Systemic lupus erythematosus : from diagnosis to prognosis

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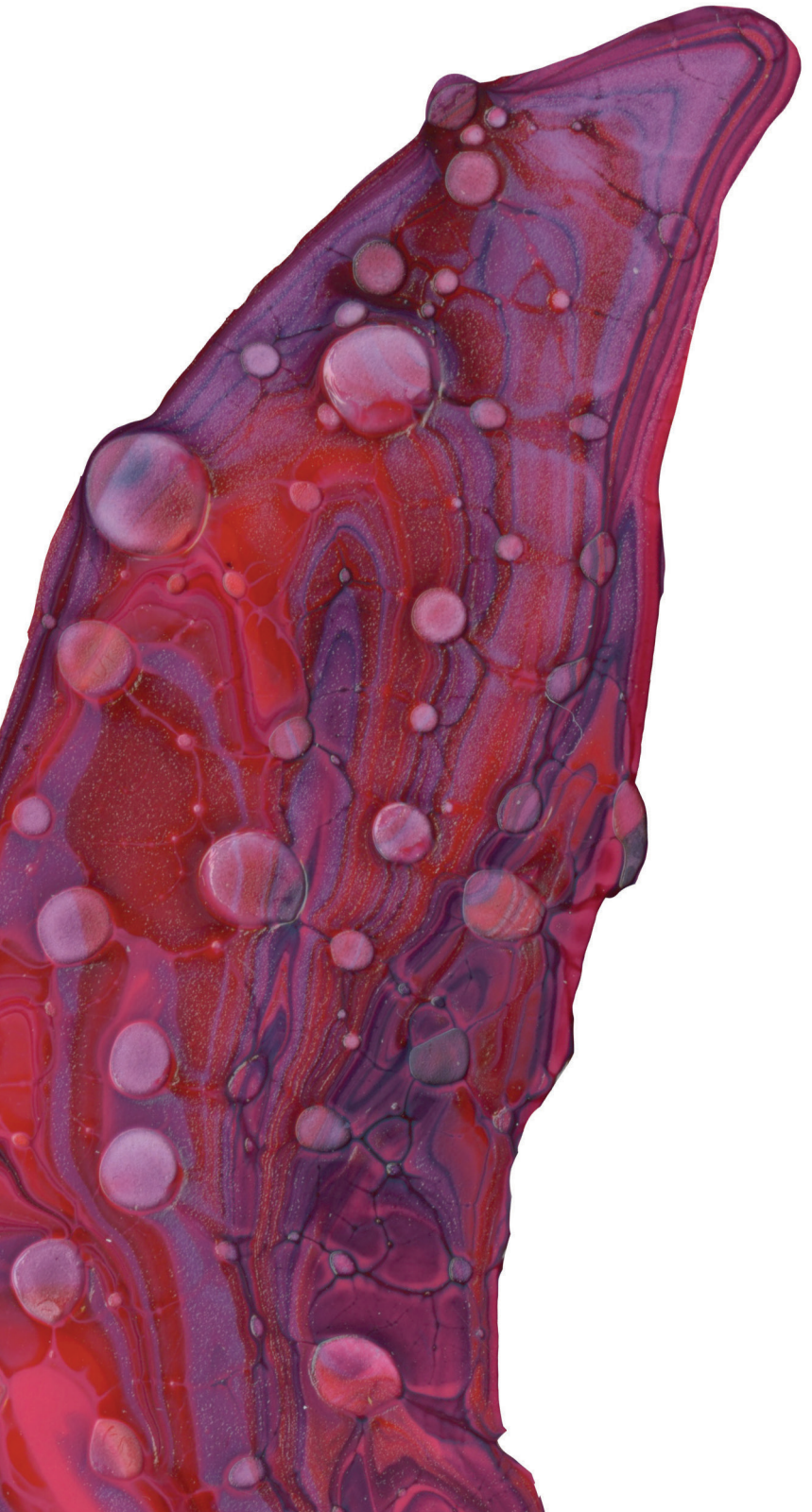

Chapter 8

Increased Microchimerism in Peripheral Blood of Women with Systemic Lupus Erythematosus

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ABSTRACT

Background

Microchimerism may play a role in the pathogenesis of systemic lupus erythematosus (SLE). Using an innovative technique permitting more sensitive and specific detection of chimeric cells than previous studies, we aimed to determine the origin and amount of microchimerism in peripheral blood of women with SLE and controls.

Methods

We investigated the relationship between microchimerism and disease onset, activity, and damage accrual. We included 11 SLE patients and 22 controls, their children, and if possible, their mothers. Quantitative PCR for insertion-deletion polymorphisms and null alleles was used to detect microchimerism in peripheral blood mononuclear cells and granulocytes.

Results

Microchimerism was detected more often in patients than controls (54.4% vs. 13.6%, P=0.03). In 50% of SLE patients with microchimerism, it originated from multiple relatives, whereas in controls microchimerism was always derived from one relative. Microchimerism was mostly of fetal origin, and the median number of fetal chimeric cells was $5/10^6$ in patients and $2.5/10^6$ in controls (P=0.048). We found no relationship between microchimerism and clinical or laboratory parameters.

Conclusions

Apart from demonstrating that microchimerism occurs more frequently in SLE patients than in controls, this study provides novel, thought-provoking evidence that microchimerism in SLE can be derived from multiple relatives.

INTRODUCTION

Microchimerism refers to the presence in an individual of a small number of genetically distinct cells of any type, originating from a different zygote. The most common (physiologic) source of microchimerism is pregnancy.[1] During pregnancy, fetal cells can enter the maternal circulation leading to fetal microchimerism in the mother. When maternal cells cross the placental barrier to the fetus, this can lead to maternal microchimerism. Pregnancies of all terms, including both miscarriages and pregnancies resulting in (live) birth, may lead to microchimerism.[2-5]

The role of microchimerism in health and disease is unclear. Microchimerism has been suggested to play a role in several autoimmune diseases, including systemic lupus erythematosus (SLE).[6-9] SLE is an autoimmune disease that mainly affects women and has a peak incidence in the reproductive years.[10] Studies in mice showed that, in selected parent-to-F1 combinations, injection of parental lymphocytes in their offspring led to a graft-versus-host response and a lupus-like disease.[11, 12] These data suggest that pregnancy-acquired microchimerism may be of pathogenic significance in the development of SLE.

Women with SLE have a significantly higher prevalence of fetal Y chromosome-positive chimeric cells in tissue than healthy controls.[13-15] A number of studies showed that also in peripheral blood, there is an increased frequency of fetal microchimerism in SLE patients compared to controls,[16, 17] whereas other studies showed no differences between patients and controls.[18, 19] Previous studies on fetal microchimerism in SLE were limited to the detection of male microchimerism, thereby underestimating the total amount of microchimerism. Furthermore, because microchimerism was mostly studied in whole blood, the phenotype of the chimeric cells could not be determined. Maternal microchimerism in SLE in peripheral blood was studied by Kanold *et al.*, and they did not find a difference between patients and controls.[20] However, their sensitivity of detecting chimeric cells was relatively low. In none of these studies were fetal and maternal microchimerism investigated together.

The aim of our study was to determine the presence and amount of microchimerism in peripheral blood of SLE patients and compare it to healthy controls. We studied peripheral blood mononuclear cells (PBMCs) and granulocytes separately to determine if microchimerism was present in either subset, or both. In our study we used insertion-deletion polymorphisms (indels) or null alleles for the detection of microchimerism, thus enabling us to study the origin of the chimeric cells: either fetal, maternal, or both. Also, we were able to establish whether microchimerism was derived from one relative or from multiple relatives. Furthermore, in order to find a clue as to the role of microchimerism in SLE, we investigated the relationship between disease activity and accumulated damage since the onset of SLE, and the presence of microchimerism. Finally, the temporal relationship between the chimerism-causing pregnancy and disease onset in SLE patients was studied.

METHODS

Ethics statement

This study was approved by the Medical Ethics Committee of the Leiden University Medical Center (LUMC) (P09.047). All research was conducted in accordance with the ethical research standards of the LUMC and in compliance with the Declaration of Helsinki. Informed consent was obtained from all participants. The parents of minors gave written consent on their behalf.

Patients and controls

Eleven female SLE patients and 22 female controls were studied. From 2010 to 2015 SLE patients were recruited from four hospitals in the Netherlands: University Medical Center Groningen, Radboud University Medical Center Nijmegen, Bronovo Hospital The Hague, and Meander Medical Center Amersfoort. All included patients fulfilled at least four of the 1998 revised American College of Rheumatology Criteria for the classification of SLE.[21] SLE disease activity was determined using the SLE Disease Activity Index 2000 (SLEDAI-2K).[22] Accumulated damage since SLE onset was measured using the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SDI).[23] The control group consisted of women without a history of autoimmune disease. For inclusion in the study, probands (SLE patients and controls) were required to have at least one child of at least 18 years old. The probands' children, as well as their mothers, were invited to participate. Peripheral blood samples were gathered from the probands, as well as either peripheral blood samples or buccal mouth swabs from their children and mothers. All probands were asked to fill out a questionnaire including their age, ethnicity, reproductive history, history of blood transfusion, use of immunosuppressive medication, and medical history.

Isolation of peripheral blood subsets

Peripheral venous blood samples were drawn in sodium-heparin solution vacutainer tubes (Becton Dickinson, Franklin Lakes, New Jersey) and processed to isolate peripheral blood mononuclear cells (PBMCs) by Ficoll amidotrizoate (pharmacy LUMC) with density gradient centrifugation 1.077 g/mL. Erythrolysis (Qiagen, Hilden, Germany) was performed to remove the erythrocytes from the remaining granulocytes. Samples were stored in 10% dimethyl sulfoxide in fetal bovine serum at −180 °C until DNA extraction.

DNA extraction

DNA was extracted from PBMCs and granulocytes using the QIAamp DNA Blood Mini Kit (Qiagen) according to the manufacturer's instructions with modifications. Briefly, we added 40 µL of proteinase K to $5*10^6$ cells suspended in 200 µL phosphate-buffered saline. After adding 400 µL AL buffer, the suspension was incubated for 30 min (PBMCs) or overnight (granulocytes) at 56 °C. After adding 200 µL of ethanol, the mixture was applied to the Mini spin column. Buffers AW1 and AW2 were used to wash the column,

after which 100 µl AE buffer was added and incubated at 70 °C for 10 minutes to elute the DNA. The eluate was reapplied for an optimal yield. All DNA samples were stored at 4 °C until quantitative PCR (qPCR). DNA extraction from buccal sterile OmniSwabs (GE Healthcare Life Sciences, Little Chalfont, United Kingdom) was performed with the same kit and according to the manufacturer's instructions.

Allele informativity and genotyping

For the detection of fetal and maternal microchimerism a set of previously published indels and null alleles was used.[24-26] In order to detect fetal and maternal microchimerism in the proband, and to discriminate between the proband's children and mother, informative alleles to distinguish the different family members were required. Maternal DNA was available for six of 11 patients and eight of 22 controls. There was no fetal DNA available for any of the miscarriages. Genotyping by qPCR was performed using the same protocol as described below, but with a DNA input of 20 ng. Of the published sets of null alleles and indels, 19 were informative in our study population: GSTM1, GSTT1, SRY, RhD (null alleles), and S01a, S01b, S03, S04a, S04b, S05b, S07b, S08b, S09b, S10a, S10b, S11a, S11b, Xq28 and R271 (indels). The primer sequences are listed in **Appendix 8.1**.

Detection of chimerism by qPCR

Fetal and maternal microchimerism were detected and quantified by qPCR. In all assays iQ SYBR Green Supermix (Bio-Rad, Hercules, California) was used, with 7.5 µM of each amplification primer. The amplification and melting conditions for all primers consisted of incubation at 96.5 °C for 10 min, followed by 44 cycles of 96.5 °C for 30 s and 60 °C for 1 min. The melting curve started at 65 °C for 5 s followed by 0.2 °C incremental increase, each lasting 5 s, to 95 °C. Amplification and melting data were collected by a Bio-Rad CFX96 detector and analysed by Bio-Rad CFX Manager version 3.1.

Primer specificity was ensured by Sanger sequencing of the amplification product and comparing the sequences to known genomic DNA sequences. Sensitivity was determined by testing serial dilutions of DNA positive for the indel or null allele in a background of DNA negative for the respective indel or null allele. A sensitivity of one genome equivalent (gEq, based on 6.6 pg DNA content per cell) in 100,000 gEq was reached for all primersets. Four aliquots, each containing 660 ng DNA (100,000 gEq), were tested in each subset (PBMC or granulocytes) for every proband. A standard curve for the specific assay was included to quantify the chimeric cells and validate the assay on each plate. The standard curve consisted of 100, 10, and 1 gEq spiked DNA per 100,000 gEq background DNA. Every sample was also tested for the housekeeping gene GAPDH. Results were expressed as the gEq of chimeric cells per one million gEq (gEq/10^6). Negative controls consisting of either a water control or background DNA not carrying the indel or null allele tested, were included in each qPCR plate. Negative controls were consistently negative across all experiments. If there was any doubt as to the specificity of the amplification product, the length of the PCR product was compared to that of the positive control using QIAxcel Advanced System (Qiagen) according to the manufacturer's protocol.

Anti-contamination procedures

Strict anti-contamination procedures were employed during blood workup, DNA extraction, and qPCR preparation. Aerosol-resistant pipette tips and clean gloves were always used. The blood workup was performed in a laminar flow cabinet. Before DNA extraction or preparation of the qPCR, the cabinet used was thoroughly cleaned with DNA decontamination reagent (Sigma-Aldrich, St. Louis, Missouri), and irradiated with UV light for one hour. All lab consumables were certified DNA free, and also irradiated with UV light for one hour. For the qPCR, 8-well strips with individual lids were used.

Statistical analysis

For the comparison of categorical data, Fisher's exact tests were used (history of blood transfusion, presence of microchimerism). Student's *t*-tests were used to compare normally distributed data (age proband, age eldest child, age youngest child, SDI). For the comparison of non-normally distributed numerical data, Mann-Whitney U tests were used (number of pregnancies, number of children, number of chimeric cells, SLEDAI-2K). A *P*-value <0.05 was considered statistically significant. All analyses were performed using SPSS Statistics 20.0 (IBM, Armonk, New York).

RESULTS

Baseline characteristics of patients and controls are shown in **Table 1**. SLE patients had microchimerism more often than controls (54.4% vs. 13.6%, $P=0.03$). When microchimerism was present, the median total number of fetal chimeric cells per proband was higher in the patient group than in the control group (5 gEq/10^6 vs. 2.5 gEq/10^6 [$P=0.048$]) (**Table 2**). When comparing patients and controls with and without microchimerism, there was no difference in age, number of children, number of pregnancies, history of blood transfusion, disease activity (SLEDAI-2K) or accumulated damage since disease onset (SDI) (**Table 3**). Also, there was no difference in the use of immunosuppressive medication between patients with and patients without microchimerism (data not shown).

All patients and controls, except one, with detectable microchimerism had fetal microchimerism (**Table 4**). Of the eight controls with maternal DNA available, one had detectable maternal microchimerism. Of the six SLE patients with maternal DNA available, one had detectable maternal microchimerism, three did not, and of two the possible maternal microchimerism was indistinguishable from the fetal microchimerism that was present, due to an overlap in indels and null alleles.

Table 1 Baseline characteristics of SLE patients and controls.

Characteristic	SLE patients (n=11)	Controls (n=22)	*P*
Age proband, y	56.6 ± 5.5	57.2 ± 5.5	0.79[a]
Age eldest child, y	31.4 ± 5.2	28.9 ± 5.7	0.24[a]
Age youngest child, y	27.5 ± 5.3	24.4 ± 4.9	0.11[a]
Number of children	2 (1)	2 (1)	0.60[b]
Number of pregnancies	3 (2)	2.5 (1)	0.37[b]
History of blood transfusion, %	72.7	13.6	0.001[c]
SLEDAI-2K	0 (4)	-	n/a
SDI	2.2 ± 2.3	-	n/a

Results are shown as mean ±SD or as median (interquartile range), unless otherwise specified. *P*-values were assessed with [a] Student's *t*-test, [b] Mann-Whitney U test, or [c] Fisher's exact test. SLE, systemic lupus erythematosus; SLEDAI-2K, SLE Disease Activity Index 2000; SDI, Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index.

Table 2 Microchimerism in SLE patients and controls.

Parameter	SLE patients (n=11)	Controls (n=22)	*P*
Microchimerism present in PBMCs or granulocytes, %	54.5	13.6	0.03[a]
Microchimerism present in PBMCs, %	36.4	9.1	0.15[a]
Microchimerism present in granulocytes, %	40.0 (n=10)	10.0 (n=20)	0.14[a]
Total number of fetal chimeric cells/proband, when microchimerism is present, gEq/10^6 gEq	5 (8.1) (n=6)	2.5 (n/a) (n=3)	0.048[b]

Results are shown as median (interquartile range), unless otherwise specified. *P*-values were assessed with [a] Fisher's exact test or [b] Mann-Whitney U test. gEq, genome equivalents; n/a, not applicable because number of cases is too low to provide an interquartile range; PBMCs, peripheral blood mononuclear cells; SLE, systemic lupus erythematosus.

Table 3 Comparison of SLE patients and controls with and without microchimerism.

	SLE patients			Controls		
Characteristic	No micro-chimerism (n=5)	Micro-chimerism (n=6)	*P*	No micro-chimerism (n=19)	Micro-chimerism (n=3)	*P*
Age proband, y	57.4 ± 5.2	56.0 ± 6.3	0.70[a]	57.6 ± 5.6	54.3 ± 4.3	0.33[a]
Number of children	2 (1)	2.5 (1.25)	0.84[b]	2 (1)	3 (n/a)	0.44[b]
Number of pregnancies	2 (1.5)	3.5 (2)	0.33[b]	2 (1)	3 (n/a)	0.71[b]
Blood transfusion, %	60.0	83.3	0.55[c]	10.5	33.3	0.37[c]
SLEDAI-2K	0 (2.5)	2 (5.5)	0.37[b]	-	-	-
SDI	1.8 ± 2.0	2.5 ± 2.6	0.64[a]	-	-	-

Results are shown as mean ± SD or as median (interquartile range), unless otherwise specified. *P*-values were assessed with [a] Student's *t*-test, [b] Mann-Whitney U test, or [c] Fisher's exact test. n/a, not applicable because number of cases is too low to provide an interquartile range; SLE, systemic lupus erythematosus; SLEDAI-2K, SLE Disease Activity Index 2000; SDI, Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index.

Table 4 Origin of microchimerism in patients and controls.

Subject	Relatives[a]	Chimerism in PBMCs	
		Origin	*Amount, gEq/10⁶*
Patients			
1	Mother, daughter 1, miscarriage (n/a), daughter 2, son	Mother	16.5
		Daughter 1 or 2	2.5
		Son	2.5
2	Mother, daughter 1, 2 and 3	Daughter 1	5
3	Mother, daughter, son	Son	2.5
		-	
4	Mother (n/a), miscarriages 1, 2 and 3 (n/a), daughter	-	-
5	Mother (n/a), daughter 1 (deceased, n/a), miscarriage (n/a), daughter 2 (n/a), daughter 3	-	-
6	Mother (n/a), son, daughter	Daughter	5
Controls			
1	Mother (n/a), daughter 1 and 2, son	Daughter 2	2.5
2	Mother (n/a), daughter, son	-	-
3	Mother, daughter 1, daughter 2, son	Mother	2.5

[a] Chronologically from old to young based on year of birth/miscarriage. [b] due to technical problems with the material from the first blood draw, a second blood draw was done approximately 1 year later. gEq, genome equivalent; n/a, no DNA available; PBMCs, peripheral blood mononuclear cells; SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index 2000; SDI, Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index.

Also, we determined if the microchimerism originated from one relative or more. In all three controls with detectable microchimerism, it originated from one relative (either one child or mother). In contrast, in at least three of the six SLE patients the microchimerism originated from more than one relative, either from multiple children or from a child and mother (**Table 4**).

Finally, in patients with detectable microchimerism, we did not find a pattern in the temporal relationship between chimerism-causing pregnancies and the start of symptoms or diagnosis of SLE. One patient had her first symptoms prior to her first pregnancy, and experienced an exacerbation of symptoms during her first pregnancy, which resulted in a spontaneous miscarriage. Two patients experienced their first symptoms during their second pregnancy. In one of these patients, this pregnancy resulted in a spontaneous miscarriage. Therefore, it could not be determined if this pregnancy resulted in long-lasting microchimerism. In the other of the two patients, both her first and second pregnancy resulted in long-lasting microchimerism. Finally, three of six patients experienced their first symptoms one, seven, and ten years after the birth of their youngest child of whom microchimerism was detected.

Chimerism in granulocytes		Blood transfusion history	SLEDAI-2K	SDI
Origin	*Amount, gEq/10^6*			
n/a	n/a	Yes	10	4
Daughter 3	7.5	Yes	4	0
Daughter 2 or mother Possibly daughter 1	2.5			
Daughter, and possibly mother	2.5	Yes	0 (1st blood draw[b])	2
-			0 (2nd blood draw[b])	2
Daughter	15	Yes	4	7
Daughter 3	5	Yes	0	1
-	-	No	0	1
Daughter 2	2.5	Yes	-	-
Daughter	2.5	No	-	-
-		No	-	-

DISCUSSION

Our study demonstrates that female SLE patients have detectable microchimerism in their peripheral blood more often than female controls. In almost all cases with detectable microchimerism, the origin of the chimeric cells was fetal. Additionally, in one patient and one control, maternal microchimerism was detected. The median total number of fetal chimeric cells in individuals with detectable microchimerism was higher in patients than in controls. Also, SLE patients often had chimeric cells originating from multiple relatives, in contrast to the controls, in whom the chimeric cells originated from only one relative.

In accordance with our results, two previous studies found fetal microchimerism in peripheral blood more often in SLE patients than in controls,[16, 17] while two other studies did not.[18, 19] Differences in the blood compartment tested (PBMCs or whole blood), the specificities and sensitivities of the different techniques used, and the numbers of patients and controls included, may account for these conflicting results. In contrast to our study, previous studies did not demonstrate a difference between SLE patients and controls

in the number of chimeric cells present. However, a limitation of these studies was that exclusively the presence of the Y chromosome was investigated, thereby only detecting male microchimerism. Our approach allowed us to detect both male and female fetal microchimerism, as well as maternal microchimerism. If, in our present study, we had only investigated microchimerism using the Y chromosome in women with at least one son, we would not have found a statistically significant difference in the occurrence of microchimerism between patients and controls (data not shown). Only one study investigated the presence of maternal microchimerism in SLE and found no difference between patients and controls with maternal microchimerism occurring in 6% and 3%, respectively.[20] This low prevalence of maternal microchimerism is in accordance with our results.

In the literature, there are indications that within one individual some sources of microchimerism lead to persistent microchimerism, while others do not. In one case report about a woman with hepatitis C, the detected chimerism in the liver seemed to originate from only one of her five pregnancies.[27] Also, after blood transfusions, it was shown that in the majority of cases with transfusion-associated microchimerism, there was evidence of only one or two non-recipient HLA-DR alleles, suggesting that the microchimerism commonly involves only one donor despite some patients receiving blood products from multiple donors.[28] However, in women with multiple children, it has not been systematically studied if there is a "favored-child" with regard to the persistence of microchimerism, i.e. if fetal microchimerism usually originates from one of the children, or from more children. Because we used indels and null alleles for the detection of microchimerism, we were able to show that at least half of the patients had persistent microchimerism from multiple relatives while all controls only had persistent microchimerism from one relative. The cause of this phenomenon is largely unknown. Studies in animals have demonstrated that syngenic or congenic matings resulted in more chimerism than allogenic matings, suggesting a role for HLA (mis)matches.[29, 30] In humans, in certain autoimmune diseases mothers and children were shown to have fewer HLA disparities,[31, 32] but these have not yet been significantly correlated to the presence of microchimerism.[31] Nevertheless, having a certain HLA allele (HLA DQA1*0501) appears to be associated with the presence of fetal microchimerism.[33, 34] Interestingly, HLA DQA1*0501 has also been associated with SLE.[35]

The phenotype of a chimeric cell may affect the potential of a chimeric cell to result in to persistent microchimerism. We detected microchimerism in both PBMCs and granulocytes. Considering the relatively short half-life of granulocytes,[36] it is likely that the chimeric cells detected in this compartment are derived from stem cells. The existence of chimeric fetal progenitor cells was demonstrated in several studies (reviewed by Seppanen *et al.*[37]). A higher prevalence of microchimerism in SLE patients than in controls can either mean that (i) more chimeric cells were acquired during pregnancy, (ii) more chimeric cells persisted after pregnancy, (iii) chimeric stem cells gave rise to more chimeric cells due to an unknown trigger, or (iv) a combination of aforementioned possibilities.

SLE patients had a blood transfusion in their history significantly more often than controls. However, within the groups of SLE patients and controls, we did not find a difference in blood transfusion history between subjects with and subjects without detectable microchimerism. In the literature, persistent chimerism was only described after blood transfusion following traumatic injury (reviewed by Bloch *et al.*[38]), which was not the indication for a blood transfusion in any of our subjects. Furthermore, a recent study in patients having received a blood transfusion in the peripartum period, like some of our subjects, did not show microchimerism at six weeks and six months after pregnancy.[39] Therefore, it is unlikely that the difference in blood transfusion history between patients and controls explains our results.

In our study there was no difference in disease activity (SLEDAI-2K) or accumulated damage (SDI) between patients with and without microchimerism. The former result is in line with a previous study.[19] Possible reasons for these findings are the absence of such associations or the small sample size. Also, many of the SLE patients were in clinical remission.

A limitation of our study is that we did not have maternal DNA available for all subjects. This means that we could not exclude a maternal source of the microchimerism in all cases. In a few patients of whom we did have maternal DNA available, it was not always possible to distinguish maternal microchimerism from the detected fetal microchimerism, due to an overlap in genetic markers. Furthermore, it was not possible to formally exclude all possible sources of microchimerism, such as unrecognised pregnancies or spontaneous abortions of which DNA was unavailable.

In summary, we detected microchimerism in peripheral blood more often and in higher numbers in female SLE patients than in female controls. The microchimerism detected was predominantly fetal in origin and was found in both PBMCs and granulocytes. Interestingly, this study provides the first evidence that SLE patients can have chimeric cells from multiple relatives, while all of the chimeric controls had chimeric cells from only one relative. Attempts to explain this phenomenon are speculative. It may depend on the immune response evoked by specific chimeric cells, possibly relating to HLA, or on the activation status of the immune system of the recipient in general. Future studies addressing the immunologic aspects of this phenomenon are called for. The exact role of chimeric cells in SLE is still unknown, but our data substantiate the hypothesis that chimeric cells do play a role in SLE.

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REFERENCES

1. Walknowska J, Conte FA, Grumbach MM. Practical and theoretical implications of fetal-maternal lymphocyte transfer. Lancet. 1969; 293(7606): 1119-22.
2. Sato T, Fujimori K, Sato A, Ohto H. Microchimerism after induced or spontaneous abortion. Obstet Gynecol. 2008; 112(3): 593-7.
3. Peterson SE, Nelson JL, Guthrie KA, Gadi VK, Aydelotte TM, Oyer DJ, *et al.* Prospective assessment of fetal-maternal cell transfer in miscarriage and pregnancy termination. Hum Reprod. 2012; 27(9): 2607-12.
4. Ariga H, Ohto H, Busch MP, Imamura S, Watson R, Reed W, *et al.* Kinetics of fetal cellular and cell-free DNA in the maternal circulation during and after pregnancy: implications for noninvasive prenatal diagnosis. Transfusion. 2001; 41(12): 1524-30.
5. Rijnink EC, Penning ME, Wolterbeek R, Wilhelmus S, Zandbergen M, van Duinen SG, *et al.* Tissue microchimerism is increased during pregnancy: a human autopsy study. Mol Hum Reprod. 2015; 21(11): 857-64.
6. Artlett CM, Smith JB, Jimenez SA. Identification of Fetal DNA and Cells in Skin Lesions from Women with Systemic Sclerosis. N Engl J Med. 1998; 338(17): 1186-91.
7. Reed AM, Picornell YJ, Harwood A, Kredich DW. Chimerism in children with juvenile dermatomyositis. Lancet. 2000; 356(9248): 2156-7.
8. Nelson JL, Gillespie KM, Lambert NC, Stevens AM, Loubiere LS, Rutledge JC, *et al.* Maternal microchimerism in peripheral blood in type 1 diabetes and pancreatic islet beta cell microchimerism. Proc Natl Acad Sci U S A. 2007; 104(5): 1637-42.
9. Johnson KL, McAlindon TE, Mulcahy E, Bianchi DW. Microchimerism in a female patient with systemic lupus erythematosus. Arthritis Rheum. 2001; 44(9): 2107-11.
10. Lisnevskaia L, Murphy G, Isenberg D. Systemic lupus erythematosus. Lancet. 2014; 384(9957): 1878-88.
11. Via CS, Shearer GM. T-cell interactions in autoimmunity: insights from a murine model of graft-versus-host disease. Immunol Today. 1988; 9(7-8): 207-13.
12. Gleichmann E, Van Elven EH, Van der Veen JP. A systemic lupus erythematosus (SLE)-like disease in mice induced by abnormal T-B cell cooperation. Preferential formation of autoantibodies characteristic of SLE. Eur J Immunol. 1982; 12(2): 152-9.
13. Kremer Hovinga I, Koopmans M, Baelde HJ, van der Wal AM, Sijpkens YW, de Heer E, *et al.* Chimerism occurs twice as often in lupus nephritis as in normal kidneys. Arthritis Rheum. 2006; 54(9): 2944-50.
14. Kremer Hovinga I, Koopmans M, Baelde HJ, de Heer E, Bruijn JA, Bajema IM. Tissue chimerism in systemic lupus erythematosus is related to injury. Ann Rheum Dis. 2007; 66(12): 1568-73.
15. Florim GM, Caldas HC, de Melo JC, Baptista MA, Fernandes IM, Savoldi-Barbosa M, *et al.* Fetal microchimerism in kidney biopsies of lupus nephritis patients may be associated with a beneficial effect. Arthritis Res Ther. 2015; 17(1): 101.
16. Kekow M, Barleben M, Drynda S, Jakubiczka S, Kekow J, Brune T. Long-term persistence and effects of fetal microchimerisms on disease onset and status in a cohort of women with rheumatoid arthritis and systemic lupus erythematosus. BMC Musculoskelet Disord. 2013; 14: 325.
17. da Silva Florim GM, Caldas HC, Pavarino EC, Bertollo EM, Fernandes IM, Abbud-Filho M. Variables associated to fetal microchimerism in systemic lupus erythematosus patients. Clin Rheumatol. 2016; 35(1): 107-11.
18. Gannage M, Amoura Z, Lantz O, Piette JC, Caillat-Zucman S. Feto-maternal microchimerism in connective tissue diseases. Eur J Immunol. 2002; 32(12): 3405-13.

19. Mosca M, Curcio M, Lapi S, Valentini G, D'Angelo S, Rizzo G, *et al.* Correlations of Y chromosome microchimerism with disease activity in patients with SLE: analysis of preliminary data. Ann Rheum Dis. 2003; 62(7): 651-4.
20. Kanold AMJ, Svenungsson E, Gunnarsson I, Götherström C, Padyukov L, Papadogiannakis N, *et al.* A Research Study of the Association between Maternal Microchimerism and Systemic Lupus Erythematosus in Adults: A Comparison between Patients and Healthy Controls Based on Single-Nucleotide Polymorphism Using Quantitative Real-Time PCR. PLoS One. 2013; 8(9): e74534.
21. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1997; 40(9): 1725.
22. Gladman DD, Ibanez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. J Rheumatol. 2002; 29(2): 288-91.
23. Gladman D, Ginzler E, Goldsmith C, Fortin P, Liang M, Urowitz M, *et al.* The development and initial validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index for systemic lupus erythematosus. Arthritis Rheum. 1996; 39(3): 363-9.
24. Alizadeh M, Bernard M, Danic B, Dauriac C, Birebent B, Lapart C, *et al.* Quantitative assessment of hematopoietic chimerism after bone marrow transplantation by real-time quantitative polymerase chain reaction. Blood. 2002; 99: 4618-25.
25. Jimenez-Velasco A, Barrios M, Roman-Gomez J, Navarro G, Buno I, Castillejo JA, *et al.* Reliable quantification of hematopoietic chimerism after allogeneic transplantation for acute leukemia using amplification by real-time PCR of null alleles and insertion/deletion polymorphisms. Leukemia. 2005; 19(3): 336-43.
26. Pujal JM, Gallardo D. PCR-based methodology for molecular microchimerism detection and quantification. Exp Biol Med (Maywood). 2008; 233(9): 1161-70.
27. Johnson KL, Samura O, Nelson JL, McDonnell MdWM, Bianchi DW. Significant fetal cell microchimerism in a nontransfused woman with hepatitis C: Evidence of long-term survival and expansion. Hepatology. 2002; 36(5): 1295-7.
28. Utter GH, Owings JT, Lee TH, Paglieroni TG, Reed WF, Gosselin RC, *et al.* Blood transfusion is associated with donor leukocyte microchimerism in trauma patients. J Trauma. 2004; 57(4): 702-7; discussion 7-8.
29. Khosrotehrani K, Johnson KL, Guegan S, Stroh H, Bianchi DW. Natural history of fetal cell microchimerism during and following murine pregnancy. J Reprod Immunol. 2005; 66(1): 1-12.
30. Bonney EA, Matzinger P. The maternal immune system's interaction with circulating fetal cells. J Immunol. 1997; 158: 40-7.
31. Nelson JL, Furst DE, Maloney S, Gooley T, Evans PC, Smith A, *et al.* Microchimerism and HLA-compatible relationships of pregnancy in scleroderma. Lancet. 1998; 351(9102): 559-62.
32. Stevens AM, Tsao BP, Hahn BH, Guthrie K, Lambert NC, Porter AJ, *et al.* Maternal HLA class II compatibility in men with systemic lupus erythematosus. Arthritis Rheum. 2005; 52(9): 2768-73.
33. Lambert NC, Evans PC, Hashizumi TL, Maloney S, Gooley T, Furst DE, *et al.* Cutting edge: persistent fetal microchimerism in T lymphocytes is associated with HLA-DQA1*0501: implications in autoimmunity. J Immunol. 2000; 164(11): 5545-8.
34. Reed AM, McNallan K, Wettstein P, Vehe R, Ober C. Does HLA-dependent chimerism underlie the pathogenesis of juvenile dermatomyositis? J Immunol. 2004; 172(8): 5041-6.
35. Martin-Villa JM, Martinez-Laso J, Moreno-Pelayo MA, Castro-Panete MJ, Martinez-Quiles N, Alvarez M, *et al.* Differential contribution of HLA-DR, DQ, and TAP2 alleles to systemic lupus erythematosus susceptibility in Spanish patients: role of TAP2*01 alleles in Ro autoantibody production. Ann Rheum Dis. 1998; 57(4): 214-9.
36. Tak T, Tesselaar K, Pillay J, Borghans JA, Koenderman L. What's your age again? Determination of human neutrophil half-lives revisited. J Leukoc Biol. 2013; 94(4): 595-601.
37. Seppanen E, Fisk NM, Khosrotehrani K. Pregnancy-acquired fetal progenitor cells. J Reprod Immunol. 2013; 97(1): 27-35.

38. Bloch EM, Jackman RP, Lee TH, Busch MP. Transfusion-associated microchimerism: the hybrid within. Transfus Med Rev. 2013; 27(1): 10-20.

39. Bloch EM, Busch MP, Lee TH, Montalvo L, Matthews Y, Bird A, *et al.* Microchimerism in the transfused obstetric population. Vox Sang. 2014; 107(4): 428-30.