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Full title

A higher incidence of chromosomal aberrations in operators performing a large volume of endovascular procedures

Running title (50 characters max):

Chromosomal aberrations in endovascular operators

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Summary (120 characters max):

Higher frequency of radiation-related chromosomal aberrations identified in endovascular operators.

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All other authors: none

A HIGHER INCIDENCE OF CHROMOSOMAL ABERRATIONS IN OPERATORS PERFORMING A LARGE VOLUME OF ENDOVASCULAR PROCEDURES

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Cardiovascular interventions using X-ray guidance are increasing in both volume and complexity. The long-term biological effects of chronic low dose radiation exposure in operators performing these procedures are, however, largely unknown. Occupational safety limits are based on physical dosimetry only and do not consider individual biological sensitivity to radiation. We previously reported DNA damage in lymphocytes isolated from operators performing endovascular aortic repair (EVAR) (1). Expression of γ-H2AX and phosphorylated ataxia telangiectasia mutated (pATM), which are markers of acute DNA damage/repair, rose immediately after performing EVAR and normalised the following day. These markers, however, do not reflect the effects of chronic exposure, including chromosomal aberrations that may herald genomic instability and predisposition to malignancy. Here we report important findings pertaining to these aberrations in an international group of operators performing a large volume of complex endovascular interventions, including branched and fenestrated EVAR (B/FEVAR).

Peripheral blood was collected from endovascular operators (n=12, 11 male) and radiation-naïve general surgeons as controls (n=6, 5 male), all of whom gave informed consent. The study was approved by our institutional review committee (Reference:16/LO/1111). The median age of endovascular and control operators [(50 (36-55) vs 47 (36-52), respectively, p=0.37] and years in practice [13.5 (3-20) vs 10.5 (5-16) respectively, p=0.19] were comparable. There were no inter-group differences in radiation exposures for personal health reasons, smoking history or medications. Two endovascular operators had cancer, a squamous skin and renal lesion, both curatively treated at age 49 and 16, respectively. Endovascular operators performed a median of 35 (20-100) standard EVARs and 70 (30-100) B/FEVARs annually with a median annual personal radiation dose of 0.96mSv (0.22-13.64) in the three years prior to blood sampling. All endovascular operators wore lead gowns and thyroid shields. Lead leg shields, headcaps and goggles were used by 58%, 42% and 92% of operators, respectively. Two operators used a ceiling suspended radiation protection suit and one used scatter radiation absorbing drapes.

Giemsa-stained metaphase preparations were used to analyse the full complement of chromosomes in at least 3,000 lymphocytes per operator (Figure 1A). Semi-automated scoring found a dicentric chromosome frequency of 0.11 (0.03-0.16) per 100 cells in the endovascular operators compared with 0.04 (0-0.06) in controls (p=0.002, Figure 1B). There was no correlation between age of operator and dicentric frequency [Pearson coefficient r=0.04 (-0.44 - 0.50, p=0.876)].

More than 2,000 lymphocytes from nine operators (5 exposed, 4 control) were analysed by multiplex fluorescence in situ hybridisation (m-FISH) using fluorescent probes hybridised to metaphase chromosomes. The frequency of unstable, complex exchanges which involve three or more breaks in two or more chromosomes (0.48 vs 0.24 per 100 cells, Mann-Whitney U test, p=0.32) and stable, reciprocal translocations (0.86 vs 0.59 per 100 cells, Mann-Whitney U test, p=0.38) trended higher in endovascular operators (Figure 1C-F). Stable exchanges can be passed onto subsequent cell generations during mitosis and are, therefore, particularly useful for monitoring cytogenetic effects of chronic radiation exposures. Aneuploidy, which refers to abnormal loss of chromosomes, was more frequent in radiation exposed operators (Wilcoxon Signed Rank test, p=0.004, Figure 1G-H), with a median difference of 0.35 per chromosome.

Dicentric chromosomes, formed by cleavage and incorrect repair of double-stranded DNA, indicate genomic instability and reflect radiation exposure during the lymphocyte's lifespan, which is approximately 3 years (2). Their frequency increases proportionally to cumulative radiation exposures (<~5Gy), allowing their use for biological assessment of chronic exposures (2). The dicentric frequencies we observed fall below the threshold that allows reliable inference of effective exposure dose using current nomograms. Nevertheless, we found an almost 3-fold higher incidence of dicentrics in endovascular operators compared with radiation naïve controls. The dicentric count in the latter group was comparable to that of the general population, which is generally quoted as approximately 0.06 per 100 cells (2). Our findings are corroborated by a recent report of higher dicentric frequency in interventional radiologists (3). These data highlight the need to investigate whether partial body irradiation to unshielded areas such as the legs may contribute to this level of chromosomal damage over time (1).

The chromosomal aberrations detected in the present study using m-FISH, whilst not necessarily caused by occupational radiation exposure, are also associated with cancer (4). These increase the burden of genetic alterations which can cause defects in cell proliferation, induce proteotoxic stress, and promote tumorigenesis, but uncertainties remain around linking these actions to cancer risk. The impact of chronic low dose occupational radiation exposure on the health of medical workers is uncertain and requires extensive epidemiological and mechanistic studies to inform (5). Our exploratory findings are hypothesis generating and strengthen the case for larger scale prospective studies that accurately record radiation doses to all body parts, capture health events, and relate these to cytogenetic markers of chronic exposure.

Data, materials, and methods will be made available to researchers through direct communication with the corresponding author.

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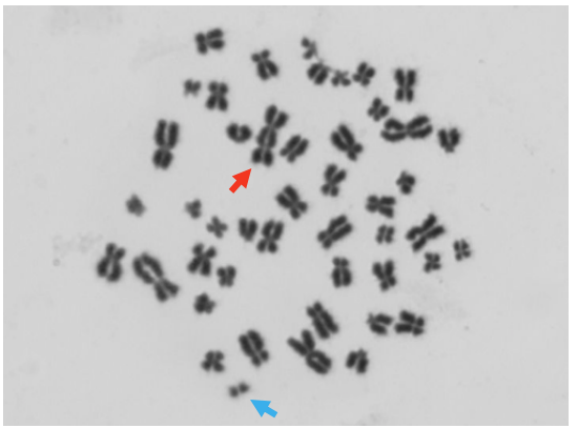
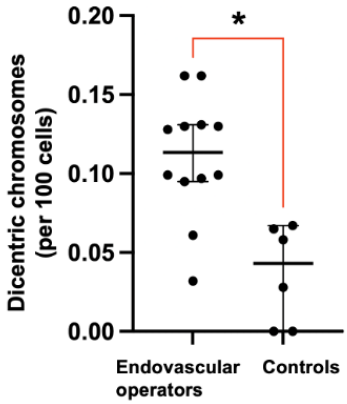
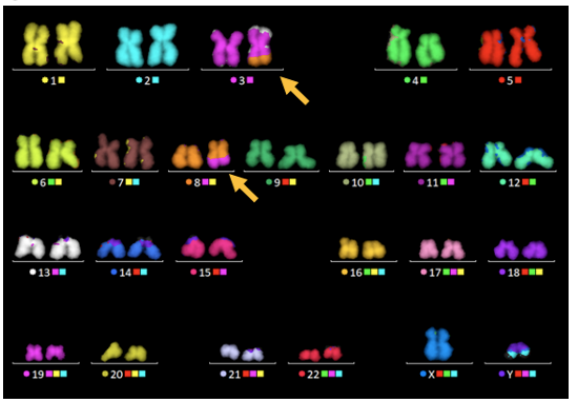
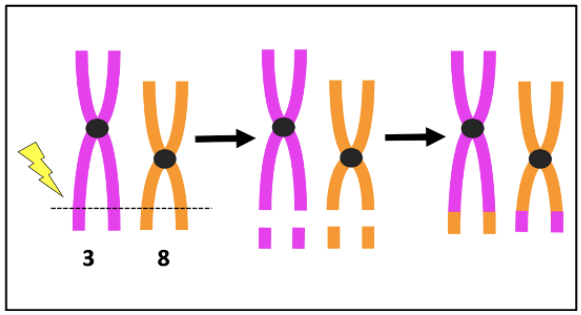
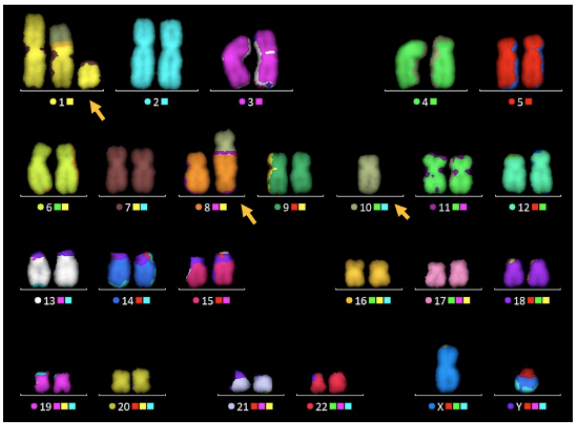
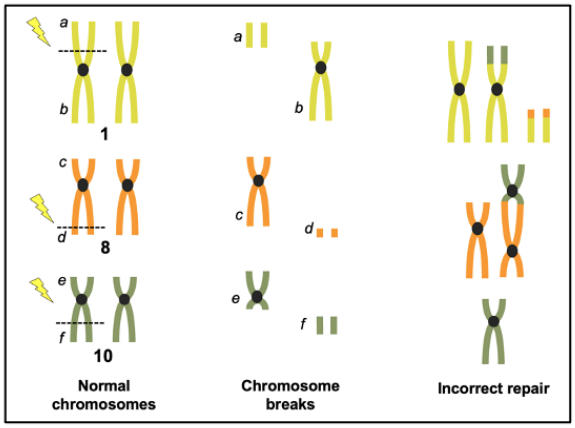


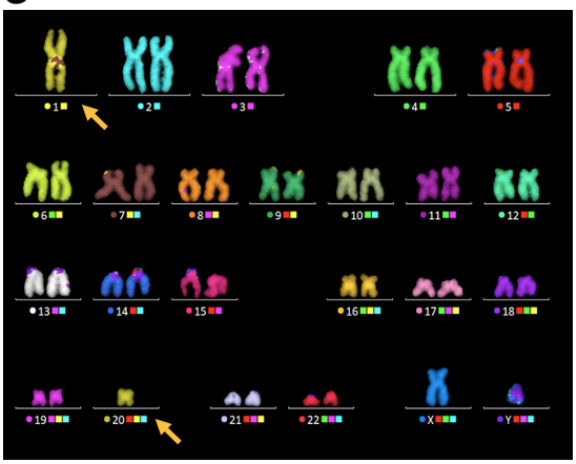

0.20
0.15
0.10
0.05
0.00
*
Dicentric chromosomes (per 100 cells)
Endovascular operators
Controls




3
8




a
b
1
c
d
8
e
f
10
Normal chromosomes
Chromosome breaks
Incorrect repair



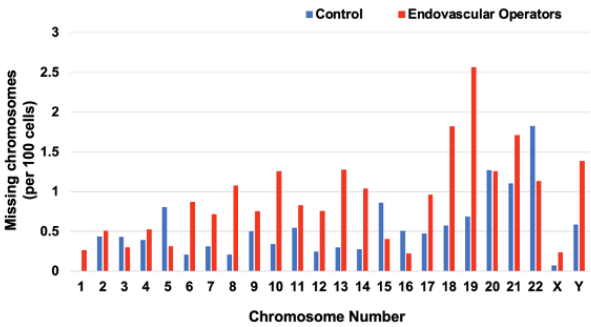

Control
Endovascular Operators
Missing chromosomes (per 100 cells)
3
2.5
2
1.5
1
0.5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y
Chromosome Number

Figure 1. Chromosomal aberrations in exposed endovascular operators versus controls.

A, Chromosome spread of a lymphocyte in metaphase, visualised by Giemsa staining, showing a dicentric chromosome (red arrow) and an acentric fragment (blue arrow). In this aberration, breaks in two chromosomes followed by incorrect repair have resulted in the formation of a single chromosome containing two centromeres and a chromosome fragment containing no centromeres. **B,** Frequency of dicentric chromosomes per 100 cells in endovascular operators compared with radiation naïve control operators [0.11 vs 0.04, respectively (*Mann-Whitney U test, p=0.002)]. **C,** Multiplex fluorescence in situ hybridisation (m-FISH) demonstrating a chromosome spread of 22 pairs of autosomes and single X and Y chromosomes. A simple reciprocal translocation between chromosomes 3 and 8 is highlighted by the yellow arrows. **D,** An illustration depicting the formation of the reciprocal translocation seen in C, where ionising radiation has caused breaks in chromosomes 3 and 8, which have then been repaired incorrectly such that a fragment of chromosome 8 is attached to chromosome 3 and vice versa. **E,** A complex, unstable, non-transmissible chromosome rearrangement visualised by m-FISH, with yellow arrows highlighting the chromosomes (1, 8 and 10) affected by breaks and incorrect repair. **F,** Illustration depicting the formation of the complex rearrangement seen in E, where ionising radiation has caused breaks in three chromosomes followed by incorrect repair. The centromere-containing portion of chromosome 10 has attached to chromosome 8, whilst the acentric portion has attached to chromosome 1. Acentric portions of chromosomes 1 and 8 have also attached to form an acentric fragment. **G,** A chromosome spread with aneuploidy visualised by m-FISH. Aneuploidy refers to the abnormal loss or gain of chromosomes within the cell. In this instance, the yellow arrows highlight the missing chromosomes 1 and 20. **H,** Bar chart showing the abnormal loss of each chromosome (aneuploidy) per 100 cells in endovascular operator samples (red) compared with the radiation naive controls (blue) after analysing a total of over 2,000 cells by m-FISH, median of differences 0.35, Wilcoxon Signed Rank test p=0.004.