Joint UKBTS Professional Advisory Committee ([1])

Position Statement

X-ray irradiation of blood components

June 2020

Approved by: Standing Advisory Committee on Blood Components

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Historical Background

Cellular components are irradiated for patients at risk of developing Transfusion-Associated Graft versus Host Disease (TA-GVHD) following transfusion of blood components containing viable lymphocytes. Traditionally this has been achieved through the use of gamma irradiation.

In 2007/8 JPAC reviewed a variety of information on the use of x-irradiation as an alternative to gamma in order to facilitate UK Blood services moving away from the use of gamma sources due to ongoing concerns regarding the potential for bio-terrorist threat relating to gamma sources (documented in JPAC papers 2007-20, 2008-50 and 2008-75).

Data in the literature and advice from biophysicists at the time suggested that gamma and x-irradiation at a given dose are likely to have a similar biological effect. Validation data from NHSBT and Sweden on blood component quality did not show significantly worse data following x-irradiation compared with gamma, and x-irradiation of components was already in routine use in various centres internationally. On the basis of this information, JPAC accepted that x-irradiation of cellular components was a suitable alternative to gamma irradiation, and as a result both the Red Book and BCSH guidelines on the use of irradiated components were changed to that effect.

Consequently, Raycell x-irradiators were first installed in Filton and were in use for a short time before being moved to Oxford due to reliability concerns at the higher throughput site. The Raycell x-ray irradiators were in use for 10 years at the Oxford NHSBT site and are currently being decommissioned having exceeded their working life.

Requirement for recent revalidation
Since the initial introduction of x-irradiators by NHSBT, a second generation of devices has been produced to improve on some of the shortcomings of the first generation instruments. Additionally, the Government has asked NHSBT to consider whether the use of gamma sources could be phased out altogether, by investigating alternatives. Thus NHSBT has set about validating second generation instruments to assess the feasibility of removal of gamma sources. Whilst gamma and x-irradiation are likely to be equivalent at a given dose, due to differences to the exact nature of the device and process, NHSBT undertook to validate red cell quality to ensure that data were as expected prior to purchase and implementation. Red cells were assessed since irradiation has limited effect on platelets or granulocyte function, but a profound effect on red cells. In addition, a full operational assessment was undertaken to ensure such aspects as the dose of radiation delivered.

This paper describes the recent data obtained in NHSBT, in relation to previous data presented to JPAC.

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Details of irradiators assessed

Studies in 2007/8 were conducted using the Raycell X-irradiator (the 'RS 3000 shielded cabinet x-ray source', manufactured by Rad-Source and later sold to Nordion). This was granted a marketing license by the FDA to market the device as 'substantially equivalent' to a gamma irradiator and was CE marked (for conformance to electrical specifications - CE mark does not deal with the effects of ionising radiation on blood components).

Studies in 2018/19 were undertaken using the Rad Source RS3400. The Rad Source RS3400 is a self-contained, purpose built, x-ray blood irradiator. It is FDA 510(k) approved and CE marked (CE0086). The irradiator uses six individual canisters, fixed on a carousel, that rotate around a central (patented, Quastar) x-ray tube. The tube emits a 360 degree x-ray radiation cloud within the shielded irradiation chamber. The x-ray emission is different from a standard 'imaging' x-ray tube design owed to an elongated filament (initiating the cathode stream of electrons) and a specially designed cylindrical anode. The RS3400 production of x-rays is more efficient than that of a standard x-ray tube and therefore a self-contained cooling system (distilled water) can be employed. There is a fail-safe door interlock safety feature to protect operators from accidental exposure.

The RS3400 system is in use within other hospitals and blood services internationally. The American Red Cross reported successful and sustainable use of the device and The Doctors Laboratory, London have also recently validated the machine in the UK.

Blood component data

Efficacy for prevention of TA-GVHD and biological equivalence.

Several publications state that x-ray irradiation is equivalent to gamma irradiation (Janatpour, K. *et al.* 2005; Klein, H.G. 2006; Hirayama, J. *et al.* 2005). Moroff and Luban state that "Two types of ionising radiation, γ rays and x-rays, inactivate T lymphocytes. Both can be used to irradiate blood and blood components. At a given absorbed dose, both γ and x-rays are equivalent in their ability to inactivate T lymphocytes"(Moroff, G. and Luban, N.L. 1997).

Janatpour et al compared x-ray with gamma irradiation using the Raycell irradiator. They performed a small study on lymphocyte function as part of this work. They reported as follows: "Lymphocytes isolated from both gamma- and x-ray-irradiated (25 Gy) portions of one unit showed an identical lack of proliferation when stimulated with mitogen or with allogeneic leucocytes. One cell division was observed in the cultures with PHA, no cell division was observed in the MLC cultures. Lymphocytes from the control portion showed expected proliferation in both assays".

The only other reference to relative efficacy of x-ray and gamma irradiation we found was an earlier study which reported that there was no difference between the effects of X-ray irradiation, cobalt and 45 MeV electron irradiation on lymphocyte response when equivalent doses were given (Herva, E. and Kiviniity, K. 1975.

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In 2007, advice to JPAC from a senior radiation physicist (JPAC 2007-20) was that in general, x-rays should have very similar effects to gamma-rays on blood components as the energy disposition mechanisms are similar, provided the doses delivered are equivalent. X-rays however are attenuated more rapidly therefore dose distributions can be variable – dosimetry therefore needs to be checked carefully.

More recently, the Head of Radiation Effects at the Public Health England Centre for Radiation and Environmental Hazards (CRCE), reviewed the biological equivalence of gamma and rays in response to a request from NHSBT, 'Gamma rays from a caesium source (Cs-137) and x-rays are both low LET (linear energy transfer) radiations and quite similar in biological effectiveness. Cellular experiments generally report that x-rays are slightly more effective in inducing chromosomal damage and cell killing than Cs-137, but the differential is not great, around 1.5-fold. So the x-irradiation certainly should inactivate white cells to the same or a slightly greater degree than the Cs-137.' Additionally when asked about the likely biological effect on red cells 'On the basis of the few publications I have been able to find it seems that the relative effectiveness differences between x-rays and other irradiations are small when considering membrane damage; for example, one study suggests an RBE of 1.4 – 1.5 for carbon ions by comparison with x-rays. For comparison the radiation weighting factor for heavy ions is 20 (and this is based more on DNA damage-related endpoints). So as with the previous reply, one might expect some slightly increased effectiveness of x- rays by comparison with gamma, and in terms of membrane damage the differences would be expected to be smaller.'

Current studies using the RS3400 device show that a consistent absorbed dose within UK specification of 25-50Gys can be achieved throughout the irradiation field. The absorbed dose delivered to components was confirmed to be within specification by placing dosimeters directly on to the surface of blood components and completing an irradiation cycle. Full dose mapping of the RS3400 showed a similar dose distribution pattern to that of the gamma irradiators in use within NHSBT. This is unsurprising in view of the proximity of the source within these irradiator models. Dose mapping of the RS3400 was first performed using Thermoluminescent Dosimeters (TLD) but the high measure of uncertainty associated with these dosimeters (10%) proved unsustainable in terms of an upper and lower limit margin of error. NHSBT has therefore employed an Alanine dosimetry method with a lower measure of uncertainty (3.9%) to allow for a more comfortable margin of error. In addition, given the loading capacity of the RS3400 can be variable (minimum load one canister and maximum load six canisters) the routine dosimetry measured every six months also includes full and partial load dosimetry testing.

Component quality

The majority of published literature on x-irradiation, and validation data from NHSBT, relates to red cells. This reflects the well-established negative effect of irradiation per se on red cells in comparison to limited effect on platelets and granulocytes. The effect of irradiation of platelets has been reviewed previously (JPAC 17-24). There are no published studies that have directly compared gamma and x-irradiation of platelets. A study on washed platelets indicates that x-irradiation has minimal impact on markers of platelet function (Hirayama et al 2014). This is consistent with data provided to JPAC by the Karolinska Institute when the use

of x-irradiation was initially approved by JPAC (JPAC 08-75) that concluded that the effect of x-irradiation on *in vitro* platelet characteristics was "negligible".

Janatpour and co-workers compared red cell quality after irradiation with gamma and x-rays at 2 doses (25 Gy and 35Gy) using non-leucodepleted red cells in CPDA-1 (not used in the UK). The recommended dose in the UK is a minimum of 25 Gy and maximum of 50 Gy. They looked specifically at free plasma haemoglobin (Hb), and extracellular potassium levels, as these had previously been shown to be parameters affected by irradiation (Janatpour, K. *et al.* 2005).

They found that at 25 Gy, X-ray irradiated units had slightly higher levels of free plasma Hb, whereas at 35 Gy, gamma irradiated units showed higher levels of extracellular potassium. They concluded that small differences in red cell membrane permeability were found between γ irradiated and x-irradiated units, but that these differences were not likely to be clinically important.

NHSBT has undertaken a variety of studies between 2008-19 on red cell quality following X or gamma irradiation. These are summarised as follows, full reports can be obtained on request from NHSBT:

Studies in 2008 (previously reviewed by JPAC):

The following were assessed:

- Standard red cells in SAGM irradiated day 14 and stored for 14 days (10 units) compared with historical data on 20 gamma irradiated units at same time points.

- Exchange RCC units (10 X-ray, 20 gamma, unpaired), irradiated day 4, stored to day 5

- IUT RCC units (20 X-ray, 20 gamma, unpaired), irradiated day 4, stored to day 5

Studies in 2018/19:

The following were assessed:

1) A pooled and split study design comparing n=10 units in each of four arms:
   - standard RCC in SAGM gamma irradiated day 14 and stored for 14 days
   - standard RCC in SAGM x irradiated day 14 and stored for 14 days
   - neonatal splits irradiated on day 5 (gamma or x), stored to day 19 (14 days post-irradiation)
   - neonatal splits irradiated on day 14 (gamma or x), stored to day 28 (14 days post-irradiation)

2) A pooled and split study design comparing n=10 units in each of four arms:
   - IUT (intrauterine transfusion) gamma irradiated day 4 and stored for 24 hours
   - IUT x irradiated day 4 and stored for 24 hours
   - Exchange red cell units gamma irradiated day 4 and stored for 24 hours
   - Exchange red cell units x irradiated day 4 and stored for 24 hours

Levels of haemolysis were not significantly different in any of the studies performed on standard red cells, neonatal splits or IUT's between x and gamma irradiation. However, there was a trend towards higher levels following irradiation using x rather than gamma and this was significant for exchange units (40% increase in haemolysis 24 hours post irradiation with x compared with gamma, the highest haemolysis value was 0.19%). Likewise, ATP levels were slightly lower in standard and neonatal split red cells following x-irradiation (on average 3-11%). Potassium leakage was significantly higher in standard red cells and neonatal splits following x rather than gamma irradiation although the absolute effect was small and therefore not considered clinically significant (on average 2-5%); this was not the case for IUT or exchange units. 2,3 DGP levels were reduced by a small amount in IUT units 24 hours following irradiation at day 4. Additionally, red cell microvesicles were higher in neonatal split components that were x-irradiated as opposed to gamma early in shelf life (day 5) and stored to day 19.

Taken together these 2018/19 NHSBT data suggest that there is a small, but significantly worse effect of x-irradiation over gamma for the same dose of irradiation (in Gy). This suggests that x-irradiation has a greater biological effect for a given dose. The size of this effect is small at the doses studied here (at a central dose of 32.6Gy, min 27.8Gy, max 40.0Gy) and not consistent across different types of red cell. It is unclear whether small differences in haematocrit or storage media (SAGM v plasma) fully explain these differences.

International context of 2018/19 study results

The UK has tighter guidelines for irradiation compared to others. BCSH and Red Book guidelines stipulate standard red cells must be stored for a maximum of 14 days prior to irradiation plus 14 days following irradiation. The AABB recommended end of shelf life following irradiation is the original expiration or 28 days whichever is the sooner. i.e. you can irradiate on day 1 and store to day 28 or up to day 14 and store to day 42. Council of Europe stipulate you can irradiate up to day 28 but must transfuse no later than 14 days after irradiation and no later than 28 days after collection. The small difference in x compared with gamma irradiation observed in NHSBT studies is likely to be far less than the difference between red cells at the maximum shelf-life following irradiation permitted by UK vs AABB guidelines. A recent international study suggests that haemolysis levels at day 28 are nearly 0.8 fold higher if you irradiate day 1 v day 14 (i.e. at the extremes of those permitted in AABB v UK standards, de Korte, D. *et al.* 2018). Likewise, a study from Canada on hundreds of units of leucocyte depleted red cells in SAGM shows that red cells irradiated using current AABB guidelines are much worse than anything in the UK, in terms of effect on haemolysis of red cells (Serrano, K. *et al.* 2014). Studies on the *in vivo* recovery of red cells units reinfused of the extremes of shelf-life following irradiation permitted by the AABB (irradiated day 14 and stored to day 42 or day 1 and stored to day 28) show acceptable levels of recovery (Dumont, L.J. and AuBuchon, J.P. 2008). SACBC have previously reviewed whether the guidance around maximal shelf-life of red cells prior to and following irradiation should be relaxed. They came to the view that this was unwarranted since there was no real operational gain in doing so and that this would result in a significant worsening of component quality. The paper from Canada held the UK as an exemplar and called for US guidelines to be tightened. Nonetheless, the differences observed here between x and gamma irradiation are likely to be

small compared with red cells irradiated at the maximum length of time permitted in the USA currently.

Taken together the NHSBT data indicate that irradiation of red cells results in increased potassium leakage and haemolysis of red cells, as is already well known; this is slightly worse for a given dose of x rather than gamma irradiation. It is unlikely that such changes would appreciably affect the survival of red cells, based on acceptable levels of red cell recovery at the extreme of shelf-life permitted by the AABB. Further we sought advice from Dr Lesley Bruce, a red cell Biologist at NHSBT Bristol, who advises 'both forms of irradiation cause such significant damage that the difference between the two seems insignificant. RBCs following either treatment will never fully regain their cation gradients, but ATP levels will probably be restored quite quickly and the cells will be functional'.

Overall, the small decrease in red cell quality from x-irradiation compared to gamma must be balanced against the benefits that x-irradiation may bring in terms of reduced bioterrorist threat. Colleagues in Australia have undertaken similar *in vitro* studies on x-irradiation of red cells in SAGM with similar results and are currently considering implementation.

Recommendation

The recommendation from JPAC based on review of this new data is that x-irradiation remains acceptable.

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