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Automated scoring of Dicentric Chromosomes differentiates increased radiation sensitivity of young children after low dose CT exposure in vitro

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Automated scoring of Dicentric Chromosomes differentiates increased radiation sensitivity of young children after low dose CT exposure *in vitro*

Abstract:

Purpose

Automated detection of dicentric chromosomes from a large number of cells was applied to study age-dependent radiosensitivity after *in vitro* CT exposure of blood from healthy donors

Materials and Methods

Blood samples from newborns, children (2–5 y.) and adults (20 - 50 y.) were exposed *in vitro* to 0 mGy, 41 mGy and 978 mGy using a CT equipment. In this study automated scoring based on 13,000–31,000 cells/dose point/age group was performed. Results for control and low dose points were validated by manually counting about 26,000 cells /dose point/age group

Results

For all age groups, the high number of analyzed cells enabled the detection of a significant increase in the frequency of radiation induced dicentric chromosomes in cells exposed to 41 mGy as compared to control cells. Moreover, differences between the age groups could be resolved for the low dose: young donors showed significantly increased risk for induced dicentrics at 41 mGy compared to adults.

Conclusions

The results very clearly demonstrate that the automated dicentric scoring method is capable of discerning radiation induced biomarkers in the low dose range (< 100 mGy) and thus may open possibilities for large scale molecular epidemiology studies in radiation protection.

Running title: Automated scoring of dicentric chromosomes after CT application

Keywords: Dicentric chromosomes, automated scoring, radiosensitivity, CT, age dependency, low dose

Introduction

Biomarkers are important tools to investigate effects of exposure to ionizing radiation and differences in radiosensitivity in humans (Pernot et al. 2012) and also to estimate the individual dose in unknown radiation scenarios. Depending on study design and objectives biomarkers have to meet certain criteria to provide valid and reproducible results. Of utmost importance for dose estimation in unknown exposure conditions is a high specificity of the biomarker to ionizing radiation to minimize the influence of confounding factors. A biomarker on the cytogenetic level with a long and successful history of application as biomarker of exposure to ionizing radiation is the dicentric chromosome. Despite the long-lasting history of application, the dicentric chromosome assay up to now is the most specific method to detect radiation induced DNA damage in human peripheral blood samples and is still regarded as the “gold standard” for biodosimetry after recent radiation exposure (Pernot et al. 2012; Hall et al. 2017). Furthermore, the dicentric assay enables differentiations between partial and whole body exposure or high LET and low LET exposure (IAEA 2011). The minimum resolvable dose of the method enables the estimation of average whole body doses of low LET radiation down to about 100 mGy based on the analysis of 1000 cells ideally within a few days after exposure (Wilkins et al. 2008; Ainsbury et al. 2011).

The statistical uncertainty of whole body dose estimation is given with a generally accepted 95% confidence interval which includes the uncertainties of the dose effect curves as well as the uncertainties of the dicentric measurements (IAEA 2011). The uncertainty of the dose estimation, confidence limits and as a consequence also the minimum resolvable dose are markedly influenced by the number of cells analyzed. Chromosome analysis is considered as a very labor intensive method requiring well-trained staff to perform the analyses. For the routine dose estimation assay, which is able to distinguish a dose of 100 mGy, the number of analyzed cells that can be analyzed in a reasonable amount of time is limited and thus it is

currently not suitable for the needs of most molecular epidemiological studies. To maximize the throughput of analyzed cells, several attempts have been started since 1980 to develop automated scoring systems (Finnon et al. 1986; Loerch et al. 1989). In the last years, considerable technical improvements (Schunck et al. 2004; Romm et al. 2013) enabled the development of electronic image analysis systems to optimize automated chromosome analysis including the detection of dicentric chromosomes (IAEA 2011).

Automated scoring of dicentric chromosomes in peripheral human blood lymphocytes has clearly enhanced the capacity and speed of the method compared to manual scoring. As a consequence, technical advances provide new opportunities to use the dicentric assay for more sophisticated research issues, for instance in the low dose range (< 100 mGy) where high cell numbers are necessary to achieve reliable results. Health effects induced by low radiation doses is an emerging topic in radiation protection. In this respect, the growing use of Computer Tomography (CT) has increased the concerns about its potential human health hazards during the last years (Mettler et al. 2009; Berrington de Gonzalez et al. 2016; Nekolla et al. 2017). More and more research is focussed on paediatric CT examinations and the associated risks for infants and young patients to develop cancer. Longer life expectancy in children in comparison to adults and the higher sensitivity of children due to developing organs and tissues have a major impact on late radiation effects (ICRP 2013). Further studies are urgently needed to identify the underlying biological mechanisms and the factual influence of age on the type and frequency of damage in response to ionizing radiation (Bakhtmutsky et al. 2014; Gomolka et al. 2018).

In a European multinational collaborative study (Epidemiological study to quantify risks for paediatric computerized tomography and to optimize doses, EPI-CT), paediatric patients undergoing CT scans were investigated with respect to cancer risks and the underlying biological effects. . The main objective was to evaluate radiation risks conferred by delivered doses from paediatric CT scans especially with respect to leukemia and brain cancer (Bosch

de Basea et al. 2015). Earlier, a feasibility study was conducted to investigate age-dependent radiosensitivity in blood samples taken from healthy individuals representing three different age groups: newborns, young children (2-5 years) and adults (20-50 years). Individual blood samples from each of the three groups were exposed in vitro to 41 mGy and 978 mGy X-rays in a CT scanner. The low dose point (41 mGy) represents approximately an organ dose from a typical CT examination (Brenner and Hall 2007). The high dose point (978 mGy) was used as a positive control of any existent age effect. The feasibility study showed an age dependent increase in the frequency of dicentric chromosomes between newborns, children and adults for the dose of 978 mGy based on 2000 – 2400 cells per age group (Gomolka et al. 2018). However, the number of analyzed cells did not provide sufficient statistical power to draw valid statistical conclusions for the low dose of 41 mGy. Based on the results sample size calculations were performed to determine the number of individuals per age group and the number of cells needed per individual and age group to resolve an age dependent effect at the low dose level (Gomolka et al. 2018). Based on the calculations, the cell number for the individuals included in this study was increased from approximately 200 to approximately 2000 cells per individual. The ten fold increase of analysed cells in a feasible timeframe was achieved by applying the automatic scoring of dicentric chromosomes. The automatic assay decreased the dose detection limit of dicentrics and thus enabled the comparison of adults and young individuals at the low dose of 41 mGy. Moreover, manual scoring of approximately 2000 cells per individual at control level (0 mGy) and 41 mGy was performed to validate the findings from the automatic scoring approach.

Materials and Methods

A detailed description of blood donors used is given in the pilot study of Gomolka et al. 2018. In addition 7 donors were added to the group of adult persons for the current investigations. An overview of the materials and procedures is given below.

Blood Donors

Peripheral blood samples used for chromosome analyses were obtained, with informed consent, from healthy donors, in accordance with ethics approval no. 11083 (Ethik-Kommission der Bayerischen Ärztekammer). Three different age groups (newborns, young children (2 – 5 years) and adults (20 - 50 years)) including 10 to 19 individuals were considered. To exclude gender effects only male individuals were investigated. After blood taking (3-9 ml) using Lithium – Heparin coated collection systems by Sarstedt or Becton Dickinson (BD) all samples were kept constantly at room temperature (20°C) and carried immediately within 1 h to the CT scanner irradiation facility (Klinikum Großhadern/LMU Munich). Each donor, or in case of the children or umbilical cord blood (newborns), one of their parents was informed in advance by a medical doctor about the background of the study and signed their consent. Health questionnaires concerning age, diseases, medication, smoking habits and radiation exposure (e.g. CT and X-ray diagnostic in the last 12 months; former radiation treatment) were answered by each subject or one of the parents.

Group I: Adult Blood samples (20-50 Years of Age)

Healthy adult donors were recruited from the employees of the Federal Office for Radiation Protection (BfS). Individuals showed no occupational radiation exposures and had no history of radiation therapy, chemotherapy, cancer or other severe diseases. In total 12 individuals had been included for the dicentric chromosome assay of the feasibility study. Seven

additional healthy donors within the same age range and radiation background were recruited and included for extended chromosome analysis in the low dose range.

Group II: Umbilical Cord Blood Samples from Full-Term Newborns (in the following called Newborns)

The umbilical cord blood samples were collected in two university hospitals in Munich (Klinikum Rechts der Isar, TU Munich and Klinikum Großhadern/LMU Munich). Blood from 11 male subjects was taken from umbilical cords within 24 h after birth with prior consents of the parents. Blood samples were excluded if mothers were exposed to radiation during pregnancy due to diagnostic examinations.

Group III: Children Blood Samples (2-5 Years of Age)

Blood samples were collected in a private Paediatric Surgery Practice in Munich from healthy male children prior to surgery for circumcision or hernia. Prior to venipuncture, the children were usually given pain relief (in the form of liquid or suppository: Ibuprofen, Nurofen) and an anaesthetics (Midazolam, Propofol, Remifentanyl, Alfentanil, Sevofluran). Exclusion criteria were X-ray or CT diagnostics in the last 12 month and severe diseases such as cancer. In total blood samples from 10 children were collected.

Irradiation of Blood Samples

Heparinized blood samples were irradiated in vitro under well-defined dosimetric conditions. In brief, blood samples were transported to the CT scanner irradiation facility at the Klinikum Großhadern / LMU Munich. The samples from each individual were divided in 6 x 1.2 ml aliquots using sterile tubes and irradiated in a uniquely designed phantom in a CT scanner (Spiral-CT-Scanner Toshiba Aquilion/LB Modell TSX-201A/1K). The doses applied to the blood samples were 0 Gy (control exposure), 41 (± 0.5) mGy and 978 (± 16) mGy. The phantom enabled simultaneous exposure of eight sample aliquots and one parallel control

measurement using a LiF thermoluminescence dosimeter. Scanner voltage was set to 120 kVp, and current settings of 400 mA for the high dose and 50 mA for the low dose were applied. A dynamic scan was performed within 15 seconds for the low dose and 3 x 15 seconds for the high dose. A homogeneous dose distribution to the sample was ensured by the nominal scan width of 32 mm which covered the size of the cryotube applied in the experiment.

Immediately after irradiation (between 30 seconds and 1.5 minutes), blood samples were incubated for 2 h at 37°C in a transportable incubator for repair procedures.

Sample processing

Cell culturing and preparation were performed according to well established procedures provided by the IAEA recommendations (IAEA 2011) and ISO (ISO 19238 - 2014, 21243 - 2008).

After irradiation and a repair time of 2 h at 37°C, 0.5 ml whole blood was transferred to culture tubes containing RPMI-1640 culture medium (Biochrom, Berlin) supplemented with 10% FCS (Biochrom, Berlin), 2% PHA (Biochrom, Berlin) and antibiotics (Biochrom, Berlin). For cell cycle controlled scoring long term Colcemid treatment (Roche, Mannheim) with a final concentration in culture of 0.08 µg/ml was added 24 h after culture set up. Blood samples were cultured in total for 48 h. The hypotonic treatment of cells was carried out with 75 mM KCl. Cells were then fixed in methanol:acetic acid (3:1) three times and the suspension was stored in the freezer (−18°C). For slide preparation the cell solution was concentrated according to the cell yield. The quality and quantity of the metaphases were checked under the microscope prior to Giemsa staining. The slides were covered by cover slips and fixed with Eukitt.

Chromosome aberration analyzes

Different scoring modes were used to analyze coded slides of the blood samples.

1. *Manual scoring of a low number of metaphase spreads / “manual low”: (200 cells / individual, i.e. 2000 - 2400 cells / dose point / age group)*

Slides from each individual were analyzed at the BfS laboratory and also sent to STUK/Helsinki, Finland for parallel analyses of chromosome aberrations. In each laboratory 100 metaphases per individual/dose point (0, 41 and 978 mGy) were manually analyzed according to a harmonised protocol and previously well trained procedure. Only metaphases that contained the full set of chromosomes were scored for dicentric chromosomes. Dicentric scoring of the two laboratories was pooled (Tab. 1) [Table 1 near here]. In this manual scoring procedure slides were scanned in a low magnification (10x) using the metaphase finding software module (MSearch) on the Metafer 4 platform from Metasystems / Germany. The analysis procedure and detection of dicentric chromosomes was performed by a well trained and experienced human scorer at 63x or 100x magnification at the microscope by eye. The dicentric chromosomes were additionally validated by the use of a karyotyping software module (Ikaros, Metasystems) and only metaphase spreads with 46 centromeres were scored. In total 200 metaphases per donor and age group were scored resulting in a total of 2000 to 2400 cells depending on the age group.

2. *Automated scoring / “automatic” (2000 cells / individual, i.e. 13,000 – 31,000 cells /dose point / age group)*

In this scoring mode the detection of dicentric chromosomes was performed on a software based procedure for 0 mGy, 41 mGy and 978 mGy. The details of this multistep procedure including the parameters of the classifier (BfS – classifier) used were published in Romm et al. 2013. Briefly, all slides were analyzed using the automatic scoring system Metafer 4 by

MetaSystems (Altussheim, Germany) including the software modules for metaphase finding (MSearch) to detect the metaphase spreads. In a second step additional software tools were applied for auto-capturing of high resolution images at 63x magnification (with oil) (AutoCapt) and automatic detection of dicentric candidates (DCScore). In a third step, a human scorer evaluated the automatically detected dicentric candidates on the screen of the PC, thus resulting not in a full but in a semi-automated scoring approach. (Romm et al. 2014). In comparison to the conventional manual scoring, the scoring procedure for dicentric chromosomes in the automatic mode is restricted to evaluate dicentric candidates detected by the software. Thus, the human intervention of an experienced scorer is very short and involves only the decision if the detected dicentric candidate (which is marked by the software with a red frame), should be confirmed as a dicentric chromosome or rejected as a false positive. Further information such as undetected dicentrics (false negatives), numbers of acentric fragments, or completeness of cells is not recorded.

3. *Manual scoring of a high number of cells / “manual high” (2000 cells / individual, i.e. 26,000 cells/dose point/ age group):*

In this scoring procedure the number of cells for the control (0 mGy) and the low dose point (41 mGy) was increased to about 2000 cells / individual / age group to increase statistical power for the low dose range.

The scoring procedure was identical to the procedure described above for the manual scoring of a lower number of metaphase spreads. Because of the limited amount of blood for all groups it was only possible to increase the number of cells for 8 individuals from the adult age group of the pilot study. Therefore, based on the sample size calculations five additional healthy donors with no radiation history comparable to the existing group were included. For the children and umbilical cord blood in total 13 individuals out of the originally collective (21 individuals) could be used for additional analyzes.

Statistical analyzes

To compare frequencies of dicentric chromosomes, the total number of dicentrics and analyzed cells per age group and dose were calculated. To test for differences in the rates of dicentric chromosomes between age groups or different doses exact Poisson tests were used. To test for differences in radiosensitivity between adults and the combined group of children and newborns, a logistic regression model of the form

$$\ln(Odds(Y)) = \ln\left(\frac{P(Y_i = 1)}{1 - P(Y_i = 1)}\right) = \beta_0 + \beta_A A + \beta_D D + \beta_{AxD}(A \cdot D)$$

was used, where Y indicates a cell with or without dicentric chromosome ($Y=1$: cell with dicentric, $Y=0$: cell without dicentric), A indicates the age group ($A=1$: young individuals, $A=0$: adults) and D indicates the dose ($D=1$: 41 or 978 mGy, respectively and $D=0$: 0 mGy). The strength of the interaction term β_{AxD} indicates whether age modifies the effect of dose on the risk to obtain dicentric chromosomes. In brief, the exponential of the interaction term (OR_{AxD}) is equal to the ratio of odds ratios of dose 41 mGy vs 0 mGy for young individuals compared to dose 41 mGy vs 0 mGy for adults:

$$e^{\beta_{AxD}} = OR_{AxD} = \frac{OR(D = 1 \text{ vs } D = 0 | A = 1)}{OR(D = 1 \text{ vs } D = 0 | A = 0)}$$

Here, a significantly increased value of OR_{AxD} suggested increased radiosensitivity for young individuals compared to adults. All statistics were performed using *R* version 3.3.1. All reported P values are two-sided.

Results

A recently published feasibility study from the EPI-CT project analyzed the age dependent radiosensitivity in blood samples of male subjects of three different age groups after in vitro exposure to three different dose points (Gomolka et al. 2018). For each age group and each dose point between 2000 – 2400 metaphase spreads were analyzed manually by eye, in total 19,750 cells could be included (Tab. 1).

This feasibility study reported a significant increase in the number of dicentric chromosomes for the low dose exposure of 41 mGy when all age groups were pooled. However, the number of analyzed cells did not provide sufficient statistical power to detect significant differences in the frequency of dicentric chromosomes between 41 mGy vs 0 mGy for adults (Figure 1 and Tab. 3 “manual low” data, rate ratio 2.25, $P=0.14$, 95% CI rate ratio: 0.72-8.27) as well as for newborns (rate ratio 6.0, $P=0.13$, 95% CI rate ratio: 0.73-275.86) and children (rate ratio 1.67, $P=0.73$, 95% CI rate ratio: 0.32-10.73). [Tab. 3 near here] Also, the control value at 0 mGy (rate ratio 2.19, $P=0.30$, 95% CI rate ratio: 0.47-11.02) as well as the low dose value at 41 mGy (rate ratio 1.79, $P=0.18$, 95% CI rate ratio: 0.70-4.56) of adult persons did not show a significant increase in dicentric chromosome frequencies compared to the corresponding values for the group of young individuals (newborns and children, Figure 1). [Figure 1 near here]

The pilot study was performed with analysis of a low number of cells (manual low), and the frequency of dicentric chromosomes was significantly increased at 978 mGy in young individuals compared to adults (rate ratio 1.54, $P<0.001$, 95% CI rate ratio: 1.34-1.76). Consequently, the effect of dose (978 vs 0 mGy) on the risk to obtain radiation induced dicentrics was significantly increased for the combined group of newborns and children compared to the adult group (Figure 2 “manual low” data, $OR_{AxD}=4.13$, $P=0.029$, 95% CI:

1.17-16.29). In comparison, this effect could not be observed for the low dose of 41 mGy vs 0 mGy (Figure 2, $OR_{AxD}=1.76$, $P=0.47$, 95% CI: 0.39-8.62). [Figure 2 near here]

With the assistance of an automated image analysis system the number of scored cells was increased by a factor of about 10 using the same slides as for manual scoring and additional ones prepared out of the same cell suspension and from additional healthy donors within the same age range and radiation background. In total 13,000 – 31,000 cells / dose point / age group were analyzed by the software module trained for the detection of dicentric chromosomes. In total more than 180,000 cells were investigated (Tab. 2) [Table 2 near here]. For the adult group as well as for the group of young individuals a significant dose effect relationship was clearly shown (Figure 3). [Figure 3 near here]

Generally the number of detected dicentrics per cell was lower for the automated than for the manual scoring mode, analysing a low number of cells (Tab. 1 & Tab. 2). This was also true for the control value of the different age groups investigated. Increasing the cell number by about 10-fold enabled the detection of a significant increase of the frequency of radiation induced dicentrics for the dose of 41 mGy vs 0 mGy for adults (Figure 1 and Tab. 3 “automatic” data, rate ratio 1.83, $P=0.004$, 95% CI rate ratio: 1.19-2.83) as well as for newborns (rate ratio 3.62, $P=0.002$, 95% CI rate ratio: 1.50-9.99) and children (rate ratio 8.86, $P<0.001$, 95% CI rate ratio: 3.22-33.97). Moreover, differences between the age groups at the low dose level could be resolved by revealing a significantly increased effect of dose (41 vs 0 mGy) on the risk to obtain radiation induced dicentrics for young donors compared to adults (Figure 2, “automatic” data, $OR_{AxD}=3.04$, $P=0.004$, 95% CI: 1.46-6.76). However, due to significantly lower control values at 0 mGy, the overall frequency of dicentrics at low doses of 41 mGy showed a non-significant tendency to be lower for the combined group of young individuals compared to adults (rate ratio 0.68, $P=0.051$, 95% CI rate ratio: 0.47-1.01). By further increasing the dose, the effect of the control value has less influence and the total

frequency of dicentrics in young individuals reaches higher values than in adults (see Figure 3).

To validate the findings from the automated scoring for the low dose level (41 mGy), the number of cells for the manual scoring procedure was increased as well (Tab. 4).[Table 4 near here]

The frequency of dicentrics was highly correlated between the manual high (increased number of cells scored for 0 mGy and 41 mGy) and the automatic scoring procedure (Figure 4, Spearman's partial rank correlation $\rho=0.73$, $P<0.001$) [Figure 4 near here]. There was a significant tendency for lower relative counts of dicentrics for the automatic procedure compared to the manual procedure (Figure 4, Wilcoxon signed-rank test $P=0.029$). Similar to the automatic scoring procedure a significantly increased frequency of radiation induced dicentrics was detected for 41 mGy vs 0 mGy in adults (Figure 1 and Tab. 3 "manual high" data, rate ratio 1.74, $P=0.004$, 95% CI rate ratio: 1.18-2.58) as well as in newborns (rate ratio 4.57, $P=0.001$, 95% CI rate ratio: 1.70-15.39) and children (rate ratio 5.25, $P=0.001$, 95% CI rate ratio: 1.77-21.03). Moreover, the observation of an increased effect of dose (41 vs 0 mGy) on the risk to develop dicentrics was confirmed for the group of young individuals compared to adults (Figure 2 "manual high" data, $OR_{AxD}=3.29$, $P=0.006$, 95% CI: 1.47-8.15).

To evaluate the saving of time, the effort for manual scoring and automatic scoring was compared. A well trained scorer is able to analyze about 200-300 cells per day. In the presented study, more than 104,000 cells were analyzed by two experienced human scorers (Tab. 5),[Table 5 near here] with about 52 weeks of work. Taking into consideration weekends, holidays and failures due to illness, 1 year for 2 persons is needed to analyze such a huge number of cells. In comparison to the manual scoring the automated analysis was performed by one trained scorer in about 4 weeks including the whole procedure of metaphase finding, auto capture, detection of dicentrics and validation by a human scorer.

Discussion

Within the EPI-CT project different biomarkers for radiation exposure were compared and their sensitivity tested to clarify the biological mechanisms behind low dose hypersensitivity observed in CT examined paediatric patients. The work was divided into a number of distinct and complementary tasks which allowed to study the effects of CT exposure using a variety of approaches, including assessment of radiation induced DNA damage. In our previous paper we proved that age dependent radiation sensitivity can be resolved by dicentric chromosome analysis but not by the gamma H2AX assay (Gomolka et al. 2018). The dicentric chromosome method was applied because of its high specificity to radiation, low background, comparability of *in vivo* and *in vitro* results and low inter- and intra-individual variations. The minimum resolvable dose of the dicentric chromosome assay is 100 mGy for an acute radiation exposure based on 1000 cells analyzed in order to give a reasonably accurate estimate of dose (IAEA 2011). Several publications in the past used dicentric aberrations to detect biological effects after CT scans in *in vivo* and *in vitro* examinations of individuals or groups. Stephan et al. (2007) demonstrated in an *in vivo* study that the yield of dicentric chromosomes was significantly increased in 10 children from 0.4 –15 years after CT examinations in comparison to the level before the CT scanning procedure. The number of manually analyzed cells per individual in this study was on average 1000 cells / child before and after CT scan. A non-significant increase in dicentric chromosomes with dose was recognised. Different exposure conditions (with and without contrast medium, different examination field), age and weight of patients affect dosimetric calculations, revealing a general problem of *in vivo* studies, where several factors introduce additional uncertainties. Abe et al. (2015) investigated 10 adult patients before and after a CT scan and found a significant increase in the formation of dicentric chromosomes after a single CT scan but no

correlation to effective dose. The authors analyzed 2000 cells per individual but suggested to increase the cell number to approximately 10,000 to reduce the detection limit to about 70 mSv, which is a challenge for a single laboratory.

In an *in vitro* study of Golfier et al. (2009) a clear dose effect relationship could be established after CT scans at dose levels from 0 - 1Gy including 0.025 Gy as lowest dose point based on the blood sample of only one healthy adult donor. Altogether, about 26,000 cells were manually analysed for dicentric chromosomes to establish the complete dose effect curve. For the low dose points (0 and 0.025 Gy) about 8000 cells were analysed. Bakhmutsky et al. (2014) investigated the influence of age on the frequency and type of chromosome damage in response to ionizing radiation and found a statistically significant increase in the frequency of dicentrics for newborns in comparison to adults in the dose range > 1 Gy. Gomolka et al. (2018) clearly demonstrated that newborns and young children (< 5 years) showed a 1.5 increased level of dicentric aberrations after *in vitro* CT exposure of 978 mGy compared to adults. This was not the case for the low dose (41 mGy), where high uncertainties due to insufficient cell numbers masked the effect. A statistically meaningful quantification of low dose exposure (< 100 mGy) can only be achieved by analyzing a very high number of cells. Statistical calculations suggested that increasing the number of cells by a factor of 10 would provide sufficient statistical power to obtain statistically meaningful results in the low dose range (Gomolka et al. 2018). The time consuming procedure of scoring metaphase spreads manually by well-trained human scorers has been one of the major limiting factors to use the assay for low dose radiation exposure (< 100 mGy). Technical improvements in automatic dicentric detection markedly speed up the procedure and enable the analysis of high cell numbers in reduced time. In this study, a clear correlation between manual and automated scoring was demonstrated (Fig. 4). The observed tendency for lower counts of dicentrics for the automatic procedure is in accordance with published results ((Vaurijoux et al. 2009; IAEA 2011; Beinke et al. 2013) Furthermore, it was possible to detect an increase in the occurrence

of X-ray induced dicentrics at 41 mGy in young individuals compared to adults (Fig. 2). Here, the significantly lower control value of the young age group in comparison to the higher control value of the adult group (Fig. 3) becomes essential for the discrimination of an effect in the low dose range. Due to the increased cell numbers, it was also possible to detect a significant increase of the frequency of dicentrics at 41 mGy for all age groups (Fig. 1). This was due to the remarkable reduction of uncertainties in the frequencies of dicentrics in the unirradiated versus the irradiated cells. The results provided by the current study clearly demonstrate the benefit of the automated dicentric scoring method for the identification of influencing factors such as age-dependent radiosensitivity at a low dose level after CT exposure. Despite this progress it has to be emphasized that the sensitivity of the assay depends strongly on the uncertainty of the background level of dicentric chromosomes and further improvement is unlikely due to the background 'noise' in the assay (Lloyd 2006).

For this study workload and time necessary to produce the results could be reduced by a factor of 13 from approximately 52 weeks (about 1 year) for manual scoring (2 trained scorers) to about 4 weeks for automated scoring (1 scorer). The factor of 13 would even be twice as high if only one trained scorer was responsible for the manual scoring procedure (1 trained scorer = 104 weeks). A fully automated analysis is not possible with the system used in this study because of the high number of false positives detected in the scoring of dicentric chromosomes in the low dose range. Such falsely detected dicentric chromosomes have to be rejected by a human scorer. The involvement of a human scorer is, however, very short and takes about 10 % of the time for the whole procedure.

The automated scoring approach enables research in the low dose range using chromosome aberrations and improves our understanding of radiation sensitivity of different age groups. Moreover, it provides the possibility to investigate the relationship of chromosomal aberrations and the risk to cancer. Cells with chromosome aberrations may not necessarily

become cancerous but increased frequencies of aberrations are a known indicator of elevated cancer risk (Bonassi et al. 2008). Bonassi et al. provided results based on a pooled database including 11 national cohorts and a total of 22,358 cancer-free individuals who underwent genetic screening for bio-monitoring purpose, including the analysis of chromosomal aberrations and follow-up for cancer incidence and/or mortality. A significant association between frequency of chromosome-type aberrations in peripheral blood lymphocytes of healthy subjects and cancer risk was obvious.

The question arises whether the analysis of symmetrical translocations is more suitable for studying an age depend effect in radiosensitivity and a potential related increased cancer risk especially in children with longer lifetime than adults. The advantage of symmetrical translocations is the higher stability over time because they can pass cell division. However, compared to the results gained with dicentric chromosomes, no statistical significant differences between symmetrical translocations before and after CT scan was detected in *in vivo* studies because of the higher baseline frequencies which are related to different confounding factors (Abe et al. 2016) not characteristic for ionising radiation. New approaches investigating global gene expression with DNA microarrays are currently performed and improve the understanding in different cellular responses to low and high doses of X-rays. Preliminary results are encouraging but further research is needed on these topics (El-Saghire et al. 2013). Approaches to explain a higher cellular radiation sensitivity in children are e.g. an immature DNA damage repair or detection system of developing T-cells in infants (Anderson et al. 2000). Also differences in cell division rates of children and adults could influence the radiation sensitivity, this was discussed in more detail in our previous paper by Gomolka et al (2018).

Software based automated detection of dicentric chromosomes has continuously improved over the years with different technologies (Furukawa et al. 2010; Garty et al. 2015; Rogan et al. 2016). This enables the use of the dicentric chromosome as biomarker for research in the low dose range to be more practical, faster and easier. Analyzing a large number of cells will be too time consuming for a single laboratory and the strategy in such cases will be to share blood samples among different laboratories. In intercomparisons it becomes obvious that interlaboratory differences can exist for the yield of dicentric chromosomes scored, therefore such approach is only possible for harmonised, well coordinated scorers (Wilkins et al. 2008; Oestreicher et al. 2017). In this context, the use of automated scoring systems can help to overcome inconsistencies between the scoring procedures of different laboratories (Iwasaki et al. 2011).

Conclusions:

The results demonstrate very clearly the benefit of the automated dicentric scoring method for the detection of age-dependent radiosensitivity for low dose levels (< 100 mGy) after *in vitro* CT exposure. The study shows that remarkable increase in the number of cells for chromosomal aberration analysis reduces the uncertainties of the dicentric yields and thus enables research at low dose range and assists in differentiating radiation sensitive human groups. In conclusion, the study shows that molecular epidemiological studies in radiation research may be feasible when considerable reduction of the extensive workload is achieved.

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Disclosure statement

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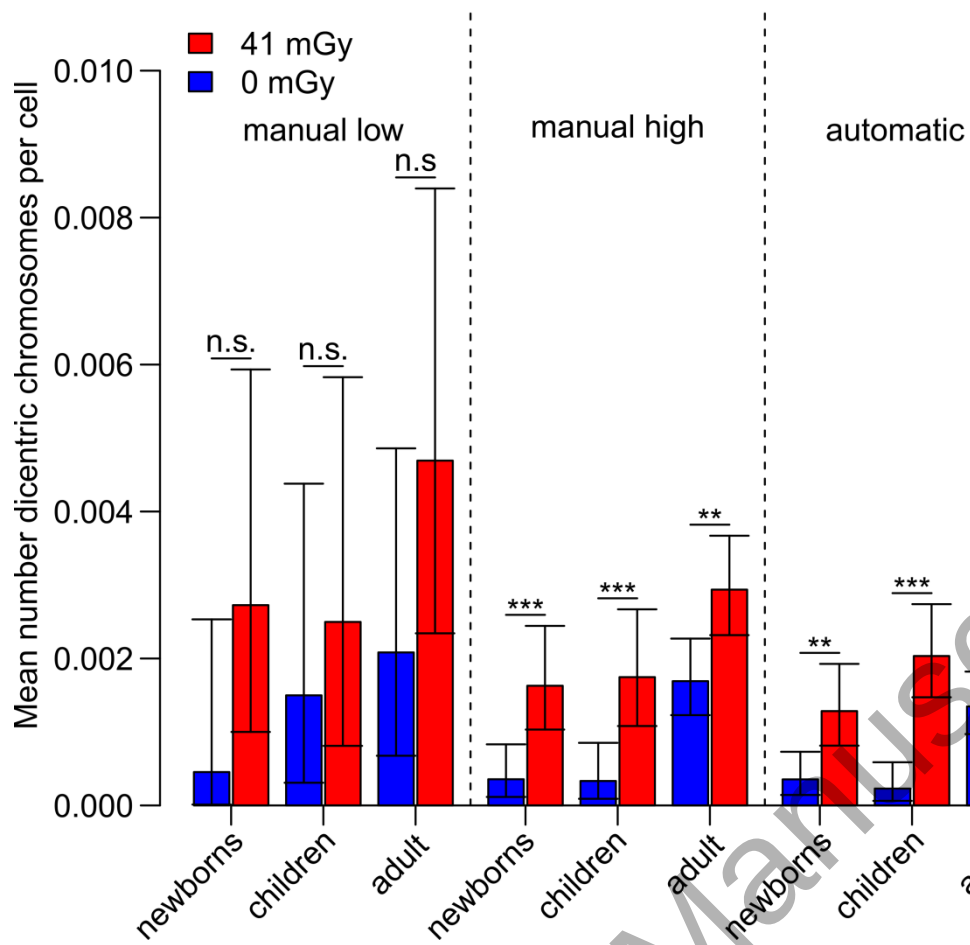
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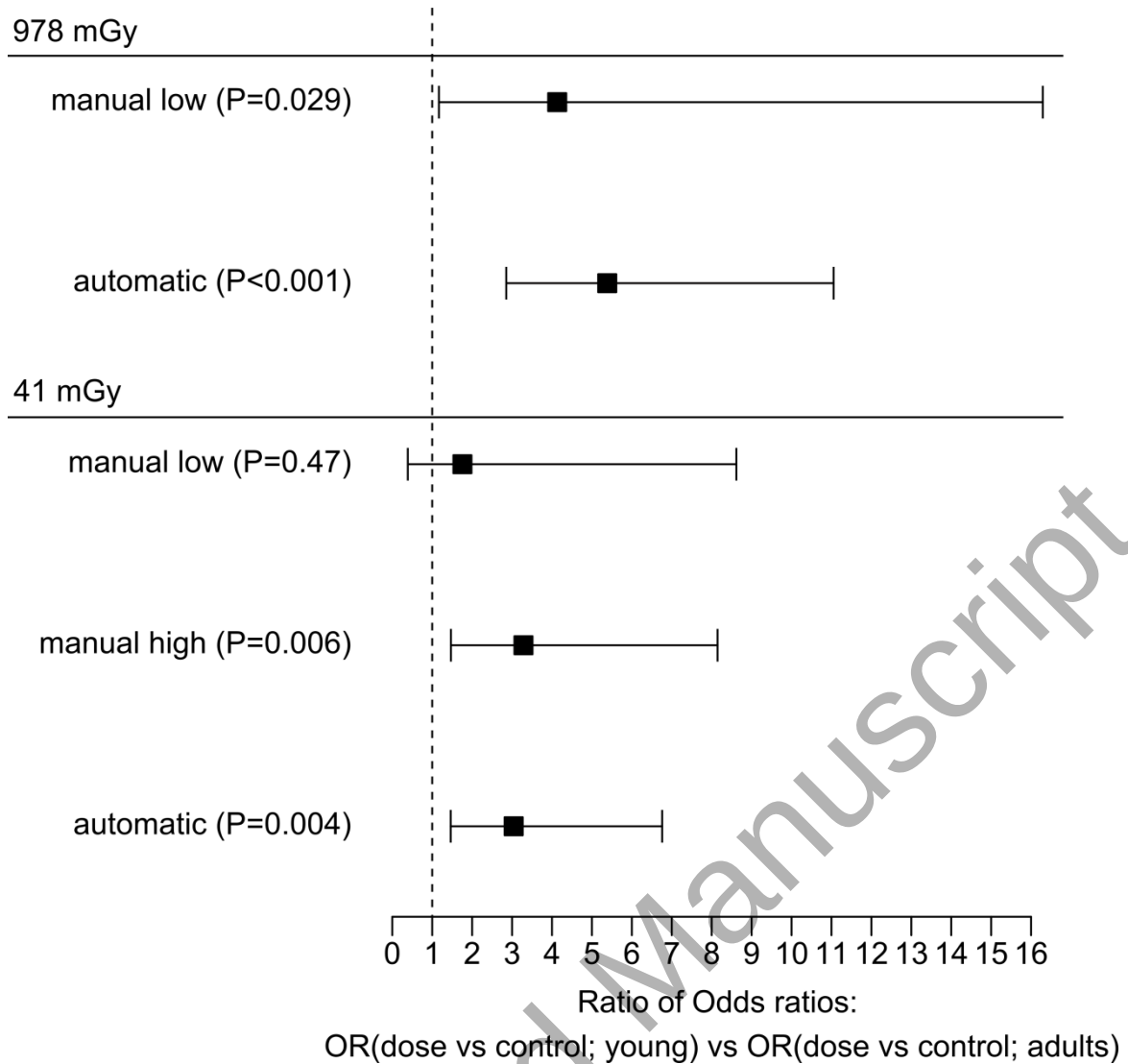
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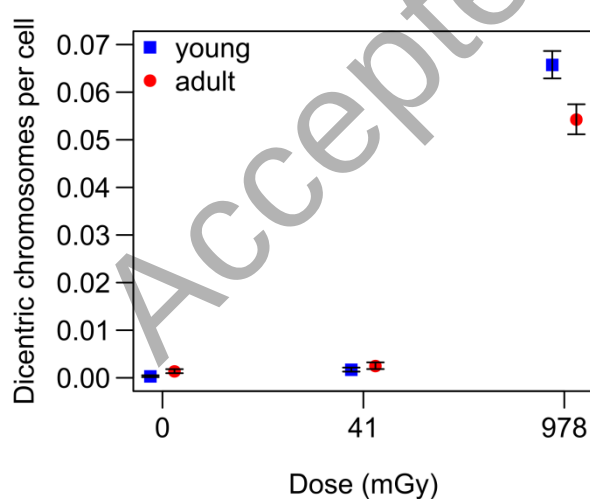
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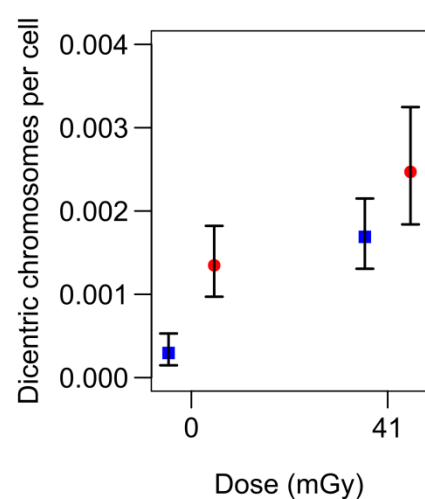


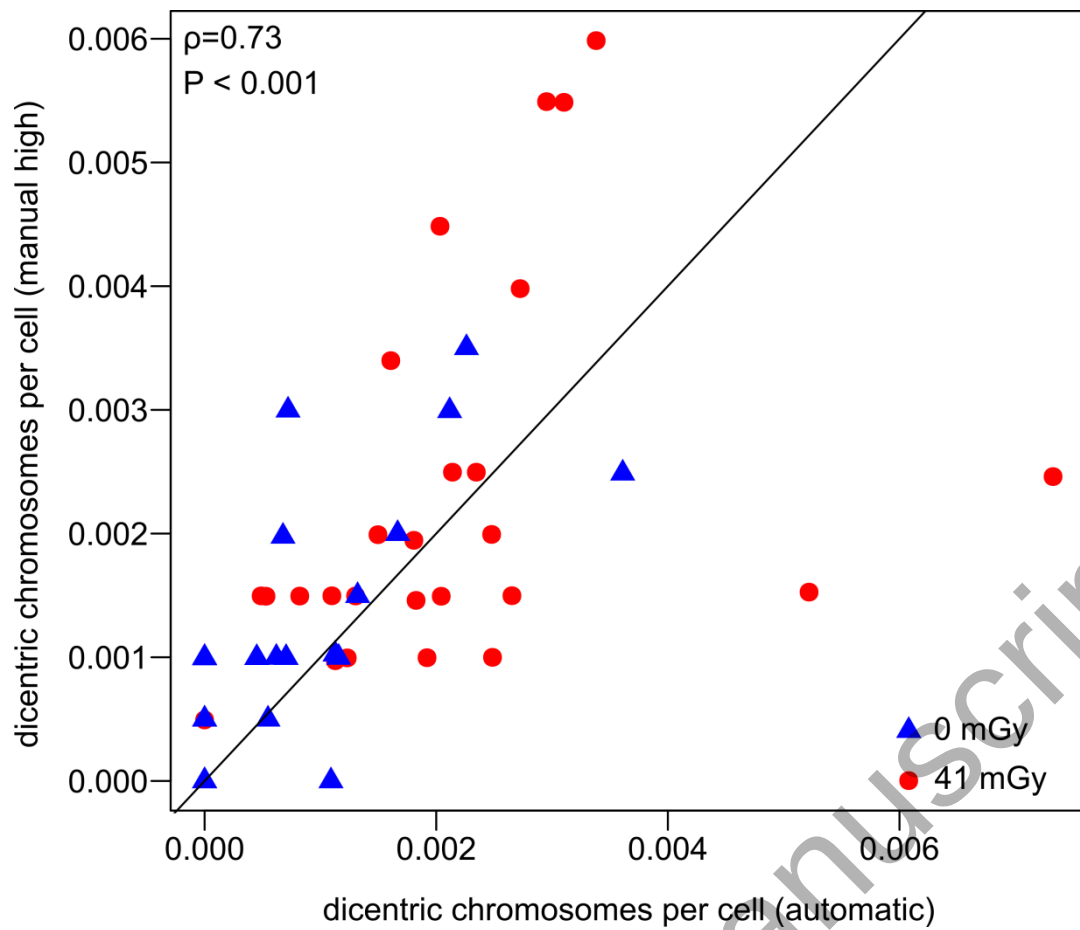


A



B





Tab. 1: Results of chromosome analysis after manual scoring of a low number of cells (manual low) in different age groups and dose points

Age group	Nr of donors	Dose (mGy)	Cells	Dic	Dic/100 cells	SE±
Adult	12	0	2401	5	0.21	0.09
		41	2344	11	0.47	0.14
		978	2400	294	12.25	0.71
Children	10	0	2002	3	0.15	0.09
		41	2002	5	0.25	0.11
		978	2000	370	18.50	0.96
Newborns	11	0	2200	1	0.05	0.05
		41	2201	6	0.27	0.11
		978	2200	420	19.09	0.93
<i>Young Donors (Children/Newborns)</i>	21	0	4202	4	<i>0.10</i>	<i>0.05</i>
		41	4203	11	<i>0.26</i>	<i>0.08</i>
		978	4200	790	<i>18.80</i>	<i>0.67</i>
Total		19750				

Tab. 2: Results of chromosome analysis after automatic scoring (automatic) in different age group and dose points

Age group	Nr of donors	Dose (mGy)	Cells	Dic	Dic/100 cells	SE±
Adult	19	0	31167	42	0.14	0.02
		41	20648	51	0.25	0.04
		978	21277	1154	5.42	0.15
Children	10	0	17416	4	0.02	0.01
		41	21143	43	0.20	0.03
		978	17153	1026	5.98	0.19
Newborns	11	0	19740	7	0.04	0.01
		41	17916	23	0.13	0.03
		978	13748	1005	7.31	0.23
Young Donors (Children/Newborns)	21	0	37156	11	0.03	0.01
		41	39059	66	0.17	0.02
		978	30901	2031	6.57	0.15
Total			180208			

Tab. 3: Rate ratios (and corresponding 95% confidence intervals) for 41 vs 0 mGy by age group and scoring mode.

	manual low	manual high	automatic
Adult	2.25 (0.72 – 8.27)	1.74 (1.18 – 2.58)	1.83 (1.19 - 2.83)
Children	1.67 (0.32 – 10.73)	5.25 (1.77 – 21.03)	8.86 (3.22 – 33.97)
Newborns	6.00 (0.73 – 275.86)	4.57 (1.70 – 15.39)	3.62 (1.50 – 9.99)

Tab. 4: Results of chromosome analysis after manual scoring of and increased number of cells (manual high) in different age group and dose points

Age group	Nr of donors	Dose (mGy)	Cells	Dic	Dic/100 cells	SE±
Adult	13	0	26017	44	0.17	0.03
		41	26216	77	0.29	0.03
Children	6	0	12021	4	0.03	0.02
		41	12026	21	0.17	0.04
Newborns	7	0	14031	5	0.04	0.02
		41	14122	23	0.16	0.03
Young Donors Children/Newborns	13	0	26052	9	0.03	0.01
		41	26148	44	0.17	0.03
Total			104433			

Tab. 5: Comparison of dicentric chromosome scoring using manual and automated method.

	Number scorers	Cells / day	Cells scored	Time needed (weeks)
Manual	2	About 400*	104433	~ 52 weeks
Automated	1	About 180208**	160268	~ 4 weeks

* Working days, manual scoring: 8 h (human staff only)

** Working days, automated scoring: 24 h, (automatic system + short human intervention)

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