MORTALITY

Male microchimerism and survival among women

Mads Kamper-Jørgensen,¹* Henrik Hjalgrim,² Anne-Marie Nybo Andersen,¹ Vijayakrishna K Gadi³,⁴ and Anne Tjønneland⁵

¹Department of Public Health, University of Copenhagen, Copenhagen, Denmark, ²Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark, ³Fred Hutchinson Cancer Research Center, Clinical Research Division, Seattle, WA, USA,⁴Department of Medicine, University of Washington, Seattle, WA, USA and ⁵Danish Cancer Society, Institute of Cancer Epidemiology, Copenhagen, Denmark

*Corresponding author. Department of Public Health, University of Copenhagen, Øster Farimagsgade 5, DK-1014 Copenhagen K, Denmark. E-mail: maka@sund.ku.dk

Accepted 9 October 2013

Background During pregnancy, woman and fetus exchange small quantities of cells, and their persistence at later times is termed microchimerism. Microchimerism is known to substantially impact on women’s later health. This study examined the survival of women according to male microchimerism status.

Methods Male microchimerism presence, measured as Y chromosome in peripheral blood samples, was determined in 272 women from the large Danish Diet, Cancer and Health cohort when aged 50–64 years during 1993–97. Women were followed up for cause-specific death in national Danish registers until the end of 2009. Survival was analysed using Cox regression.

Results A total of 190 women (70%) were male microchimerism positive. During follow-up 21 women died, of whom 11 (52%) were male microchimerism positive at enrolment and 10 were negative. Of the 21 deaths, 13 (62%) were due to cancer and 5 (24%) were due to cardiovascular disease. Male microchimerism presence was associated with a reduced hazard ratio of all-cause mortality of 0.42 (95% CI 0.17–1.03). The hazard ratio of death from cancer and cardiovascular disease was 0.24 (95% CI 0.08–0.79) and 1.66 (95% CI 0.18–15.48), respectively, among male microchimerism positive compared with negative women.

Conclusions Although the biological mechanisms are not precisely known, male microchimerism presence in peripheral blood of women is associated with substantially improved survival in women. The results also indicate that the association with male microchimerism may vary between different causes of death.

Keywords Microchimerism, survival, epidemiology, Denmark
microchimerism and women’s later health. With few exceptions, microchimerism has been associated with an increased risk of autoimmune disorders\textsuperscript{3–8} and a decreased risk of cancer.\textsuperscript{9–12} In the present investigation, we evaluated the association between male microchimerism and survival among women. The analyses are based on peripheral blood samples and questionnaire data from 272 women, collected at enrolment into the Danish Diet, Cancer and Health cohort during 1993–97 when the women were aged 50–65 years. All women were followed up for migration and cause of death in national registers until the end of 2009.

Methods

Materials

We based the present analyses on peripheral blood samples, questionnaire data, and anthropometric measures used for a recently published case-cohort study of the role of male microchimerism in developing breast and colon cancer, respectively, among women.\textsuperscript{9} Blood and data were obtained from the population-based prospective Cancer, Diet and Health cohort comprising Danish cancer-free men and women enrolled during 1993–97 when aged 50–65 years.\textsuperscript{13} At baseline, cohort participants completed a detailed questionnaire on reproductive, lifestyle and other variables, and visited a study centre where they had blood drawn and anthropometric measures taken. Originally, all participants were followed until 27 April 2006 for incident cancers in the Danish Cancer Registry\textsuperscript{14} and migration and death in the Danish Civil Registration System.\textsuperscript{15} Using the combined information from the Diet, Cancer and Health cohort and the linked registers, we used case-cohort sampling to identify a subset of female participants who developed breast or colon cancer and a subset of female controls. For the present investigation we followed the sampled controls only. We sampled women only because we had ascertained male microchimerism by Y chromosome sequences which conventionally should not be present in women outside original exchange at the maternal-fetal interface. For the present study, we used the unique Danish 10-digit identification number to obtain updated data on migrations from the Danish Civil Registration System and cause of death from the Danish Register of Causes of Death,\textsuperscript{16} up to 31 December 2009. The research protocol was approved by the Danish Data Protection Agency (journal number 2011-41-6911) and by the Danish National Committee on Health Research Ethics (journal number H-KF-01-345/93) in accordance with national laws and regulations.

Laboratory methods

From peripheral blood buffy coat specimens drawn from each woman, genomic DNA was isolated and tested for DYS14 sequences as a marker of male microchimerism, by a female technician working in a dedicated PCR-deadbox. Potential carryover contamination from prior DYS14 amplification product was mitigated against by utilizing AmpErase TaqMan chemistry (Applied Biosystems). Using a validated TaqMan polymerase chain reaction specific to the multi-copy Y chromosome gene DYS14,\textsuperscript{17} we determined male DNA presence in DNA extracted from buffy coat cells. Concentration of male microchimerism was estimated as number of chimeric cells per 10\textsuperscript{6} female genomes. Blood samples with any presence of Y chromosome were regarded male microchimerism positive. Details of the laboratory test can be found in Kamper-Jørgensen et al.\textsuperscript{9}

Statistical methods

We estimated survival among microchimerism positive and negative women using Cox regression models with delayed entry, and age as the underlying timescale. Analyses were run using the PHREG procedure in SAS version 9.2. We first fitted a crude Cox regression model to predict the unadjusted absolute survival according to attained age and male microchimerism status. Next, we fitted multivariate Cox regression models to estimate survival among microchimerism positive women relative to that of microchimerism negative women, adjusted for attained age and baseline smoking. The relative survival was expressed as hazard ratios (HR) with 95% confidence intervals (95% CI). Analyses were run separately for all-cause mortality, cancer mortality (colon, pancreatic, lung, ovarian and bladder cancer, and myeloid leukaemia) and cardiovascular mortality (essential hypertension, acute myocardial infarction and pulmonary embolism). Also, we evaluated all-cause mortality in strata of male microchimerism concentration (negative, <5 or \( \geq 5 \) male cells per 10\textsuperscript{6} female cells). Despite availability of a range of lifestyle, anthropometric and reproductive variables, we chose a priori only to adjust the estimates of relative survival for baseline smoking (never, former, current). This decision was based on previous work by our group indicating an association between baseline smoking and male microchimerism positivity\textsuperscript{18} and because of the established detrimental influence of smoking on survival. We followed women from time of interview, i.e. enrolment into the cohort, until exit, i.e. death, migration or end of follow-up, whichever occurred first. Deaths were classified according to the 10th revision of the International Classification of Diseases.\textsuperscript{19} We evaluated the proportional hazards assumption graphically by fitting a local regression curve of the Schoenfeld residuals against the age at time of death, followed by statistical tests of the interaction term between male microchimerism status and the logarithm of age at time of exit, and baseline smoking and the logarithm of age at time of exit, respectively.\textsuperscript{20}
Results

Table 1 shows characteristics of the studied women according to male microchimerism status. A total of 272 women were studied, of whom 190 (70%) were microchimerism positive. During follow-up 21 deaths occurred, distributed as 11 deaths (52%) among microchimerism positive, and 10 deaths (48%) among microchimerism negative women. Mean age at interview was 57 years (range 50–65, P-value 0.38), and mean age at exit was 70 years (range 55–80, P-value 0.75) among both microchimerism positive and negative women. Some differences between microchimerism positive and negative women were observed with regard to baseline smoking (P-value 0.06). Slightly more microchimerism positive (56%) compared with negative (52%) women had ever smoked. However, considerable differences were seen in the distribution of current and former smokers. Causes of death were as follows: colon cancer (n = 2), pancreatic cancer (n = 1), lung cancer (n = 4), ovarian cancer (n = 1), bladder cancer (n = 1), brain cancer (n = 3), myeloid leukemia (n = 1), diabetes type 2 (n = 1), Alzheimer’s disease (n = 1), essential hypertension (n = 1), acute myocardial infarction (n = 3), pulmonary embolism (n = 1) and chronic obstructive pulmonary disease (n = 1) (data not shown). Thus, the cancer mortality group comprised 13 deaths (62%), the cardiovascular mortality group included 5 deaths (24%), and the last 3 deaths (14%) were due to other causes. Causes of death were not differently distributed among microchimerism positive and negative women (P-value 0.38).

The unadjusted absolute survival of women according to microchimerism status and attained age is depicted in Figure 1. At age 80 years, survival was 85% (95% CI 75%–95%) among microchimerism positive and 67% (95% CI 50%–91%) among microchimerism negative women. After adjustment for smoking status at baseline, this translated to an all-cause mortality HR of 0.42 (95% CI 0.17–1.03) among microchimerism positive, compared with negative women (Table 2). Similar analyses of cancer and cardiovascular mortality yielded HRs of 0.24 (95% CI 0.08–0.79) and 1.66 (95% CI 0.18–15.48), respectively (Table 2).

Compared with male microchimerism negative women, women with a concentration of <5 male cells and ≥5 male cells per 10^6 female cells had HRs of 0.51 (95% CI 0.18–1.47) and 0.35 (95% CI 0.12–1.06), respectively (data not shown). Despite the dose-response-like pattern, the HR was not significantly different between women with a concentration of <5 and ≥5 male cells per 10^6 female cells (P = 0.55), and testing the trend yielded a HR of 1.00 (95% CI 1.00–1.00) for each additional male cell per 10^6 female cells (P = 0.77) (data not shown). We found no indication that the assumption of proportional hazards was not met for male microchimerism presence, whereas this was the case for baseline smoking (data not shown). This violation of the proportional hazards assumption was handled by introducing a strata statement to the model, allowing for separate baseline hazards among current, former and never smokers, respectively.

Discussion

To our knowledge, we are the first group to study the association between microchimerism and survival. We report a 60% lower all-cause mortality among male microchimerism positive compared with negative women, primarily driven by a markedly reduced risk of death from cancer. Although numbers were small, we also found some indication that the risk of dying from cardiovascular disease was elevated among microchimerism positive women, and that higher microchimerism

Table 1 Characteristics of women in the study population, according to male microchimerism status

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Microchimerism positive</th>
<th>Microchimerism negative</th>
<th>Total</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of women, (%)</td>
<td>190 (70%)</td>
<td>82 (30%)</td>
<td>272 (100%)</td>
<td></td>
</tr>
<tr>
<td>Number of deaths, (%)</td>
<td>11 (52%)</td>
<td>10 (48%)</td>
<td>21 (100%)</td>
<td>0.38</td>
</tr>
<tr>
<td>Age in years at interview, mean (range)</td>
<td>57 (50–65)</td>
<td>57 (50–65)</td>
<td>57 (50–65)</td>
<td>0.75</td>
</tr>
<tr>
<td>Age in years at exit, mean (range)</td>
<td>70 (55–80)</td>
<td>70 (55–80)</td>
<td>70 (55–80)</td>
<td></td>
</tr>
<tr>
<td>Smoking status at baseline, n (%)a</td>
<td></td>
<td></td>
<td></td>
<td>0.06</td>
</tr>
<tr>
<td>Current</td>
<td>56 (29%)</td>
<td>31 (38%)</td>
<td>87 (32%)</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>50 (26%)</td>
<td>11 (14%)</td>
<td>61 (23%)</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>84 (44%)</td>
<td>39 (48%)</td>
<td>123 (45%)</td>
<td></td>
</tr>
<tr>
<td>Cause of death, n (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.38</td>
</tr>
<tr>
<td>Cancer</td>
<td>5 (45%)</td>
<td>8 (80%)</td>
<td>13 (62%)</td>
<td></td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>4 (36%)</td>
<td>1 (10%)</td>
<td>5 (24%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>2 (18%)</td>
<td>1 (10%)</td>
<td>3 (14%)</td>
<td></td>
</tr>
</tbody>
</table>

aOne woman had no information on smoking status at baseline.

* Denotes the probability of equal distribution among microchimerism positive and microchimerism negative women based on t test for age at interview and age at exit, chi-square test for smoking status and Fisher’s exact test for cause of death.
concentrations were associated with reduced all-cause mortality in a dose-response-like pattern.

The absolute survival in the present study is better compared with national figures of survival among all 80-year-old Danish women who had survived until age 50 years, as reported by Statistics Denmark. At age 80 years, we find an absolute survival proportion of 85% among microchimerism positive and 67% among microchimerism negative women, respectively. Statistics Denmark report a survival of 55% among all 80-year-old Danish women who were alive at age 50 years during 1995. The better survival in our study should be ascribed to the fact that women recruited for the Diet, Cancer and Health cohort were cancer-free at time of enrolment and had healthier profiles compared with the general Danish female population. A recent publication by Larsen et al. showed that mortality among non-participating women was 2.29 (95% CI 2.19–2.40) times higher than that of participants in the Diet, Cancer and Health cohort. The absolute survival reported by Larsen et al. was very similar to the estimates in the current study. This selection, however, should not introduce bias in the present study because participation is very unlikely to be associated with male microchimerism status.

The use of controls sampled for a previous study of breast and colon cancer could raise concern regarding risk-free follow-up time, i.e. controls not being at risk of dying from time of enrolment into the cohort to time of identification as control. However, because we randomly identified controls at time of enrolment and had healthier profiles compared with the general Danish female population. A recent publication by Larsen et al. showed that mortality among non-participating women was 2.29 (95% CI 2.19–2.40) times higher than that of participants in the Diet, Cancer and Health cohort. The absolute survival reported by Larsen et al. was very similar to the estimates in the current study. This selection, however, should not introduce bias in the present study because participation is very unlikely to be associated with male microchimerism status.

The use of controls sampled for a previous study of breast and colon cancer could raise concern regarding risk-free follow-up time, i.e. controls not being at risk of dying from time of enrolment into the cohort to time of identification as control. However, because we randomly identified controls at time of enrolment among all cohort members i.e. the case-cohort sampling technique, controls could include women dying before end of follow-up. Among control women in the present study, 17 died before the original end of follow-up on 27 April 2006.

Biological mechanisms underlying the association between microchimerism presence and improved survival are not well established, but increased immune surveillance against malignancy and a general enhanced repair of damaged tissues induced by allogeneic cells has been proposed. From the present study, we cannot tell which biological mechanism, if any, is driving the observed association. We recently reported male microchimerism presence to have opposite associations with development of breast and colon cancer. We found male microchimerism presence to reduce the risk of breast cancer to one-third, and at the same time to increase the risk of colon cancer 4-fold. However, in a subsequent study we found indications of improved survival among male microchimerism positive compared with negative women who had developed breast or colon cancer. We therefore speculate whether microchimerism could have a general beneficial role in cancer, which in some sites may not be evident because an allogeneic maternal immune reaction hastens cancer development. Repair of damaged tissues induced by fetal cells has been proposed to play roles in both cardiovascular diseases and malignancies.

Whether presence of fetal cells in tissues is a sign of repair or, rather, a sign of disease development is debated. The present study points towards a reduced risk of death from cancers, but no reduction or possibly an increased risk of death from cardiovascular diseases, among microchimerism positive women. If true, this speaks against a general beneficial repair mechanism induced by fetal cells. To our knowledge, no other groups have studied the association between microchimerism, measured using biological material, and cardiovascular disease. A register-based study of mortality among parents of all births in Norway from 1967 to 1992, by Irgens et al. however, support the notion that microchimerism may reduce cancer mortality as well as increase cardiovascular mortality. Based on studies documenting increased cell trafficking between the pregnant woman and her fetus in pregnancies complicated by preeclampsia, preeclampsia may be regarded a reasonable proxy of microchimerism positivity. Irgens et al. report a statistically significantly increased risk of cardiovascular death, and a non-significantly reduced risk of cancer death, among women who delivered to term and who were diagnosed with preeclampsia during pregnancy, compared with women without preeclampsia. No such differences were observed among their male partners.

We found a suggestion of better survival associated with increasing concentrations of male microchimerism. Numbers were small and neither the contrasts nor the trend reached statistical significance.

![Figure 1 Absolute survival among women, according to microchimerism status and attained age](image)

Table 2 Risk of death: HR (95% CI) according to cause of death and male microchimerism status

<table>
<thead>
<tr>
<th>Status</th>
<th>All-cause mortality</th>
<th>Cancer mortality</th>
<th>Cardiovascular mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microchimerism positive</td>
<td>0.42 (0.17–1.03)</td>
<td>0.24 (0.08–0.79)</td>
<td>1.66 (0.18–15.48)</td>
</tr>
<tr>
<td>Microchimerism negative</td>
<td>1 (ref.)</td>
<td>1 (ref.)</td>
<td>1 (ref.)</td>
</tr>
</tbody>
</table>

All analyses were adjusted for smoking status at baseline and attained age.
In a previous study, we reported increasing concentrations of male microchimerism to be unrelated to the risk of breast cancer, whereas it was related in a dose-response-like pattern to increasing risk of developing colon cancer. Alternatively, male microchimerism may not be biologically relevant, but instead be closely associated with another feature with significant impact on survival among women. To the contrary, in previous work by our group we found no such association with a range of lifestyle, anthropometric, reproductive and hospital or clinic visit history variables. Only baseline use of contraceptive pills, hormone replacement therapy, and smoking showed weak associations with male microchimerism presence. Of these, only smoking notably affects all-cause mortality, and serves as the reason why we chose a priori to adjust the estimates of relative survival for smoking. For the current study, we ran a crude analysis as well as several sensitivity analyses adjusting for all combinations of smoking, contraceptive pill use and hormone replacement therapy at baseline. Regardless of which model was fitted, the association between male microchimerism positivity and reduced mortality among women persisted (data not shown).

We assumed Y sequences in blood samples to originate from male pregnancies, with 70% of women testing positive. Other studies before the present have reported male microchimerism prevalences of 43% and 24%, respectively, among healthy women. The higher frequency in our study may be the result of more unrecognized pregnancies, a different source population or the employment of a test with improved sensitivity. Because all women in the present study were healthy at enrolment, it is unlikely that other male microchimerism sources such as blood transfusion or allograft reception affected our findings. Y chromosome in women may also stem from a male twin, a vanished twin or an older male sibling. Twinning status of women in this study was unknown, but the live-birth twinning prevalence is only 1.7% in Denmark. Although not yet investigated, sexual intercourse has also been hypothesized as a source of male microchimerism. Information on sexual habits was not collected in this study; therefore we could not pursue the possibility that either current or past sexual activity confounded the observed association.

If it occurred, male contamination would be non-differential because blood draw and DNA purification was carried out several years before the women were followed up for death. We do not believe that samples were contaminated in the laboratory because all handling of the specimens was done by female technicians. Because non-differential misclassification generally biases relative association measures such as the hazard ratio towards reduced difference between the studied groups, the better survival among male microchimerism positive women will at worst be underestimated. We studied only male microchimerism because we targeted Y chromosome sequences. There are no convenient targets to distinguish a daughter’s cells, and we therefore acknowledge that we have identified only a fraction of the potential cells of fetal origin. Nonetheless, we have no reason to think that the improved overall survival associated with microchimerism positivity should differ according to whether cells originated from male or female fetuses. Following a pregnancy complicated by pre-eclampsia, the risk of breast cancer is markedly reduced after birth of a boy, whereas no such risk reduction is reported after birth of a girl. Among the vast majority of women who have normal pregnancies, however, offspring gender does not affect their later risk of breast cancer.

In conclusion, the present study is the first to establish a link between male microchimerism and survival among women. Male microchimerism positivity is associated with a considerably improved survival. Strengths include an established sequence of exposure and outcome, high quality assessment of both exposure and outcome and little risk of confounding. Limitations include a small sample size and inability to identify female cells of fetal origin. Although the biological mechanisms are not well established at present, the results suggest that the role of male microchimerism may be different for different causes of death.

**Funding**

This work was supported by the Danish Council for Independent Research, Medical Sciences (grant number 10–093896).

**Conflict of interest:** None declared.

**KEY MESSAGES**

- Mother and fetus naturally exchange cells during pregnancy.
- In many women, cells of fetal origin persist in small quantities, a phenomenon known as microchimerism.
- In women, presence of male microchimerism is associated with 60% reduced mortality.
References


