Chimerism in health, transplantation and autoimmunity

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Chimerism in kidneys, livers and hearts of normal women: implications for transplantation studies

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Abstract

Background

Tissue chimerism was recently described in transplanted organs from female donors into male recipients, by demonstration of the Y chromosome in tissue derived cells. It was claimed that these Y chromosome chimeric cells were recipient derived. To find out whether chimeric cells, derived from pregnancies of sons or blood transfusions, could have been present in solid organs before transplantation, we performed the following study.

Methods

In situ hybridization for the Y chromosome was performed on normal organs (51 kidneys, 81 livers, 69 hearts) from 75 women of the normal population, of whom the child and blood transfusion history was known.

Results

Chimeric cells were found in 13 kidneys, 10 livers, and 4 hearts, of 23 women. There was no relation between the child or blood transfusion history with the presence of Y chromosome-positive cells.

Conclusion

We have for the first time demonstrated that male cells are present in normal kidneys, livers, and hearts. Theoretically, these organs could have been used for transplantation. Therefore, our findings demonstrate that the chimeric cells thus far described in transplantation studies, are not necessarily donor derived, and could have been present in the organs before transplantation.

Introduction

Chimerism can be defined as a phenomenon in which cells from one individual are present in another individual. These cells can either be circulating or they can be integrated into the parenchyma. After the first description of its occurrence in transplanted organs in 1965,[1] chimerism has grown into a frequently discussed concept, especially in relation to transplantation. Important issues are how chimerism is induced, for instance by damage caused by rejection, and whether it may enhance recipient tolerance. In 2001, Lagaaij et al.[2] demonstrated the presence of recipient-derived endothelial cells in kidney grafts. In other studies, chimeric tubular epithelial cells were described in transplanted kidneys.[3,4] Chimerism was also reported in other transplanted organs: Chimeric endothelium, duct epithelium, and hepatocytes were found in transplanted livers;[5-7] chimeric cardiomyocytes and smooth muscle cells were found in transplanted hearts;[8,9] and chimeric bronchial epithelium and type II pneumocytes were found in transplanted lungs.[10] The differences in the reported amounts of chimeric cells in solid organs after transplantation in various studies are remarkable, ranging from no chimerism[11] to low[12,13] or even high[8] levels of chimeric cells.

After the publication of Quaini et al.,[8] reporting on chimeric cells in heart transplants, the identity and source of the chimeric cells were heavily debated. It was questioned whether the transplanted hearts could not already have been chimeric before transplantation, due to, for instance, circulating fetal cells or cells derived from previous blood transfusions. It was criticized by Bianchi[14] that fetal stem cells may persist in the peripheral blood in healthy women as long as 27 years after delivery.[15] In approximately 30% of women who had been pregnant of a son, male cells of fetal origin were found in the circulation up to 38 years after delivery.[16] It was argued, therefore, that the detected male cells in the transplanted hearts might be derived from the sons of the female donors, and not from the male recipients. Blood transfusions may be another possible source of chimerism. A study that is often cited is from Lee et al. who found male circulating cells in seven out of ten women who had received blood transfusions because of trauma.[17] Therefore, blood transfusions may be a source of chimeric cells already present in the solid organ graft before transplantation.

In most transplantation studies, normal organ controls are lacking or relatively scarce. In 16 studies published on chimerism in solid organ transplantation, the negative controls amount to a total of only 18 organs (4 kidneys and 14 hearts) of which the results are clearly described.[2-5,7-13,18-22] In three studies on liver transplantation, negative controls are mentioned to be negative, but the number of control tissues is unclear.[5,7,19] Child and blood transfusion status are never mentioned of the controls. Also of the females who donated the organs, information on child and blood transfusion status in mostly lacking.

The previously mentioned studies regard chimerism as a post-transplantation phenomenon. However, chimeric baseline levels, meaning the amount of background chimerism already present before transplantation, were never thoroughly investigated. It is necessary to investigate these chimeric baseline levels to distinguish between 'background chimerism' and 'transplantation induced chimerism' before drawing conclusions with respect to the immunological role of chimeric cells in transplanted organs.

The goal of the present study was to find out whether organs that are frequently used for transplantation, namely kidneys, livers, and hearts, may already contain chimeric cells before transplantation. Therefore, we have searched for the presence of Y chromosome-positive cells in normal kidneys, livers, and hearts of 75 women whose child and blood transfusion status were known. Our results provide essential baseline data for future research on the subject of chimerism in solid organ transplantation.

Patients and methods

Patients

Tissue specimens came from autopsies on women, performed at the Leiden University Medical Center (LUMC) between 1999 and 2001. Clinical exclusion criteria comprised: a history of autoimmune disease, solid organ transplantation, bone marrow transplantation, or stem cell transplantation.

Child status

Permission of the medical-ethical committee of the LUMC was obtained to enquire general practitioners about the child status of women on whom autopsy was performed. We enquired 117 general practitioners about the child status of 154 women, and 81% replied. A definite answer about the child status was obtained for 95 women. Of these, 53 had given birth to at least one son, 11 had only daughters, and 31 had no children. Tissue specimens of women with sons and of women without children were entered into the study.

Blood transfusion status

Data on blood transfusions were obtained from the Department of Immunohaematology and Blood Bank (the IHB) of the Leiden University Medical Center. Data were available from 1987 onward, and included the number, time, and type of transfusion. It appeared that 45 of 75 women who eventually entered the study, had received a blood transfusion. It was known how many nucleated cells are maximally present in any transfusion type, making it possible to estimate the maximum amount of nucleated cells transferred by each transfusion. As some transfusions are a combination of several donors, and as there are an equal number of female and male donors on average, an estimate of the maximum number of male nucleated cells transferred by each transfusion could be made. To obtain information on blood transfusions in the period before 1987, we contacted the general practitioners of all women in the study. 84% replied. However, they provided no additional data to those that were already known from the IHB.

Quality of samples

Tissue specimens of the heart, liver, and kidney were reviewed in the hematoxylin and eosin (HE) staining for histomorphological lesions and signs of autolysis. Tissue specimens of 9 women were consequently excluded, 7 of whom had sons, and 2 of whom had no children. Because the liver block most of the time also contained a tissue specimen from the spleen, we decided to incorporate the spleen into our study as well. All remaining specimens covered at least an area of 1 x 1 cm. To verify the quality of the tissue samples for the detection of sex chromosomes, in situ hybridization for the X chromosome was performed on a random selection of various organ samples of 46

non-autolytic specimens: all specimens were positive. Tissue specimens of 75 women were entered into the study, 46 of whom had sons, and 29 of whom had no children. Clinical data of all women are given in Table 1.

Table 1. Clinical data

	Women with sons	Women without children
Number of women	46	29
Age (years)	63 (29 - 93)	59 (10 - 89)
Cause of death		
Infectious	9	6
Cancer	14	5
Cerebral	8	6
Vascular/myocardial	12	11
Other	3, i.e. amniotic fluid embolus liver cirrhosis from alcohol abuse cachexia	1, i.e. liver failure from alcohol abuse
Blood transfusion		
Blood transfusion	30	15
No blood transfusion	16	14
Organs studied by ISH	138	77
Kidneys	31	20
Livers	34	17
Hearts	43	26
Spleens	30	14

ISH, in situ hybridization

In situ hybridization

Archived paraffin-embedded tissues of the kidney, liver, and heart from the autopsied cases were cut into 4-µm sections, and deposited onto Superfrost plus glass slides (Menzel-Glaser, Germany). The sections were dried overnight at 37°C to improve tissue adherence. A Y chromosome-specific DNA probe [23] was labeled with digoxigenin according to the standard Nick-translation protocol. After labeling, the probe was precipitated, dried and dissolved in a hybridization mixture (50% deionized formamide, 0.05 M sodium phosphate buffer pH 7.0, 2 x 0.3 mol/L NaCl, 30 mmol/L Na citrate [2*SSC] and 10% dextran sulphate). To prevent nonspecific binding of DNA, salmon sperm DNA, transfer RNA and Cot-1 DNA were added to the hybridization mixture.

Slides were deparaffinized in xylene and dehydrated in an ethanol series followed by a distilled water rinse. The sections were pretreated with 0.05 M citrate buffer (pH 6.0) at 80°C for 80 minutes, rinsed in prewarmed distilled water at 37°, followed by a 0.5% Pepsin (Serva Electrophoresis GmbH, Heidelberg, Germany) in 0.01 M HCL at 37°C for 20 minutes, for enzyme digestion. Slides were then dehydrated in upgraded ethanol and air-dried. Tissue sections on each slide were covered with a 30-µL hybridization mixture containing 5 ng/µl labeled probe. They were then denatured on a 80°C metal plate for 10 minutes and incubated at 37°C overnight. The following day, the sections were washed three times in 2*SSC/0.1% Tween at 37°C and three times in 0.1*SSC at 60°C. To visualize the Dig-labeled probe, sections were incubated consecutively with a mouse-anti-Dig monoclonal antibody (Sigma-Aldrich, St. Louis, Missouri, USA), rabbit-anti-mouse immunoglobulin-HRP (Dako, Glostrup, Denmark), and swine-anti-rabbit immunoglobulin-HRP (Dako) at room temperature. Finally, the sections were developed with Nova Red Vector for ten minutes. A hematoxylin staining served as a background.

An X chromosome-specific DNA probe[24] was hybridized according to the same method as described above. Tissue samples from either a male or a female Eurotransplant kidney that were rejected for transplantation because of technical reasons, served as positive controls for the in situ hybridization of the Y and the X chromosome, respectively.

Scoring

All slides were evaluated by at least two observers. A sample was scored positive if in one or more nuclei a red brown-stained dot was present, with a similar size and staining intensity as those of the positive controls samples. The background was clear, and the stained dots were specifically present in cell nuclei. Sporadically, a non-specific pattern of Nova Red Vector positivity was found with numerous little speckles in the nucleus, or with a blurry staining pattern. The non-specifically stained cells seem to be leucocytes, possibly plasma cells, and were not counted positive.

Polymerase-Chain-Reaction analysis for Y chromosome-positive cells

To confirm the results from the in situ hybridization we performed a nested Polymerase-

Chain-Reaction (PCR). DNA was extracted from paraffin-embedded kidney specimens with the Wizard® Genomic DNA Purification Kit (Promega) from 7 women who bared sons. Two of these specimens were scored positive by in situ hybridization, and five specimens were scored negative.

A specific Y chromosome sequence was detected by amplifying DNA from a non-functional-Sex-determining region Y (SRY) gene in a nested PCR with primers designed with the Beacon Designer (Bio-Rad laboratories, Hercules, CA, USA) software. The first amplification was done with primers Y1-1, which has the sequence 5'- CGC ATT CAT CGT GTG GTC TCG-3' and Y1-2, which has the sequence 5'- TTT TCG GCT TCA GTA AGC ATT TTC C-3' (product of 120 bp). The nested amplification was done with primers Y1-3, which has the sequence 5'- TCA GAC GCG CAA GAT GGC TC-3', and Y1-4, which has the sequence 5'- AGT AAG CAT TTT CCA CTG GTA TCC C-3' (product of 88 bp). Approximately 20 ng DNA were used in a 25-µL assay containing 2.5 µl 10 x PCR buffer, 300 pmol of each primer, 0.5 µl of 10 mM of each dNTP, and 0.1 U Amplitaq DNA Polymerase (Roche Applied Science, Indianapolis, IN, USA) The conditions for amplification were denaturation at 95°C for thirty seconds, annealing at 60°C for thirty seconds, and extension at 72°C for thirty seconds for 35 cycles. Two microliter of this PCR product was used for a nested PCR, which was performed by 45 cycles of denaturation at 95°C for thirty seconds, annealing at 60°C for thirty seconds, and extension at 72°C for thirty seconds. All PCR analyses contained a blank (water without DNA) for a negative control and a known positive sample for the Y chromosome (male DNA). A PCR for a non gender-related DNA-fragment was used to verify the quality of the isolated DNA.

To avoid contamination, all experiments were performed by a female technician. Pre-amplification steps were carried out in a separate room, in a safety hood which was sterilized each time by UV light. Furthermore, for the nested PCR, each sample was accompanied by an extra blank control.

The resulting 88 bp Y chromosome-specific fragment was identified by ethium bromide staining after electophoresis on a 3 percent agarose gel.

Sequence analysis of the PCR-amplified product

The 88 bp PCR product was sequenced to confirm its identity. The fragment was sequenced with Y1-3 forward primer. The sequence results were verified for homology with the SRY gene using BLAST search against Genbank on the NCBI website.

Table 2. Results in situ hybridization for the Y chromosome

	Women with sons	Women without children
	(n=46)	(n=29)
Organs scored positive	16	19
Women with at least one organ scored positive	14 / 46 (30%)	13 / 29 (44%)
Age of women with at least one organ scored positive	62 (29 - 93 yr)	62 (29 - 89 yr)
Women with more than one organ scored positive	1 with liver and kidney	1 with liver, kidney and spleen
	1 with liver and spleen	1 with kidney, heart and spleen
		1 with liver and kidney
		1 with liver and spleen
kidneys scored positive	6 / 31 (19%)	7 / 20 (35%)
livers scored positive	7 / 34 (21%)	3 / 17 (18%)
hearts scored positive	0 / 43 (0%)	4 / 26 (15%)
spleens scored positive	3 / 30 (10%)	5 / 14 (36%)

Results

Y chromosome-positive cells, as determined by in situ hybridization, were found in 13 kidneys, 10 livers, 4 hearts, and 8 spleens. In Table 2, the division of the positive tissue samples among women with sons and women without children is presented, showing no significant difference between these two groups. In Table 3, the division of the positive tissue samples among women with and without blood transfusions is presented, also showing no significant difference between these two groups. Combining the data from the child and blood transfusion history, there were 30 women with a positive blood transfusion history and sons, 16 women with a negative blood transfusion history with sons, 15 women with a positive blood transfusion history without children, and

14 women with a negative blood transfusions history without children. The number of women who had chimerism in any organ in these four groups respectively, were: 9 of30, 5 of 16, 7 of 15, and 6 of 14. We calculated the maximum number of male nucleated cells present in the blood transfusion, and this number showed no significant relation with the number of positive tissue samples in any of the tested organ specimens (Figure 1). There was also no statistically significant relationship between the number of positive tissue specimens of any organ with either the child or the blood transfusion status.

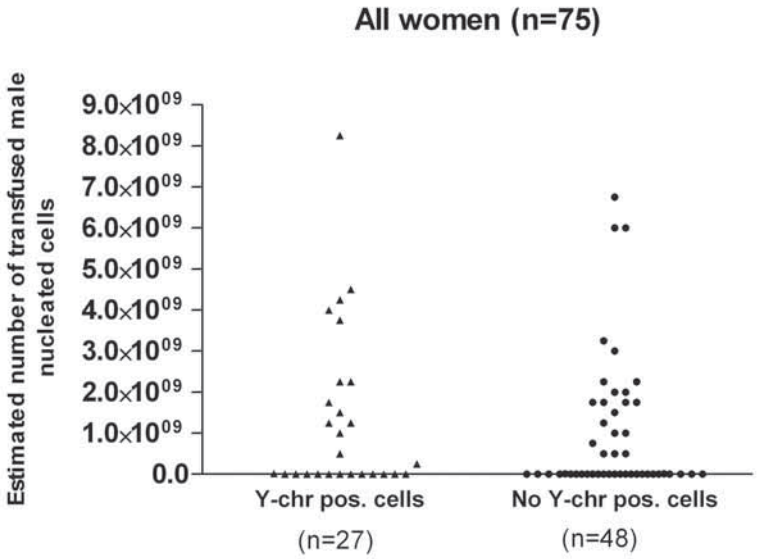

Figure 1. Overview of the estimated number of transfused male nucleated cells and ISH results.

There were 6 women with more than one organ showing positive results in the situ hybridization for the Y chromosome, but it was never the case that all the organs evaluated in one woman were all positive (Table 2). Most positive samples only contained a small number of dots. However, in two patients the kidneys had an extensive number of nuclei in which the Y chromosome was present. The results of each organ will be described separately below.

Table 3. Blood transfusion data

	Y chromosome positive cells	No Y chromosome positive cells	Total
Women with sons (n=46)			
Blood transfusion	9	21	30
No blood transfusion	5	11	16
Total	14	32	46
Women without children (n=29)			
Blood transfusion	7	8	15
No blood transfusion	6	8	14
Total	13	16	29
All women (n=75)			
Blood transfusion	16	29	45
No blood transfusion	11	19	30
Total	27	48	75

Kidney

In 13 of 51 kidney tissue samples, the Y chromosome was detected. The positive cells were present in glomeruli and tubules. In two women who had sons, up to 10% of the cells were positive (Figure 2A). These women were 77 and 81 years old, and died of sepsis and a ruptured aneurysm of the vertebral artery, respectively. The 77-year-old had had a blood transfusion, whereas the 81-year-old had received no blood transfusion. From the 77-year-old, also tissue specimens of liver, heart, and spleen were incorporated in the study, but these were negative. From the 81-year-old, a specimen from the heart was incorporated in the study, which was negative. In the other positive kidney samples, only one or two cells with Y chromosomes were found (Figure 2B). Table 4 gives a division of positive kidney samples in relation to cause of death, child status, and previous blood transfusions.

Liver

In 10 of 51 liver tissue samples, the Y chromosome was detected. The positive cells seemed to be present in hepatocytes (Figure 2C), and sometimes in the infiltrates of portal triads. Positive cells were scarce, up to 10 in each tissue sample. Table 4 gives a division of positive liver samples in relation to cause of death, child status and previous blood transfusions.

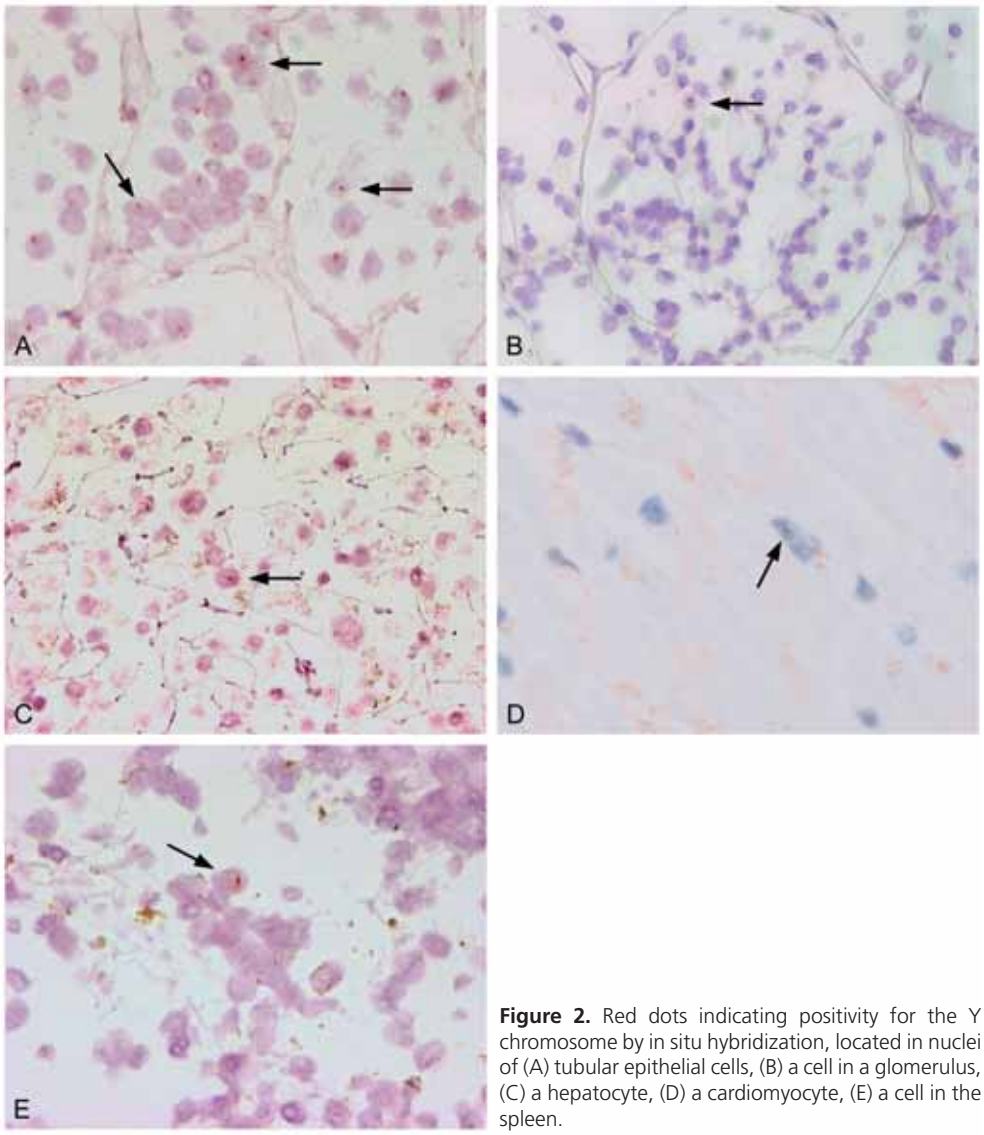

Figure 2. Red dots indicating positivity for the Y chromosome by in situ hybridization, located in nuclei of (A) tubular epithelial cells, (B) a cell in a glomerulus, (C) a hepatocyte, (D) a cardiomyocyte, (E) a cell in the spleen.

Heart

All tissue specimens from women with sons were negative (0/43). However, in the group of women without children, 4 of 26 heart specimens showed Y chromosome-positive cells, which appeared to be cardiomyocytes (Figure 2D). Table 4 gives a division of positive heart samples in relation to cause of death, child status and previous blood transfusions.

Table 4. Women with a Y chromosome identified by ISH in at least one organ

Patient	Age at death	Kidney	Liver	Heart	Spleen	Blood transfusion	Cause of death
WOMEN WITH SONS							
1	29	+	-	-	-	Yes	Adenocarcinoma of the lung
2	38	+	+	-	-	Yes	Amniotic fluid embolus
3	43	-	+	-	-	Yes	Cytomegalovirus pneumonia/sepsis
4	44	-	-	-	+	No	Saddle embolus
5	46	+	n.a.	-	n.a.	Yes	Cerebral hemorrhage
6	57	-	-	-	+	No	Neuro-endocrine carcinoma
7	65	n.a.	+	n.a.	-	Yes	Sepsis
8	67	-	+	-	+	No	Myocardial infarction
9	71	-	+	-	-	No	Pneumonia
10	74	-	+	-	-	Yes	Left ventricular fibrillation
11	76	+	n.a.	-	n.a.	Yes	Cardiac failure
12	77	+ *	-	-	-	Yes	Sepsis
13	81	+ *	n.a.	-	n.a.	No	Ruptured aneurysm of the vertebral artery
14	93	-	+	-	-	Yes	Cachexia
WOMEN WITHOUT CHILDREN							
15	29	n.a.	-	+	-	Yes	Metastasized carcinoma of the ovary
16	38	-	+	+	+	Yes	Myocardial infarction
17	43	+	-	-	+	Yes	Cerebral hemorrhage
18	45	-	+	-	n.a.	No	Myocardial infarction
19	52	n.a.	+	-	n.a.	No	Metastasized melanoma
20	57	-	-	+	-	Yes	Cytomegalovirus infection
21	66	+	+	-	+	No	Liver failure from alcohol abuse
22	68	-	-	+	n.a.	No	Myocardial infarction
23	69	n.a.	-	-	+	Yes	Sepsis
24	80	n.a.	+	-	n.a.	Yes	Pneumonia
25	81	+	+	-	-	No	Cerebral hemorrhage
26	86	-	+	-	n.a.	Yes	Cerebral hemorrhage
27	89	n.a.	n.a.	-	+	No	Sepsis

ISH, in situ hybridization; *, extensive chimerism; n.a., non available

Spleen

In 8 of 44 tissue spleen samples positive cells were found (Figure 2E). In all tissue samples, a number of cells were positive, but not more than ten. Aspecific staining occurred more in the spleen than in the other organs. Table 4 gives a division of positive spleen samples in relation to cause of death, child status and previous blood transfusions.

PCR analyses of kidney DNA

Sequencing confirmed the identity of the Y chromosome-specific-product amplified from the two DNA samples of the women with sons that were scored positive by in situ hybridization. The amplified product had homology with the SRY-sequence unique to the Y chromosome, indicating that it was a male-chromosome sequence and not an irrelevant product.

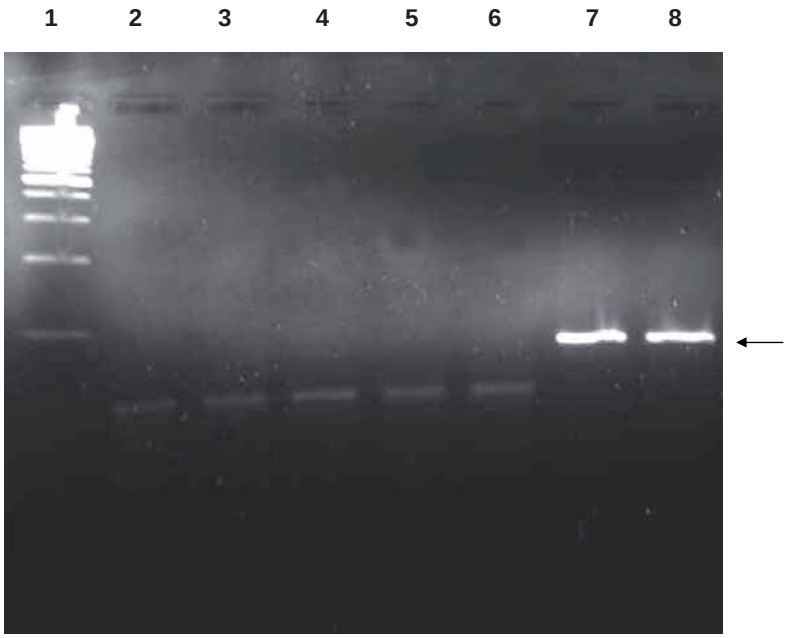

Figure 3. PCR analysis of SRY1 in DNA extracted from kidneys in women with sons. Lane 1 shows a 100-bp size marker; lanes 2, 3, 4, 5, and 6 show DNA from patients of whom the kidney was scored negative by in situ hybridization; lanes 7 and 8 show DNA from patients of whom the kidney was scored positive. The samples in lanes 7 and 8 show a band corresponding to the 88-bp product amplified from Y chromosome DNA, as is indicated by the arrow.

Y chromosome-specific DNA was detected in both kidney specimens of the two women that were scored positive after in situ hybridization, but in none of the 5 specimens that were scored negative. The results are shown in Figure 3. All blank controls were negative. All seven specimens showed comparable results for the control DNA PCR (data not shown).

Discussion

The study we performed investigated the occurrence of chimeric cells in normal hearts, kidneys, livers, and spleens from women of whom the child history and blood transfusion status was known. From our study, it becomes finally clear what the basic rate of chimeric cells in normal organs of the normal female population is, this being a point of discussion in many recent studies on chimerism in transplanted organs. Our main conclusion is that chimerism occurs as a background phenomenon in kidneys, livers, and hearts of normal women and must be interpreted with caution by those who assign it some immunologic role in the context of organ transplantation. Our results bear consequences for studies on chimeric cells in organs after transplantation.

Fifty-one kidneys, 51 livers, 69 hearts, and 44 spleens from 75 women were studied for the presence of Y chromosome-positive cells. Fourty-six women had sons, 29 women had no children. Our results show that chimerism is not related to child or blood transfusion status. Both women with sons and women without children had a considerable number of organs containing Y chromosome-positive cells. Similarly, both women with and without a history of blood transfusion had a considerable number of organs with Y chromosome-positive cells. When combining the child history data with the blood transfusion history data, no differences in the presence of chimerism in various organs became apparent. In the group of women without children and without previous blood transfusions, 6 out of 14 had chimeric cells in at least one of the organs that we investigated.

Still, our results do not rule out the possibility of Y chromosome-positive cells being derived from pregnancies. In contrast to blood transfusions where we can distinguish with a high amount of certainty those women who never had a blood transfusion from those who had, we cannot with the same certainty distinguish those women who never were pregnant of a son from those who were not. In the present study, the history of elected or spontaneous abortions could not be retrieved. However, even if we had these data, the number of unrecognized pregnancy losses would still remain unknown. Given the finding that fetal cells start circulating as soon as 4 weeks and 5 days after conception,[25] chimeric cells may for a considerable part be due to unrecognized pregnancies. Although not statistically significant, there seems to be a tendency towards more chimerism in the group of women without sons. This seems contradictory, but it is possible that these women had more recognized or unrecognized pregnancy losses than the women with sons. Firstly, it has been reported that women with fertility problems experience a relatively higher number of pregnancies, ending in early pregnancy losses, than women whithout fertility problems.[26] Secondly, it is known that at the time of pregnancy termination, a peak of chimeric cells occur in the maternal circulation.[27]

Although we have demonstrated the presence of chimeric cells in normal organs, we can only rely on their localization and histomorphology as determined by light microscopy, to define the type of cells that are positive. It seems evident histologically, that the chimeric cells in the kidney are tubular epithelial cells, in the liver hepatocytes, and in the heart cardiomyocytes. In the tissue specimens from the spleen it is uncertain with what type of cells we are dealing with; it cannot be ruled out that they are circulating cells. It seems unlikely that all the chimeric cells we detected are circulating cells, because this would have led to a more equal distribution of positive cells in all organs. For the moment, however, it is impossible to define their identity more specifically. We are currently developing new techniques, such as laser capture approaches, to study this more extensively.

What consequences bear the results of the present study for the interpretation of results from previous and future transplantation studies on chimerism? In the solid organ

transplantation studies known to us, normal organ controls are lacking or relatively scarce.[2-5,7-13,18-22] Practically all these studies investigated Y chromosome-positive cells in female donor organs that were transplanted into males. It was assumed that the Y chromosome-positive cells were recipient derived. We have for the first time demonstrated that male cells can be present in normal kidneys, hearts, and livers. Theoretically, these organs could have been used for transplantation. Therefore, our findings demonstrate that the chimeric cells thus far described in transplantation studies, are not necessarily donor derived, and could have been present in the organs before transplantation. Taking into account pre-transplantation biopsies (time zero biopsies) to establish whether chimeric cells are present before transplantation, may be useful, but the relative infrequency with which chimeric cells occur may give rise to sampling error.

In transplantation studies, it is often suggested that the chimeric cells are the result of a repair mechanism by which recipient stem cells replace parenchymal cells of the transplanted organ. In line with this hypothesis, chimerism of tubular epithelial cells was reported in transplanted kidneys following acute renal transplant failure.[3,4] Two kidneys in our study showed extensive chimerism of tubular epithelial cells. Should these kidneys have been transplanted into a male recipient, it might have been concluded unjustfully that these were from recipient origin. On the whole, however, it seems that the number of chimeric cells in the normal organs from our study is lower than the number of chimeric cells in most transplantation studies, although comparing different quantities is difficult, because many different quantification techniques are used in various studies.

In transplantation studies, efforts should be made to verify the source of chimeric cells in donor organs, because these sources may be various. Our results suggest that in transplanted organs, part of the chimeric cells may be donor derived, whereas another part may be recipient derived. Only by DNA-testing verification of the source is possible, which firstly is technically hard to perform on the few chimeric cells present in donor organs, and secondly, is encountered with ethical matters which may form a serious obstruction to the realization of these studies. Hypothetically, it is possible that Y chromosome–positive chimeric cells in a female donor organ transplanted into

a male recipient, have various sources including the recipient, the donor's sons (either recognized or unrecognized), the donor's donor of a previous blood transfusion, or another not yet identified source. It seems to be the apotheosis of effort to attempt to separate these various sources, and it may be more important to investigate the clinical implications of chimeric cells in transplanted organs. However, in doing so, taking into account the basic rate of chimeric cells before transplantation, as indicated in this study, is of the utmost importance.

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