moeilijke tijd gehad en je had alle begrip terwijl je het zelf ook moeilijk had, en hebt, met zieke dierbaren. Jouw promotie is de volgende en nogmaals, ik ben zo trots op je! Jij bent mijn eerste PhD student en ik hoop dat je snel de tweede mag inwerken voordat je aan de opleiding begint. Geloof in jezelf en wees trots, je kan het.

Ingrid: jij bent een onmisbare schakel in ons team. Het is zo fijn om jou als collega te hebben. Doortastend, menselijk en kundig. De laatste jaren klaar voor een nieuwe uitdaging die je met volle overtuiging hebt aangenomen en zoveel zal betekenen voor de resultaten van ons internationale research project voor jeugdreuma. Wie weet kan je voor mijn SLE project in de toekomst hetzelfde doen, eerst zal ik hopelijk fondsen werven en dan weet ik waar de expertise zit. Hou me scherp in de chaos!

Mariken: ik heb zoveel respect voor jou, je bent een alleskunner. Wat een dappere stap om later in je carrière voor een subspecialisatie te gaan, en ik ben zo blij dat je dat deed. Jouw visie op hoe patiëntenzorg hoort te zijn klopt precies met hoe Merlijn, Ingrid en ik dat zien. Je nam een rol aan als fellow op een leeftijd die ongebruikelijk is met al jouw ervaring en expertise op andere vakgebieden waar wij ook weer van kunnen leren. Dit viel samen tot kinderreumatologische zorg zoals het moet zijn. Dus: don’t ever leave us.

Marja Pannekoek: je weet het zelf niet maar jouw observaties van ‘extravasaties’ liggen ten grondslag aan dit proefschrift. Dit wekte namelijk mijn nieuwsgierigheid en heeft geleid tot dit boekje en tot inmiddels een volledige onderzoekslijn. Je werkt niet meer in het AMC maar ik zal je opsporen om je persoonlijk te kunnen bedanken.

Dear Maurizio: your atlas of capillaroscopy was the basis of my research. The findings of my studies in cSLE were sometimes surprising for you (no, these patients did not have overlap disease!). Thank you for being a part in my learning process and I hope that I can add some more knowledge to a future atlas.

Beste KIRI collega’s Martijn, Dasja, Marceline, Michael en Henriette. We zijn 1 vakgroep maar werken ook veel gescheiden. Toch horen we echt bij elkaar. Bedankt voor jullie steun en feedback over de jaren waarin ik kon groeien als fellow naar volwaardig staflid met een eigen research lijn. Martijn: jou wil ik extra bedanken voor je steun voor onze kinderreumatologie-groep. Volledig dankzij jou heb ik een fonds gekregen van het zeldzame ziektefonds. Hoop nog lang tegen je aan te mogen zeuren als kamergenoot, en vice versa natuurlijk.

Ik wil vanzelfsprekend al mijn collega’s bedanken in het Emma Kinderziekenhuis en dat zijn er teveel om op te noemen. Toch wil ik nog extra de kindernefrologen benoemen. Tonny, Michiel, Rik, Arend en Jaap: wat hebben we een fijne samenwerking voor onze patiënten. We leveren topzorg op maat met onze combinatie-consulten en we zijn niet voor niets expertisecentrum geworden.

Pina (aka Jennifer): heldin. In je eentje de kinderdermatologie op zo’n hoog niveau uitvoeren. We gingen met Amara samen naar Gent en kwamen als garnalenvrouwtjes terug. Hopelijk lukt het om samen de zorg voor onze sclerodermie patiënten nog verder te verbeteren. Het is een groot voorrecht om met jou te werken en ik hoop dat nog heel lang kan doen. Blijf en hou vol!

Ook wil ik specifiek enkele oud-‘verdiepers’ noemen. Beste Koen, Daniel, Giske, Daan, Veronica, Cornelis, Laurens, Anne, Daria, Ilan en Marein: door jullie kon ik mijn wetenschaps-dagen ook echt efficiënt gebruiken. Het is echt mede door jullie dat dit proefschrift er daadwerkelijk ligt, ik ben jullie heel erg dankbaar. Het was ook erg gezellig en leuk om te zien hoe jullie allemaal werden gegrepen door de kinderreumatologie, wat een fantastisch vak he?

Alle balie- en secretariaat-medewerkers, we werken door drukte zo vaak langs elkaar heen en daarom uit ik mijn waardering voor jullie onvoldoende, zeker in een fase waarin ik het erg zwaar had. Katenka, Simone W, Hester, Lilian, Anja, Madeleine, Simone H, Inez, Sabine, Mirthe en Monique: jullie zijn echt onmisbaar in het dagelijkse reilen en zeilen van onze afdeling, bedankt voor alles.

Hans van Goudoever: bedankt voor je persoonlijke bericht en aandacht in een zware periode. Dat heb ik echt enorm gewaardeerd.

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Petra (Leiden) en Joost (Utrecht): ik wil jullie apart noemen want jullie hebben mij de richting van kinderreumatologie op gewezen die zo goed bij me past. Ik ben daar erg dankbaar voor want ik had het anders nooit overwogen. Het is nog extra leuk omdat we nog steeds zoveel kunnen samenwerken. Sylvia, ik herinner me een etentje op een koude winteravond bij Merlijn thuis waarin we hebben uitgesproken volledig samen te gaan werken met onze cohorten, en zo geschiedde. Bedankt dat je me meeneemt in je projecten, fondsaanvragen en netwerken. Hoe je alle ballen hoog houdt is mij een raadsel, ik ben zo blij met je steun. Alle overige collega's binnen de kinderreumatologie: Leontien, Wineke, Elizabeth, Esther, Ellen, Nico, Bas, Marc, Simone, Gijs, Danielle, Marleen en Amani: het is kostbaar dat we zo close samenwerken op landelijk niveau. Laten we dat goed vasthouden en verder uitbouwen voor al die zeldzame ziektebeelden die we behandelen.

Javad: je bent nu al professor in de dop. De ene na de andere onderzoeksprijs sleep je binnen, en terecht. Wat leuk en fijn dat we konden samenwerken, en nieuwe projecten zijn al gaande. Sterkte met afronding van ook jouw proefschrift binnenkort en succes met de opleiding tot kinderarts. Terecht dat je bent aangenomen en zo leuk dat je een collega wordt. We houden ongetwijfeld veel contact.

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Lieve jaarclub vriendinnen Hester, Kim, Meike, Suzan, Merle en Anna: al 25 jaar een trouwe vriendschap, door weer en wind, met meestal een schaterlach maar soms ook met een traan. We hebben al vele levensfasen met elkaar doorlopen. We blijven trouw aan onze reisjes die inmiddels door geheel Europa gingen, misschien moeten we inmiddels meer intercontinentaal gaan denken. Ook jullie gaven toestemming om jullie eigen kinderen te onderzoeken. Terwijl jullie tijdens ons weekend weg lekker samen aan het borrelen waren, was ik helaas als een monnik aan de keukentafel hard aan het werk met mijn apparaat, maar het was het waard. Jullie zijn voor mij HET voorbeeld dat onze generatie vrouwen veel kansen kan pakken voor een goede carrière. We zijn allemaal hardwerkende vrouwen die deze kansen met beide handen hebben aangegrepen om expert te worden in hun eigen vakgebied. Dit zo'n groot verschil met de generaties voor ons. Nu is het tijd voor feest, en als er iemand weet hoe je moet feesten dan zijn jullie het wel. Let's go girls!

Kate en Berry: jullie vriendschap is alles waard. Jullie kunnen naar de andere kant van de wereld verhuizen maar onze band blijft toch ijzersterk. We konden leef en leed nog steeds intens met elkaar delen, was het niet via beeld dan toch wel met jaarlijkse vakanties "halverwege". Wat waren we blij dat jullie eindelijk echt naar Nederland terugkwamen, en het moment waarop kon ook niet beter. Laten we er een feestje van maken zoals in Shanghai, het dak mag eraf!

Lieve Caroline, Deborah en Hester: alle drie zo anders maar mij stuk voor stuk zo dierbaar met een hechte vriendschap al vanuit het begin van onze studententijd. Daarna alle mijlpalen van ons leven samen doorlopen. Door die tropenjaren met jonge kinderen zien we elkaar niet zoveel als daarvoor maar onze vriendschap is tijdloos en rotsvast. Jullie waren een grote reddingsboei toen ik jullie zo hard nodig had. Bedankt voor al die jaren vriendschap, jullie zijn voor mij onmisbaar.

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Lieve Mindy, sommige mensen zijn familie maar anderen *worden* familie. Je bent zo'n belangrijk onderdeel van ons gezin, niet alleen praktisch als reddende engel maar ook qua emotionele band. Onze kinderen houden zielsveel van je en jij van hen. Je gedachten over opvoeding sluiten volledig aan bij die van ons. Dankzij jou had ik de rust om dit proefschrift te kunnen maken. Ik kan me geen leven zonder jou voorstellen.

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Lieve Marije en Bas! Mijn paranimfen, wat een woord he. Het voelt goed om daar straks te staan, als een blok, met zijn drieën. Ik zal daar zoveel steun aan hebben. Het is zoveel rijkdom om een grote familie te hebben. Door het leeftijdsverschil waren we soms met volledig andere levensfasen bezig, als tieners gingen we onze eigen weg maar nu is dat anders. Onze beroepskeuze is divers maar de gemeenschappelijke eigenschap is de serieuze en volhardende aanpak in ons werk. En Eva, als schoonzus pas je daar dus ook perfect tussen! We zien onze kinderen samen opgroeien en onze groepsapp wordt dagelijks druk gebruikt. Het meest ondenkbare gebeurde in Maart 2020 maar samen staan we sterk. Ik hou van jullie!

Lieve papa met je eindeloze oneliner quotes waarmee je ons hebt opgevoed: 'brutalen hebben de halve wereld'. 'Nee heb je, ja kan je krijgen'. 'Begrijp je het niet, verwonder je dan'. 'Niet klagen maar dragen' (het stukje bidden voor kracht liet je weg). 'Soms gaat het niet om wat je kan, maar wie je kent". Je hebt me geleerd wat doorzettingsvermogen is en dat hard werken loont. Hier ligt het resultaat. Lieve mama, altijd met de vleugels beschermend om ons heen om het kwaad van de wereld buiten de deur proberen te houden. Zo moeilijk, met al die reislustige kinderen. Je staat altijd klaar voor ons, wil ieder pijntje of verdriet overnemen. Wat bijzonder dat je de omslag hebt kunnen ontwerpen. Je vond het heel spannend (want ik heb mijn perfectionisme niet van een vreemde) maar het is zo mooi en persoonlijk geworden. Ik ben trots en hopelijk ben jij dat ook! Jullie zijn zelf opgegroeid in een traditioneler gezin uit een hele andere tijd, eentje waar een vrouwelijke carrière niet werd nagestreefd. Jullie hebben ons wat anders meegegeven, en stimuleerden ons allemaal om ons eigen pad te zoeken. Al jaren is de vrijdag jullie oppasdag, dat begon 14 jaar geleden met jullie eerste kleinkind en is tot de dag van vandaag doorgegaan. Het is zo waardevol dat jullie zo'n sterke band opbouwen met alle kleinkinderen. Ik ben dankbaar voor mijn fijne jeugd en ook voor het feit dat jullie er altijd zijn voor ons, wat er ook gebeurt. Ik ben echt heel trots op jullie!

Allerliefste Willemijn: we missen je zo erg. Het blijft onwerkelijk en het zal nooit wennen. Met de veertjes zien we je terug, het is heel troostend om dat echt zo te voelen. Wat zou je trots op mij zijn geweest, je kon zo genieten voor een ander. Zelfs in de laatste periode had je altijd interesse voor anderen, in wat zij voelden of beleefden. Onze levensles is de enorme dankbaarheid die je uitstraalde waardoor we met gevoelens van geluk en rust het verdriet enigszins lijken te kunnen dragen. We zullen op dit soort dagen van mijlpalen de leegte weer extra voelen. Bij een concert van The Script waar we samen waren zong je huilend mee: “turn the pain into power”. Ik heb het laten terugkomen in mijn stellingen en er naar geleefd de afgelopen jaren. Een van de vele laatste dingen die je tegen me zei was: wees lief voor jezelf. Wat kende me je goed. Ik zal genieten, en ik zal aan je denken en jou zo missen maar visualiseren dat je erbij bent met je stralende sprankelende lach.

Mijn lieve prachtige kids, ik hou zielsveel van jullie en vond het elke keer zo moeilijk om na zwangerschapsverlof weer te gaan werken, maar alles kwam goed. Ik ben zo benieuwd naar jullie toekomst maar ik geniet van elke fase. Ik heb de eer om jullie te zien opgroeien tot nu al zulke zelfstandige en sociale personen vol energie (net als papa). Ieder met uniek karakter en ook samen echt een team (met steeds minder ruzie). Het is uniek dat jullie bij mijn promotie kunnen zijn, dat is dan weer een groot voordeel dat ik er zo lang over heb gedaan. Mijn werk vergt soms veel energie en tijd maar jullie zijn zo lief en begripvol en snappen mijn ambitie. Siebe: alleskunner, doorzetter en zo lief en begripvol voor een ander. Het is altijd EN EN in plaats van OF OF, net als je moeder. Dat gaat je ver brengen, maar vergeet niet af en toe uit te rusten! Douwe: kleine grappige netwerker van me. Jij kent iedereen en iedereen kent jou. Ik probeer te leren van jouw relativeringsvermogen. Je moet niet overal je spullen vergeten maar verander verder niet! Mijntje: blije pittige kletskous. De creatieve wereld ligt aan je voeten, daar ga jij zeker je eigen weg in vinden. Blijf altijd vragen stellen zoals je nu doet. Je bent altijd bezig met creëren en hebt een talent voor presenteren en theater. Je kan niet wachten tot je groot bent maar blijf nu nog maar even ons kleine lieve meisje.

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ABOUT THE AUTHOR

Dieneke Schonenberg-Meinema was born on 23 February 1977 in Rijnsburg where she grew up as the oldest of 4 siblings. In 1995 she completed high school at the Rijnlands Lyceum in Oegstgeest. From 1995-1997 she first studied ‘Medical Biology’ for two years at the ‘Vrije Universiteit’ in Amsterdam. In 1997 she started to study Medicine. In 2004, she graduated cum laude (with honor). She did the last intern ship in a rural hospital in Cameroon where she also started the foundation ‘Give Milk Stop Aids’ with one of her best friends Caroline. This foundation has helped many families with HIV-positive mothers during a decade. In 2004 she started her first job as a resident in pediatrics in the ‘Groene Hart Ziekenhuis’ in Gouda after which she specialized in pediatrics in the ‘Leids Universitair Medisch Centrum’. In 2011, she started a fellowship Pediatric Rheumatology in Amsterdam UMC. This specialization was finished in 2016, from that time up till present she is a staff member of the Emma Children’s Hospital from Amsterdam UMC.

In 2015 she observed abnormalities in her patients with nailfold capillaroscopy. She reached out for collaboration with experts in this field from Belgium and Italy. This subsequently led to the beginning of many research projects. In 2016 she received a research grant from the Emma Foundation for a pilot study. In 2017 her longitudinal cohort study proposal was approved by the medical ethical committee of Amsterdam UMC. The first results are written in this thesis, a second PhD student is working on the following ongoing projects. In 2018 Dieneke started a very fruitful collaboration with Sylvia Kamphuis, a pediatric rheumatologist from Erasmus MC Rotterdam. They decided to work closely together and work towards a national database of these patients which has been executed in 2022.

Dieneke is married to Aloys Meinema since 2007, together they have three children: Siebe (2008), Douwe (2010) and Mijntje (2013) and two cats Queen W (2019) and Winston Churchill (2021). In her free time she likes to spend time at the dinner table with friends and family or look at the sport activities from her children. She also likes to do running, fitness, tennis and skiing and loves to travel with her family, especially to Asia. Her youngest sister passed away in March 2020 due to metastatic breast cancer, at the young age of 33. She would say: “Vier het leven zonder spijt”, which is the last quote of this PhD thesis, in her reminiscence.