Systemic lupus erythematosus : from diagnosis to prognosis

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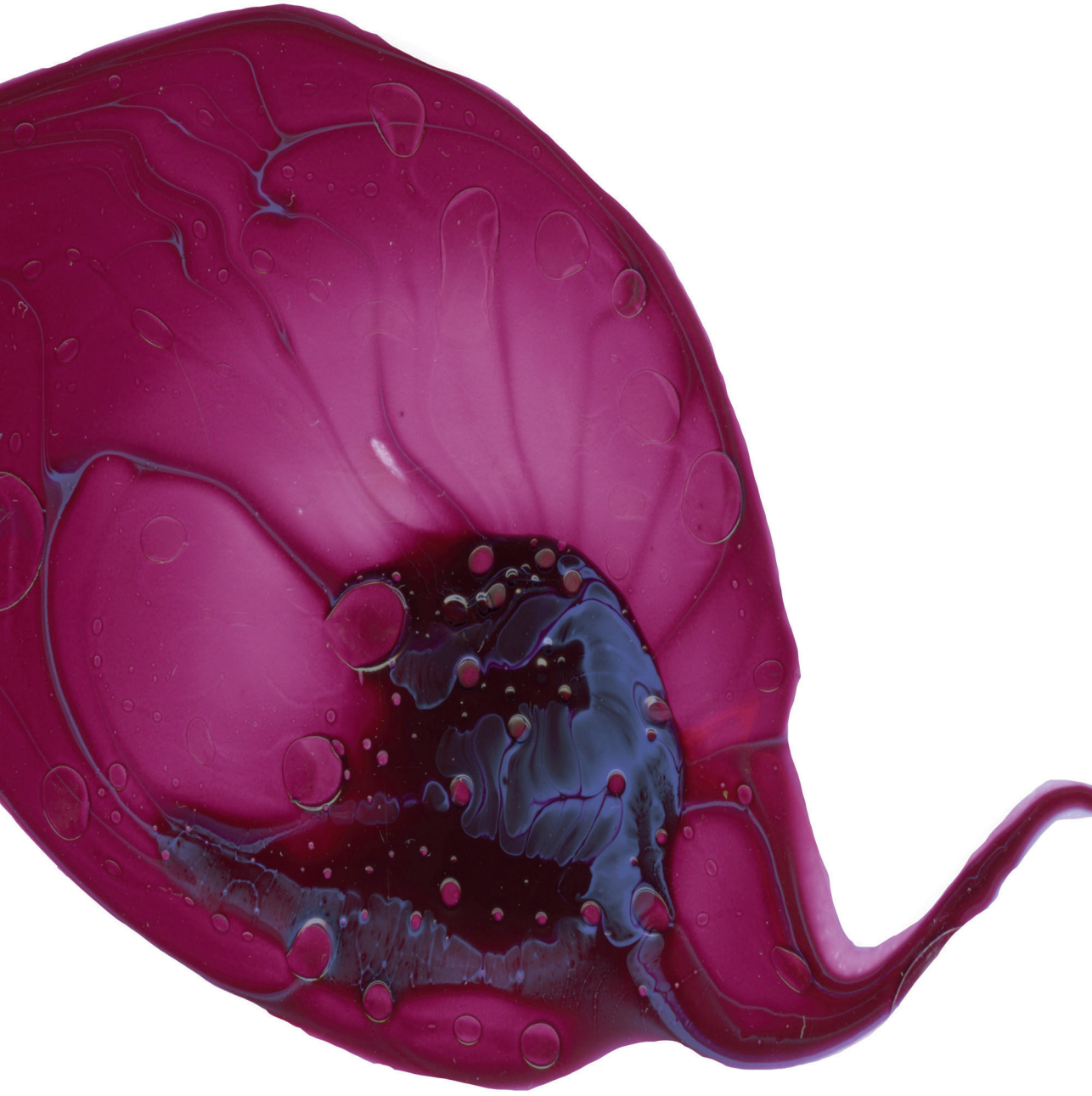

Chapter 7

Tissue Microchimerism is Increased During Human Pregnancy: A Human Autopsy Study

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ABSTRACT

Background

Microchimerism is the occurrence of small populations of cells with a different genetic background within an individual. Tissue microchimerism is considered to be primarily pregnancy-derived and is often studied relative to female-dominant autoimmune diseases, pregnancy complications, malignancies, response to injury, and transplantation outcomes. A particular distribution pattern of chimeric cells across various organs was recently described in a model of murine pregnancies. Our aim was to determine the frequency and distribution of tissue microchimerism across organs during and after pregnancy in humans.

Methods

We performed in situ hybridization of the Y chromosome on paraffin-embedded autopsy samples of kidneys, livers, spleens, lungs, hearts, and brains that were collected from 26 women who died while pregnant or within one month after delivery of a son. Frequencies of chimeric cells in various tissues were compared to those of a control group of non-pregnant women who had delivered sons.

Results

Tissue microchimerism occurred significantly more frequently in lungs, spleens, livers, kidneys, and hearts of pregnant women than non-pregnant women (all $P<0.01$). We showed that some of the chimeric cells were CD3+ or CD34+. Corrected for cell density, the lung was most chimeric (470 Y chromosome-positive nuclei per million nuclei scored), followed by the spleen (208 Y+/10^6 nuclei), liver (192 Y+/10^6 nuclei), kidney (135 Y+/10^6 nuclei), brain (85 Y+/10^6 nuclei), and heart (40 Y+/106 nuclei).

Conclusions

Data from this unique study group of women who died while pregnant or shortly after delivery provide information about the amount and physiologic distribution of chimeric cells in organs of pregnant women. We demonstrate that during pregnancy, a boost of chimeric cells is observed in women, with a distribution across organs that parallels findings in a mouse model.

INTRODUCTION

Microchimerism is defined as the occurrence of a small number of cells in an individual that originate from another individual.[1] One possible source of naturally occurring microchimerism is pregnancy. Knowledge about the source and dynamics of naturally occurring tissue microchimerism is relevant for interpreting its contribution to the pathogenesis of autoimmune disease, pregnancy complications, and malignancies; and for studying its role in response to injury and transplantation outcomes. Because of the association between certain autoimmune diseases and pregnancy, as well as their increased incidence in women of fertile age, the aim of many studies has been to investigate the role of microchimerism in the onset of autoimmune disease.[2] An adverse effect of pregnancy-acquired microchimerism has also been suggested in kidney transplantation. Here, female recipients of a spousal donor kidney who had not been pregnant had better graft survival than those who had been pregnant.[3] On the other hand, a decreased incidence of malignancy in association with increasing parity has been found for various malignancies,[4] suggesting a protective role for pregnancy-acquired microchimerism. To assess the significance of pregnancy-derived tissue microchimerism in autoimmune disease, malignancy, and other pregnancy-related conditions, however, its occurrence during pregnancy in humans must be established first.

During pregnancy fetal cells enter the maternal circulation (fetal microchimerism) and maternal cells enter the fetal circulation (maternal microchimerism). Fetal cellular microchimerism in the maternal circulation has been detected as early as seven weeks of gestation[5] and remains detectable in maternal blood up to 27 years postpartum.[6] Male cells of presumably fetal origin can differentiate and be incorporated into maternal tissues after pregnancy.[7] We previously demonstrated that microchimerism, as measured by in situ hybridization of the Y chromosome, is present in the thyroid gland, lungs, skin, lymph nodes, kidneys, livers, and hearts of healthy non-pregnant women.[8, 9] Remarkably, these studies showed no significant association between microchimerism and parity, yet pregnancy is often assumed to be the main source of tissue microchimerism in women. Most studies have focused on microchimerism in relation to pregnancy in blood samples.[10-14] Studies on microchimerism during pregnancy at the tissue level are limited to the demonstration of presumably fetal male microchimerism in affected tissues of pregnant women with polymorphic eruptions of pregnancy,[15] pregnancy-associated breast cancer,[16] melanoma,[17] and appendicitis.[18] However, no studies have systematically investigated human microchimerism in relation to pregnancy in healthy tissues.

Studying murine pregnancies, Fujiki *et al*. detected fetal cells in tissues of mouse dams after mating wild-type females with green fluorescent protein (GFP)-transgenic males.[19] Interestingly, these authors found a particular distribution pattern of chimeric cells across various organs, identifying the greatest concentrations in the lungs, followed by the spleen, liver, and kidney. Remarkably, microchimerism in maternal lungs exceeded that found in other

organs 10- to 100 fold. Fujiki *et al.* suggested that the high rate of blood flow through lung tissue might explain this high rate of microchimerism in the lungs, in addition to the fact that the lung contains the first capillary bed a fetal cell crosses after entering the maternal uterine venous circulation. To further unravel the impact of microchimerism on maternal health by studying mouse models, the validity of these models to represent the human situation must be confirmed by studying the distribution of tissue microchimerism during human pregnancy.

The aim of the present study was to investigate the frequency and distribution of tissue microchimerism in lungs, spleens, livers, kidneys, brains, and hearts from women pregnant with sons and the influence of the postpartum interval on the amount of microchimerism. Our results provide information about the physiologic distribution of chimeric cells across the organs of pregnant women, allowing comparisons to animal models, and support a role for pregnancy as a source of tissue microchimerism.

METHODS

Patient selection and ethical approval

Tissue specimens came from autopsies performed on women in the Netherlands between 1990 and 2006. Samples were searched using the Dutch PALGA database, a histo- and cytopathology network and archive to which all pathology laboratories within the Netherlands contribute.[20] The search strategy included the parameters "autopsy", "women", and "age between 18 and 45 years". All patients were included who died while pregnant with male fetuses or who died within one month after delivery of a son.

With this search strategy, we obtained formalin-fixed, paraffin-embedded tissue samples from 26 women, including samples from 19 lungs, 15 spleens, 19 livers, 19 kidneys, 17 hearts, and 5 brains. The number and availability of organ samples varied because of differences between laboratories. Also, some samples were excluded because of poor quality due to autolysis. A previously described group of non-pregnant women with sons served as historic controls.[8,9] Patient characteristics of the pregnant women included in the current study and those of the historic control group are shown in **Table 1**.

Clinical data were obtained from autopsy reports and from the records of the National Maternal Mortality Committee of the Dutch Society of Obstetrics and Gynaecology. All samples were coded and handled anonymously in accordance with the Dutch National Ethics Guidelines (Code for Proper Secondary Use of Human Tissue, Dutch Federation of Medical Scientific Societies). In addition, this study was approved by the ethics committee of the Leiden University Medical Center (P12.107). **Table 2** shows pregnancy-related characteristics of the 26 pregnant women included in this study.

Table 1 Characteristics of pregnant women and controls.

Characteristic	This study	Koopmans *et al.* 2005	Koopmans *et al.* 2008
	Pregnant women with sons	*Non-pregnant women with sons*	*Non-pregnant women with sons*
Number of women	26	45	50
Age in years, mean (range)	32 (20–45)	64 (29–93)	64 (29–93)
Cause of death			
Infectious	0	9	11
Malignancy	0	14	13
Cerebral	1	8	10
Vascular/myocardial	14	12	13
Other	11, i.e. pre-eclampsia/ eclampsia (6), amniotic fluid embolism (2), liver cirrhosis, EUG, sarcoidosis	2, i.e. liver cirrhosis, cachexia	3, i.e. liver cirrhosis (2x), cachexia
Blood transfusion			
Yes	1	29	30
No	25	16	20
Organ samples studied by ISH			
Kidney	19	30	-
Liver	19	33	-
Heart	17	42	-
Spleen	15	29	-
Thyroid	-	-	43
Lung	19	-	37
Skin	-	-	20
Lymph node	-	-	6
Brain	5	-	-

EUG, extra-uterine gravidity; ISH, in situ hybridization.

In situ hybridization

The same protocol for in situ hybridization of the Y chromosome was used as previously described for our historic control group.[8, 9] Formalin-fixed, paraffin-embedded samples were cut into 4 µm slices, transferred to Superfrost plus glass slides (Menzel-Glaser, Germany), and dried overnight at 37 °C. A Y chromosome-specific DNA probe[21] was labelled with digoxigenin (DIG) according to the standard Nick-translation protocol. After labelling, the probe was precipitated, dried, and dissolved in hybridization mixture (50% deionised formamide; 0.05 M sodium phosphate buffer, pH 7.0; 0.3 mol/L NaCl; 30 mmol/L Na citrate (2×SSC); and 10% dextran sulphate). Salmon sperm DNA, transfer RNA, and Cot-1 DNA were added to the hybridization mixture to prevent nonspecific binding of DNA.

Table 2 Pregnancy-related characteristics of cases.

Characteristic	Pregnant women (n=26)
Death postpartum, n	11
GA at birth, weeks	37.4 (29.9–39.1)
Death postpartum, hours	36.0 (0.2–432)
Death during pregnancy, n	15
GA at death, weeks	29.0 (10–40.3)
Death to autopsy time, hours	24.0 (1–48)
Sex of fetus	
Male, %	100
Female, %	0
Gravidity	2.2 (1–10)
Parity	1 (0–5)
Eclampsia/preeclampsia, n	6

Data are presented as mean (range), unless indicated otherwise. GA, gestational age.

The slides were deparaffinised in xylene and rehydrated in an ethanol series followed by a distilled water rinse. The slides were prepared with 0.05 M citrate buffer (pH 6.0) at 80 °C for 80 minutes and rinsed in prewarmed distilled water at 37 °C. Enzyme digestion was performed using 0.5% pepsin (Serva Electrophoresis GmbH, Heidelberg, Germany) in 0.01 M HCl at 37 °C for 20 minutes. The slides were dehydrated in upgraded ethanol and air-dried. Tissue sections on each slide were covered with 30 µL of hybridization mixture containing 5 ng/µL labelled probe. The slides were denatured for 10 minutes on a metal plate at 80 °C and incubated at 37 °C overnight. The following day, the slides were washed three times in 2×SSC/0.1% Tween at 37 °C to remove the cover slips and three times in 0.1×SSC at 60 °C. To visualise the DIG-labelled probe, the sections were incubated consecutively with a mouse anti-DIG monoclonal antibody (Sigma-Aldrich, St. Louis, Missouri, USA) and anti-mouse Envision-HRP (Dako, Glostrup, Denmark), followed by development with Nova Red Vector for 10 minutes. Haematoxylin staining was used as a background.

We confirmed by nested PCR and sequencing that the probe was specific for the Y chromosome.[8] As a positive control, we used a male tissue sample in which the sensitivity of the Y chromosome signal was 58%. As a negative control, a male tissue sample was used on which the complete hybridization protocol was performed using the hybridization mixture without the Y chromosome probe.

Double staining

To study the phenotype of the chimeric cells in pregnant women, we performed CD3 and CD34 immunohistochemical staining in combination with in situ hybridization of the Y chromosome as described previously.[22]

Scoring

Slides were scanned using a Panoramic Digital Slide Scanner (3DHISTECH Kft., Budapest, Hungary) and scored using Panoramic Viewer (3DHISTECH Kft., Budapest, Hungary). A predefined random area of 50 mm^2 was scored at 400× magnification by two observers. The Y chromosome was considered to be present when a red dot was visible within nuclei of similar size, colour, and intensity to those in positive controls. The degree of microchimerism in positive samples was scored quantitatively by counting the number of positive nuclei and expressing them as the number of positive nuclei per mm^2. To correct for differences in cell density among organs, image analysis using ImageJ (NIH) was performed to estimate the number of nuclei scored on each slide individually.

Because of the potential relationship between tissue injury and microchimerism, the degree of injury in each organ was scored semi-quantitatively by a pathologist. Haematoxylin and eosin-stained sections of the same tissue blocks on which in situ hybridization of the Y chromosome was performed were analysed by light microscopy. A score for active injury was composed of the following criteria: (0) no foci of inflammation, haemorrhage, and congestion; (1) minor focal inflammatory infiltrates, small foci of haemorrhage, and/or mild congestion; (2) more diffuse inflammatory infiltrates, diffuse signs of haemorrhage, and/or moderate congestion; (3) extensive inflammatory infiltrates, extensive haemorrhage, and/or extensive signs of congestion. Moreover, a score for chronic injury was based on the following criteria: (0) no calcifications, fibrosis, and steatosis; (1) minor foci of calcifications, fibrosis, and/or steatosis; (2) moderate signs of calcifications, fibrosis, and/or steatosis; (3) diffuse calcifications, fibrosis, and/or steatosis.

Statistical analyses

Fisher's exact tests were used to compare proportions of organs from pregnant women and non-pregnant women that were scored as positive. To correct for correlations among organ measurements within a patient, we used a linear mixed model to compare quantities of Y chromosomes in different organs. In our mixed model, we performed analyses with organ type and gravidity as fixed effects, as well as conducting separate analyses for organ type and maternal death either ante- or postpartum, organ type and gestational age, and organ type and either vaginal delivery or Caesarean section. Visual inspection of the distribution of residuals was performed to assess normality assumptions. We assessed correlations between active and chronic injury scores and number of Y chromosome-positive nuclei per million nuclei by calculating Spearman correlation coefficients (ρ). *P*-values <0.05 were considered significant. All analyses were done using SPSS Statistics 20.0 (IBM Corp., Armonk, New York).

RESULTS

Frequency of male cells in maternal organs

We performed in situ hybridization of the Y chromosome on 19 lungs, 15 spleens, 19 livers, 19 kidneys, 5 brains, and 17 hearts. Chimeric cells were found in all organs studied. **Table 3** shows that the proportion of women with male cells in their lungs, spleens, livers, kidneys, and hearts was significantly higher in this study of pregnant women with sons than in a historic control group of non-pregnant women with sons.[8,9]

Table 3 Fractions of chimeric organs in pregnant versus non-pregnant women.

Organ	Pregnant women with sons (%)	Non-pregnant women with sons (%)	*P*
Kidney	16/19 (84)	5/30 (17)	<0.0001
Liver	18/19 (95)	6/33 (18)	<0.0001
Spleen	15/15 (100)	3/29 (10)	<0.0001
Heart	5/17 (29)	0/42 (0)	<0.01
Lung	19/19 (100)	10/37 (27)	<0.0001
Brain	5/5 (100)	-	-

Organ distribution of male cells

For the quantitative analyses of tissue microchimerism, the numbers of Y chromosome-positive cells per section were corrected for area and cell density. The spleen showed the most microchimerism per area with, on average, 0.45 positive Y chromosomes per mm^2, followed by the lung (0.30/mm^2), kidney (0.27/mm^2), liver (0.23/mm^2), brain (0.06/mm^2), and heart (0.02/mm^2). However, after correction for cell density, the lung was most chimeric, with an average of 470 Y chromosome-positive nuclei per million nuclei scored. The lung was followed by the spleen (208 Y+/10^6 nuclei), liver (192 Y+/10^6 nuclei), kidney (135 Y+/10^6 nuclei), brain (85 Y+/10^6 nuclei), and heart (40 Y+/10^6 nuclei). **Figure 1** shows the organ distribution of chimeric cells.

Lungs contained significantly more Y chromosome-positive cells corrected for cell density than any of the other organs studied (lung vs. any organ: $P<0.001$). Furthermore, the spleen and liver were significantly more chimeric than the heart ($P=0.032$ and $P=0.026$, respectively).

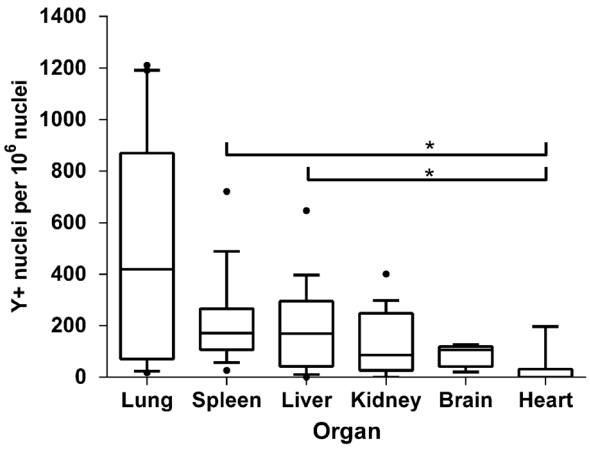
Location and phenotype of chimeric cells

Chimeric cells appeared to be both parenchymal cells and hematopoietic cells. **Figure 2** shows photographs of slides with chimeric cells as observed in the various organs. In lungs, chimeric cells were incorporated into the alveolar septa, bronchiolar walls, and blood vessels. In the spleen, chimeric cells seemed to be part of the hematopoietic cell population while in livers, chimeric cells resembled hepatocytes and were found in blood vessels. In

the kidney, chimeric cells occurred in tubules, glomeruli, and the endothelium of blood vessels. Male microchimerism was present in the cerebral cortex of all five women whose brain autopsy material was available. In the brain, chimeric cells appeared to be glial cells and possibly neurons and were also found in blood vessel endothelium. In hearts, chimeric cells resembled cardiomyocytes.

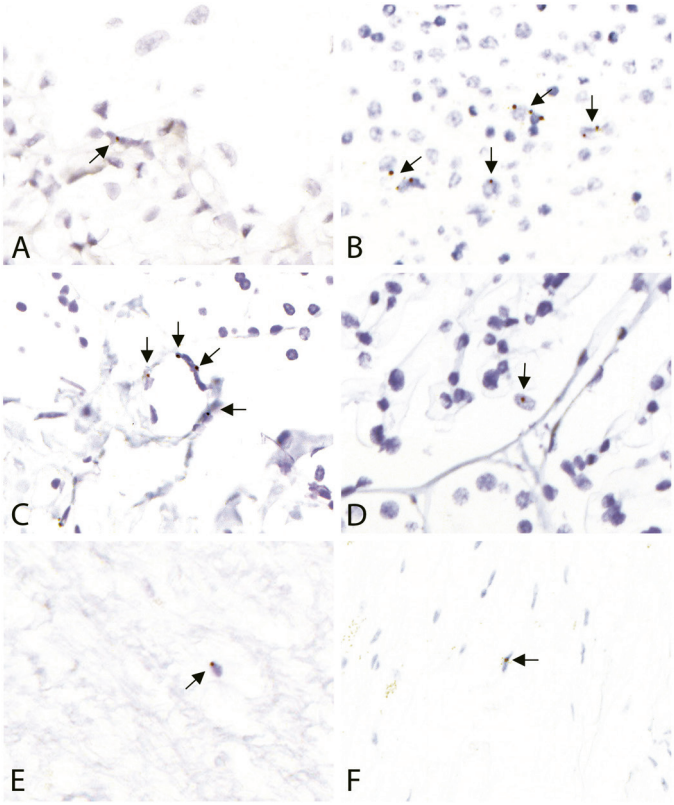
Double staining was performed on tissue sections showing high positivity after in situ hybridization of the Y chromosome from 2 lungs, 1 spleen, 1 liver, 2 kidneys, 1 heart, and 1 brain. In the lung, spleen, and kidney, some of the chimeric cells were CD3+, and in the lung, kidney, and liver, some were CD34+ (**Figure 3**). Also, following both double staining procedures, chimeric cells were found that did not show positivity for CD3 or CD34. In some instances, CD34+ chimeric cells lined a vascular wall, which could indicate that they are endothelial cells. Some of the CD34+ chimeric cells were found alone, which could imply that they have a stem cell phenotype.

Figure 1 Graph showing the numbers of Y chromosome-positive cells in maternal organs.



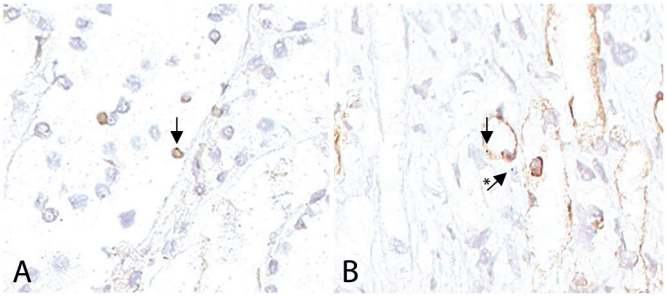
Lungs contained significantly more Y chromosome-positive cells corrected for cell density than any of the other organs studied (lung vs. any organ: $P<0.001$). * $P<0.05$. Horizontal lines represent median values, boxes represent 25th and 75th percentiles, whiskers represent 10th and 90th percentiles, circles represent outliers.

Figure 2 Y chromosome-positive nuclei in the lung (A), spleen (B), liver (C), kidney (D), brain (E), and heart (F).



Arrows indicate positively scored nuclei.

Figure 3 Double-staining by combination of in situ hybridization of the Y chromosome and immunohistochemical staining for CD3 and C34.



Cells that were both Y chromosome-positive and CD3+ (A) or CD34+ (B) were identified in kidney sections (A and B), as well as lungs, spleens, and livers (not shown). Y chromosome-positive, but CD3– (not shown) or CD34– (B, *) cells were also found after double staining.

Tissue microchimerism in relation to injury

The sections used for the in situ hybridization were given injury scores by a pathologist, who identified 15 out of 19 pulmonary sections with signs of active injury (congestion and inflammatory infiltrates), while none of the pulmonary sections had chronic injury. Of the splenic sections, six out of 15 had signs of acute congestion while none had chronic injury. A total of 15 out of 19 hepatic sections had active injury (congestion and inflammatory infiltrates), and two out of 19 had chronic injury (steatosis). Three out of 19 renal sections with active injury were identified (lymphocytic infiltrate and erythrocytes in tubuli), as well as three out of 19 sections with chronic injury (calcifications). From the hearts, five out of 17 sections contained active injury (acute ischemia), and one of 17 sections showed chronic injury (fibrosis). From the brains, one out of five sections was identified with active injury (Purkinje cell degeneration). Neither the active injury score nor the chronic injury score was significantly correlated with tissue microchimerism (as expressed by number of Y chromosome-positive nuclei per million) when all organs were analysed together (active injury: ρ=0.077, P=0.5; chronic injury: ρ=0.017, P=0.9). Active and chronic injury scores did not significantly correlate with quantities of microchimerism in organs studied individually, except for a significant correlation between active injury score and tissue microchimerism in the kidney (ρ=0.475, P=0.04).

Microchimerism in relation to pregnancy-related characteristics

The number of previous pregnancies did not associate with the amount of microchimerism (P=0.9). Women who died during pregnancy had on average more microchimerism than women who died after delivery; however, this difference failed to reach statistical significance (P=0.4). No relationship was found between gestational age and the amount of microchimerism (P=0.7), and the presence of preeclampsia/eclampsia did not differ relative to the amount of microchimerism (P=0.6). Only one woman had a known history of blood transfusion; therefore, the relationship between tissue microchimerism and blood transfusion history could not be evaluated. Women with a vaginal delivery had on average more microchimerism than women who delivered by Caesarean section; however, this difference was not statistically significant (P=0.1).

DISCUSSION

In this study, we investigated the frequency and distribution of chimeric cells in organs in a distinctive cohort of women who died during or shortly after pregnancy and of whom autopsy material was available. Male microchimerism occurred significantly more often in lungs, spleens, livers, kidneys, and hearts of women pregnant with sons than in non-pregnant women who had given birth to at least one son. We showed that some of the chimeric cells were CD3+ or CD34+. Moreover, we found a distribution pattern of microchimerism across organs of pregnant women that was similar to that identified in pregnant mice, with the lung being the organ in which microchimerism presented most

often and in greatest abundance.[19]

If microchimerism were mainly pregnancy-derived, one expected implication would be a negative correlation between the postpartum interval and the amount of tissue microchimerism. Moreover, because of the circulatory anatomical relationship between mother and fetus, the lung would be the candidate organ for having the highest amounts of microchimerism, as it contains the first capillary bed through which blood from the placenta passes. The current study has yielded two important observations that support the hypothesis that tissue microchimerism is mainly pregnancy-derived. First, tissue microchimerism occurs with higher frequency during pregnancy than in the years after pregnancy and is thus negatively correlated with the postpartum interval. Second, the lung harbours significantly more microchimerism than any other organ type after correction for cell density.

It is fascinating that we found the same distribution pattern of microchimerism across organs in our study in humans as in mice during pregnancy,[19] with the lungs being the organs in which microchimerism presented most often and in greatest abundance, followed by the spleen, liver, kidney, and lowest amounts of microchimerism found in the heart and brain. Because we corrected our data for cell density, they are comparable to the flow cytometry results for mice.[19] In mice, frequencies of microchimerism as measured by flow cytometry targeting GFP ranged from 0 to 60 GFP-positive cells per million cells. In our study, the range of observed frequencies of Y chromosome-positive cells was wider by a factor 20, from 0 to 1200 Y chromosome-positive cells per million cells. This difference could be explained by differences in pregnancies between mice and humans, such as duration, litter size, and uterine anatomy, or by variation in the sensitivity of the detection methods. In our study, the amount of microchimerism is likely underestimated because not all nucleated male cells will give a Y chromosome-positive signal: in histologic 4 µm thick sections, the Y chromosome will be present in approximately 58% of all nuclei.[9] Also, detection of tissue microchimerism from other sources in our study is limited to microchimerism derived from males. Microchimerism was also underestimated by Fujiki *et al.* using GFP in mice as a marker for microchimerism, as on average 48% of pups expressed GFP.

Several explanations are possible for the distribution of microchimerism across organs. One is that fetal cells are passively trapped in the lungs, which contain the first capillary bed that cells must cross after entering the uterine venous circulation. Another possible explanation is that the distribution of chimeric cells reflects blood flow, as expressed by percentage of cardiac output received. In fact, the fractional distribution of cardiac output corresponds to the pattern of distribution of chimeric cells across the organs of pregnant women, with lungs receiving 100% of cardiac output, followed by the liver (22.7%), kidneys (17.5%), brain (11.4%), and heart (4.0%).[23] Finally, chimeric cells could be actively accumulated in specific tissues because of favourable microenvironments, much like the "seed and soil" theory in cancer studies.[24] Accordingly, the high prevalence of microchimerism in the spleen and liver may arise from the sequestration of chimeric cells in these tissues.

In mice, allogenic fetal cells migrate to the spleen during pregnancy and may continue proliferation in the spleen long afterward.[25] Numerous studies in humans and mice have shown a preferential homing of chimeric cells to sites of injury.[26-29] Pregnancy-associated cardiovascular events often have significant effects on target tissues, such as the lung[30] It could therefore be argued that the high prevalence of microchimerism in lungs of deceased pregnant women is due to injury. A significant number of our cases died of vascular causes such as pulmonary embolism or preeclampsia, in which pulmonary injury could be anticipated. Indeed, 15 out of the 19 pulmonary sections contained signs of active injury. However, correlations between quantities of chimeric cells corrected for cell density with active injury scores were not significant in the lung sections analysed. The only organ with a significant correlation between tissue microchimerism and active injury scores was the kidney. Of interest, in gentamicin-induced kidney injury, fetus-derived chimeric cells engraft in the kidney as tubular cells, suggesting a role in the repair process.[27] In the setting of lupus nephritis, a repair function of microchimerism was recently supported by the demonstration of higher amounts of microchimerism in patients with better renal function.[31] It is therefore conceivable that the kidney in particular is an organ prone to repair in the setting of microchimerism.

Chan *et al*. recently reported the existence of male microchimerism in the brains of 37 out of 59 (63%) autopsied women as measured by real-time quantitative PCR, but the women included in that study were not pregnant and had an unknown reproductive history.[32] To our knowledge, we are the first to describe male microchimerism in the brains of pregnant women, having identified it in all 5 women studied. Because of small sample size, we cannot draw conclusions on increased prevalence of microchimerism in the brain during pregnancy. Changes in the permeability of the blood-brain barrier have been described during pregnancy and could therefore explain the establishment of a fetal cell population in the maternal brain.[33] In mice, Fujiki *et al.* did not observe fetal cells in the brain by flow cytometry.[19] However, others using PCR have detected GFP+ fetal cells in the brains of mouse dams, resembling perivascular macrophages, neurons, astrocytes, and oligodendrocytes.[34, 35]

Our results support a role for microchimerism in several pregnancy-related conditions, including autoimmune disease, pregnancy complications, and certain malignancies. In the setting of autoimmune disease, the difference between men and women in the incidence of autoimmune diseases makes pregnancy-derived microchimerism an intriguing candidate for causing these diseases. It has been suggested that chimeric CD3+ cells have a pathogenic role in systemic sclerosis by inducing a graft-versus-host-like reaction.[36] Because we found CD3+ chimeric cells in the tissues of pregnant women, it is possible that acquisition of these cells during pregnancy forms the basis for development of autoimmune diseases in which chimeric T cells are present. On the other hand, fetal CD34+ chimeric cells have been found in the circulation and tissues of healthy women many years after pregnancy, without the onset of autoimmunity,[6, 37] and may therefore also be seen as innocent bys-

tanders. Further study is necessary to unravel the impact of fetal cell microchimerism on maternal health and should include analysis of microchimerism in different immunologic background strains of mice.

Concluding, we are the first to demonstrate the abundance of microchimerism in different tissues of pregnant women. Because we had a unique opportunity to study a group of women who died while pregnant or shortly after delivery, we could demonstrate that during pregnancy, a boost of pregnancy-derived chimeric cells is indeed observed in women, with a distribution across organs paralleling the distribution identified in a previous study in mice. Whether the distribution of chimeric cells across different organs reflects the occurrence of disease in these organs, as well as the immunologic mechanisms involved in the pathogenesis, will be the subject of ongoing research.

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