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

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9 Running title (50 characters max):

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11 Chromosomal aberrations in endovascular operators

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27 Summary (120 characters max):

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

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

15
16 Peripheral blood was collected from endovascular operators (n=12, 11 male) and
17 radiation-naïve general surgeons as controls (n=6, 5 male), all of whom gave informed
18 consent. The study was approved by our institutional review committee
19 (Reference:16/LO/1111). The median age of endovascular and control operators [(50
20 (36-55) vs 47 (36-52), respectively, p=0.37] and years in practice [13.5 (3-20) vs 10.5
21 (5-16) respectively, p=0.19] were comparable. There were no inter-group differences
22 in radiation exposures for personal health reasons, smoking history or medications.
23 Two endovascular operators had cancer, a squamous skin and renal lesion, both
24 curatively treated at age 49 and 16, respectively. Endovascular operators performed
25 a median of 35 (20-100) standard EVARs and 70 (30-100) B/FEVARs annually with a
26 median annual personal radiation dose of 0.96mSv (0.22-13.64) in the three years
27 prior to blood sampling. All endovascular operators wore lead gowns and thyroid
28 shields. Lead leg shields, headcaps and goggles were used by 58%, 42% and 92%
29 of operators, respectively. Two operators used a ceiling suspended radiation
30 protection suit and one used scatter radiation absorbing drapes.

31

1 Giemsa-stained metaphase preparations were used to analyse the full complement of
2 chromosomes in at least 3,000 lymphocytes per operator (Figure 1A). Semi-
3 automated scoring found a dicentric chromosome frequency of 0.11 (0.03-0.16) per
4 100 cells in the endovascular operators compared with 0.04 (0-0.06) in controls
5 ($p=0.002$, Figure 1B). There was no correlation between age of operator and dicentric
6 frequency [Pearson coefficient $r=0.04$ (-0.44 - 0.50, $p=0.876$)].

7
8 More than 2,000 lymphocytes from nine operators (5 exposed, 4 control) were
9 analysed by multiplex fluorescence in situ hybridisation (m-FISH) using fluorescent
10 probes hybridised to metaphase chromosomes. The frequency of unstable, complex
11 exchanges which involve three or more breaks in two or more chromosomes (0.48 vs
12 0.24 per 100 cells, Mann-Whitney U test, $p=0.32$) and stable, reciprocal translocations
13 (0.86 vs 0.59 per 100 cells, Mann-Whitney U test, $p=0.38$) trended higher in
14 endovascular operators (Figure 1C-F). Stable exchanges can be passed onto
15 subsequent cell generations during mitosis and are, therefore, particularly useful for
16 monitoring cytogenetic effects of chronic radiation exposures. Aneuploidy, which
17 refers to abnormal loss of chromosomes, was more frequent in radiation exposed
18 operators (Wilcoxon Signed Rank test, $p=0.004$, Figure 1G-H), with a median
19 difference of 0.35 per chromosome.

20
21 Dicentric chromosomes, formed by cleavage and incorrect repair of double-stranded
22 DNA, indicate genomic instability and reflect radiation exposure during the
23 lymphocyte's lifespan, which is approximately 3 years (2). Their frequency increases
24 proportionally to cumulative radiation exposures ($< \sim 5\text{Gy}$), allowing their use for
25 biological assessment of chronic exposures (2). The dicentric frequencies we
26 observed fall below the threshold that allows reliable inference of effective exposure
27 dose using current nomograms. Nevertheless, we found an almost 3-fold higher
28 incidence of dicentrics in endovascular operators compared with radiation naïve
29 controls. The dicentric count in the latter group was comparable to that of the general
30 population, which is generally quoted as approximately 0.06 per 100 cells (2). Our
31 findings are corroborated by a recent report of higher dicentric frequency in
32 interventional radiologists (3). These data highlight the need to investigate whether
33 partial body irradiation to unshielded areas such as the legs may contribute to this
34 level of chromosomal damage over time (1).

35
36 The chromosomal aberrations detected in the present study using m-FISH, whilst not
37 necessarily caused by occupational radiation exposure, are also associated with
38 cancer (4). These increase the burden of genetic alterations which can cause defects
39 in cell proliferation, induce proteotoxic stress, and promote tumorigenesis, but
40 uncertainties remain around linking these actions to cancer risk. The impact of chronic
41 low dose occupational radiation exposure on the health of medical workers is uncertain
42 and requires extensive epidemiological and mechanistic studies to inform (5). Our
43 exploratory findings are hypothesis generating and strengthen the case for larger
44 scale prospective studies that accurately record radiation doses to all body parts,
45 capture health events, and relate these to cytogenetic markers of chronic exposure.

46
47 Data, materials, and methods will be made available to researchers through direct
48 communication with the corresponding author.

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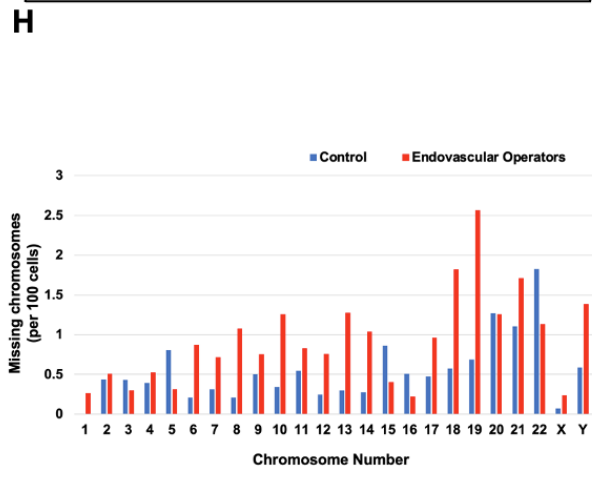
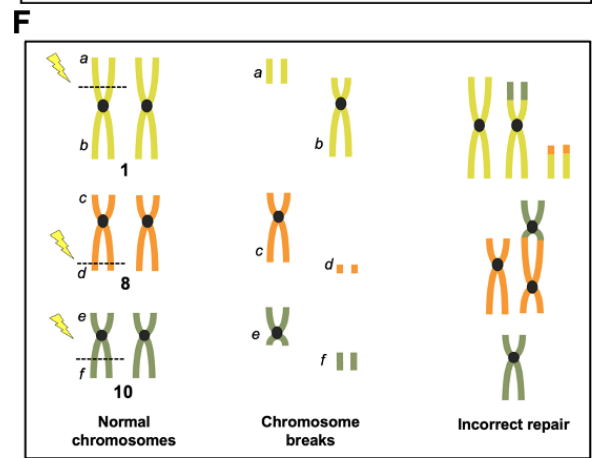
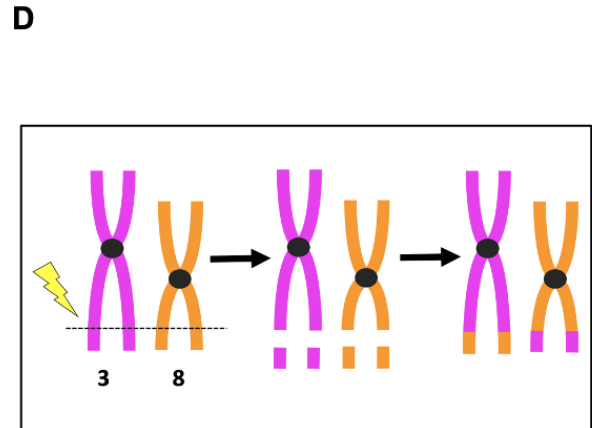
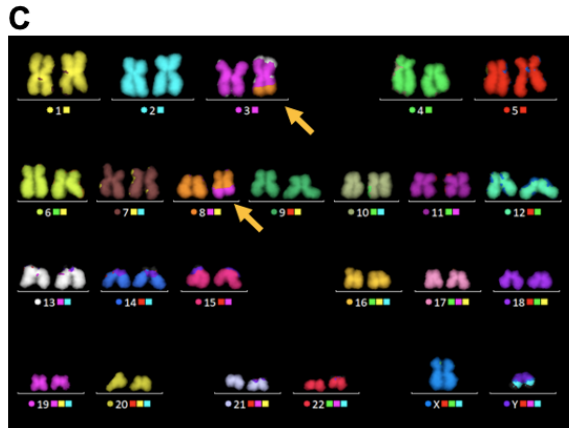
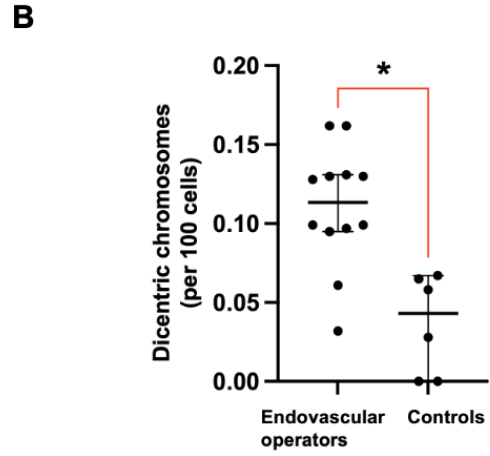
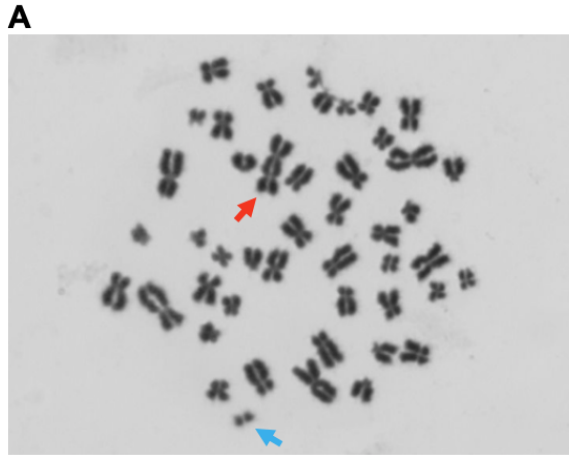


Figure 1. Chromosomal aberrations in exposed endovascular operators versus controls.

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 naïve controls (blue) after analysing a total of over 2,000 cells by m-FISH, median of differences 0.35, Wilcoxon Signed Rank test $p=0.004$.