Type of the Paper: Article Homogenous Gamma-Irradiation by a Linear Accelerator for Inactivation of viruses in Plasma Samples, a Pilot Study with HIV-1-Infected Plasma

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Abstract

BACKGROUND

The risk of the transmission of (emerging) infectious diseases via blood products is a major public health concern. Therefore, it has been a long-sought goal to find a cost-effective way to sterilise blood after collection. No pathogen inactivation (PI) techniques are available for red blood cells on the market yet, though phase III trials are currently performed. γ-irradiation could theoretically inactivate viruses in red blood cells (RBCs). The dose of γ-irradiation needed to achieve sterility causes irreversible damage to the red blood cells and is therefore not suitable for PI of RBC concentrates. Inhomogeneous irradiation has always been used in the past, but leads to inconsistent measurements and uncertainty of results. By contrast, homogeneous irradiation may achieve the sterility assurance level (SAL) at much lower doses. If a maximum of 25 Gy of homogeneous γ-irradiation is able to sterilise blood products, this would be a very attractive PI method for RBC products.

METHODS

This proof-of-concept study aims to sterilise HIV-1 infected plasma using homogeneous γ-irradiation. Six HIV-1 infected plasma samples were irradiated with 25 Gy of homogeneous γ-irradiation. HIV-1 RNA-loads before and after were compared.

RESULTS

No difference was found in the HIV-1 RNA loads within the limitations of the test used before and after the irradiation. Therefore, homogeneous γ-irradiation appears not to be feasible as a PI-technique for HIV-1 in RBC products.

CONCLUSION

This inevitably means SAL will not be achieved for all viruses with up to 25 Gy of homogeneous γ-irradiation.

1. Introduction

Over 110 million units of blood are collected yearly worldwide.[1] Notwithstanding the current screening and prevention procedures, the risk of transmission of infectious diseases via blood products and plasma derivatives is a major public health concern.[2] Safety of blood supply using donor screening and laboratory testing is limited because it requires knowledge of possible infectious agents, effective laboratory tests for each agent and application of that testing to all collected blood. Therefore, it has been a long-sought goal to find a cost-effective way to sterilise blood after collection using a PI technology. Also, the impact of global warming may have a serious impact on the necessity to implement effective PI technology. As temperatures rise, the transmission of vector-borne pathogens may increase dramatically. As a direct consequence, we may need to rely increasingly on PI technology in the future.[3]

For plasma and platelets, various costly PI techniques are available: Solvent/Detergent techniques and photochemical inactivation techniques, using methylene blue, amotosalen/UVA or riboflavin/UVB.[2] All with its own limitations like the damaging of platelets (and RBCs) by UV light [2] and risk of contamination caused by handling of RBC concentrates. No PI techniques are available on the market yet for RBC concentrates. However, phase III trials are running for Mirasol® and INTERCEPT® with RBCs, using riboflavin/UVB and a frangible anchor linker effector, respectively.[1]

γ-irradiation has been demonstrated to be able to inactivate viruses [4-11] and is routinely used for decontaminating tissue allografts.[6,7,11] However, γ-irradiation is known to damage RBCs, as well.[13,14] Certain reports address the fact that the amount of irradiation needed to achieve the SAL of viruses would be too high (15-34 kGy) for the blood products, causing too much damage to RBCs.[5-10,12] However, in these reports inhomogeneous irradiation was used which allows for inconsistent measurements and uncertainty of results. By using homogenous irradiation we hypothesise that much lower doses can be used to achieve SAL, without damaging the RBCs. RBC products are already incidentally being irradiated with 25 Gy for T-cell inactivation for certain haemato-oncological patients.[15] This, however is inhomogeneous irradiation. 25 Gy is the maximum dose of irradiation that should be used on RBC products, since studies report that 25 Gy or more of γ-irradiation can negatively affect RBC products.[13,14] If homogeneous irradiation with ≤25 Gy could achieve SAL with relative little damage to the blood product, PI of RBC can be achieved with a simple and cheap technique which could reduce health costs dramatically. Therefore, we believe it is of great interest to investigate whether homogeneous irradiation can eradicate viruses in blood in doses agreeable to preserve quality of blood products. Schmidt et al. reported that HIV-1 is resistant to irradiation. A relative high dose of inhomogeneous irradiation was needed in various media to achieve SAL for HIV-1.[5,7] Therefore it appears that HIV-1 is a good model virus to use for this pilot study. A standard dose of homogeneous irradiation should be capable to inactivate HIV-1, whereas a standard dose of inhomogeneous irradiation is not. We hypothesised, therefore, that 25 Gy of homogenous γ-irradiation is capable of eradicating HIV-1 RNA from a plasma sample.

2. Materials And Methods

Study design

This study is a single arm, prospective, non-randomised, open label, proof-of-concept study. This study aimed to explore whether homogenous γ-irradiation can be used to eradicate viruses in blood, using doses agreeable to preserve quality of blood products. We chose HIV-1 as a model virus because it has proven to be a relatively resistant virus for inhomogeneous γ-irradiation. Six anonymised HIV-1-infected rest plasma samples of patients of the Haga Teaching Hospital were used.

Intervention

After HIV-1-loads were measured, the plasma samples were pipetted in smaller containers to minimise the amount of air in the containers. The plasma samples were attached in the middle of a water container to

optimise homogeneity of irradiation (Fig.1). The distribution field of the irradiation is shown in appendix A. Within the water container, the dose of irradiation was within 95%-105% limits of the intended dose(25 Gy). At the site of placement of the plasma samples, the

dose was between 99% and 101% of the intended dose. The six samples then separately received 25 Gy of γ-irradiation using a LINAC linear accelerator. Irradiation of the plasma samples was performed by the radiotherapy department of the Haga Teaching Hospital. After irradiation, HIV-1-loads were measured again using the Abbott RealTi*m*e HIV-1 assay, which is an *in vitro* reverse transcription-polymerase chain reaction test. Viral loads were compared to assess the effect of irradiation.

Statistics

Data was reviewed for normality, a paired t-test was used for statistical analysis. Statistical analysis was conducted using SPSS (version 25.0, SPSS Inc., Chicago, USA). A p-value <0.05 (two-sided) was considered statistically significant.

Ethical approval

Ethical approval was granted by the Medical Ethical Committee Leiden-Den Haag-Delft. Approval was given by the board of the Haga Teaching Hospital. The trial was performed in accordance with the Good Clinical Practice guidelines and the Declaration of Helsinki of 2013.

3. Results

Six HIV-1-patient plasma samples with HIV-1 >2.0 Log_{10} copies/ml were anonymised and irradiated with 25 Gy of homogenous γ-rays using a LINAC. The HIV-1-RNA loads are shown in table 1.

Table 1. HIV-1-RNA load before and after irradiation

	Log_{10} copies/ml	
Sample	**Before irradiation**	**After irradiation**
1	2.12	2.38
2	4.62	4.55
3	2.10	1.96
4	2.53	2.46
5	3.16	3.21
6	4.98	5.12

A Shapiro-Wilk test showed normality of data. A paired T-test was used to compare the means of HIV-1-RNA load before (3.25 $\log_{10}$ copies/ml) and after (3.28 $\log_{10}$ copies/ml). The difference in mean, 0.03 $\log_{10}$ copies/ml, had not changed significantly (p=0.665).

4. Discussion

Key findings

The goal of this study was to investigate whether 25 Gy of homogeneous irradiation is able to sterilise HIV-1 positive plasma. Our results show that irradiation with 25 Gy is insufficient for eradicating or even reducing the HIV-1-RNA load in plasma. Mean $\log_{10}$ copies/ml before irradiation was 3.25, and after 3.28. All samples individually also failed to show a decrease (>0.5 log) in HIV-1-RNA load.

Interpretation

The reported difference in mean (+0.03 $\log_{10}$ copies/ml) is negligible as the variability of the HIV-1-RNA PCR test is 0.5 $\log_{10}$ copies/ml.[16] This means that homogeneous irradiation with 25 Gy does not influence the HIV-1 virus. It can be hypothesised that virus particles in lymphocytes were released due to the breakdown of these lymphocytes during the irradiation, increasing the HIV-1 concentration in the samples. However, all virus particles, whether in lymphocytes or not, should have been eradicated if we were to continue exploring the potential of γ-irradiation in PI. Other studies have reported that 25 Gy or more of γ-irradiation can negatively affect RBC products.[13,14] Therefore, increasing the radiation dose is not an option as an effective PI technique.

Strengths and limitations

We believe this is the first study to focus on homogeneous γ-irradiation and its hypothesised advantage over inhomogeneous γ-irradiation towards PI. Our proof of concept study however, has its limitations. First of all, there are no controls and only one dose of irradiation was tested. We intended to do a series of different doses and controls, but first wanted to verify that 25 Gy would be powerful enough - 25 Gy being the maximum dose to be used on blood products with current protocols. As this first test failed, there was no reason to continue testing with lower doses or even controls, the outcome would not have changed. Furthermore the small sample size is limiting. The last limitation is the fact that the PCR does not measure the infectiousness of the irradiated HIV-1 RNA. Theoretically, the HIV-1 RNA can be damaged by the γ-irradiation, and thereby incapacitated to cause new infections, but still be detected by the Abbott RealTi*me* HIV-1 assay. However, it is well known that HIV-1 is not very infective *in vitro*, [17] which makes it very difficult to show a reduction in infectiousness in traditional culture techniques with PHA-activated PBMC.

5. Conclusion

Homogeneous γ-irradiation of HIV-1 infected plasma with 25 Gy does not affect the viral load as measured through the Abbott RealTi*m*e HIV-1 assay. Therefore, homogeneous γ-irradiation appears not to be feasible as a PI-technique for HIV-1 in RBC products. This inevitably means the sterility assurance level will not be achieved for all viruses with up to 25 Gy of homogeneous γ-irradiation.

Declarations

Ethics approval and consent to participate

Ethical approval was granted by the medical ethics committee Leiden-Den Haag-Delft. The need for written consent was waived by the committee.

Consent for publication

Not applicable

Availability of data

All data generated or analysed during this study are included in this published article.

Competing interest

The authors declare no conflict of interest.

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Authors' Contributions

MR, HV, and RT Conceived the study design, EF, RT and EE ran the tests, RT performed the statistical analysis. All authors participated to the interpretation of the results. RT wrote the first draft. All authors reviewed the draft.

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Abbreviations

PI Pathogen Inactivation

RBCs Red Blood Cells

SAL Sterility Assurance Level

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Figures

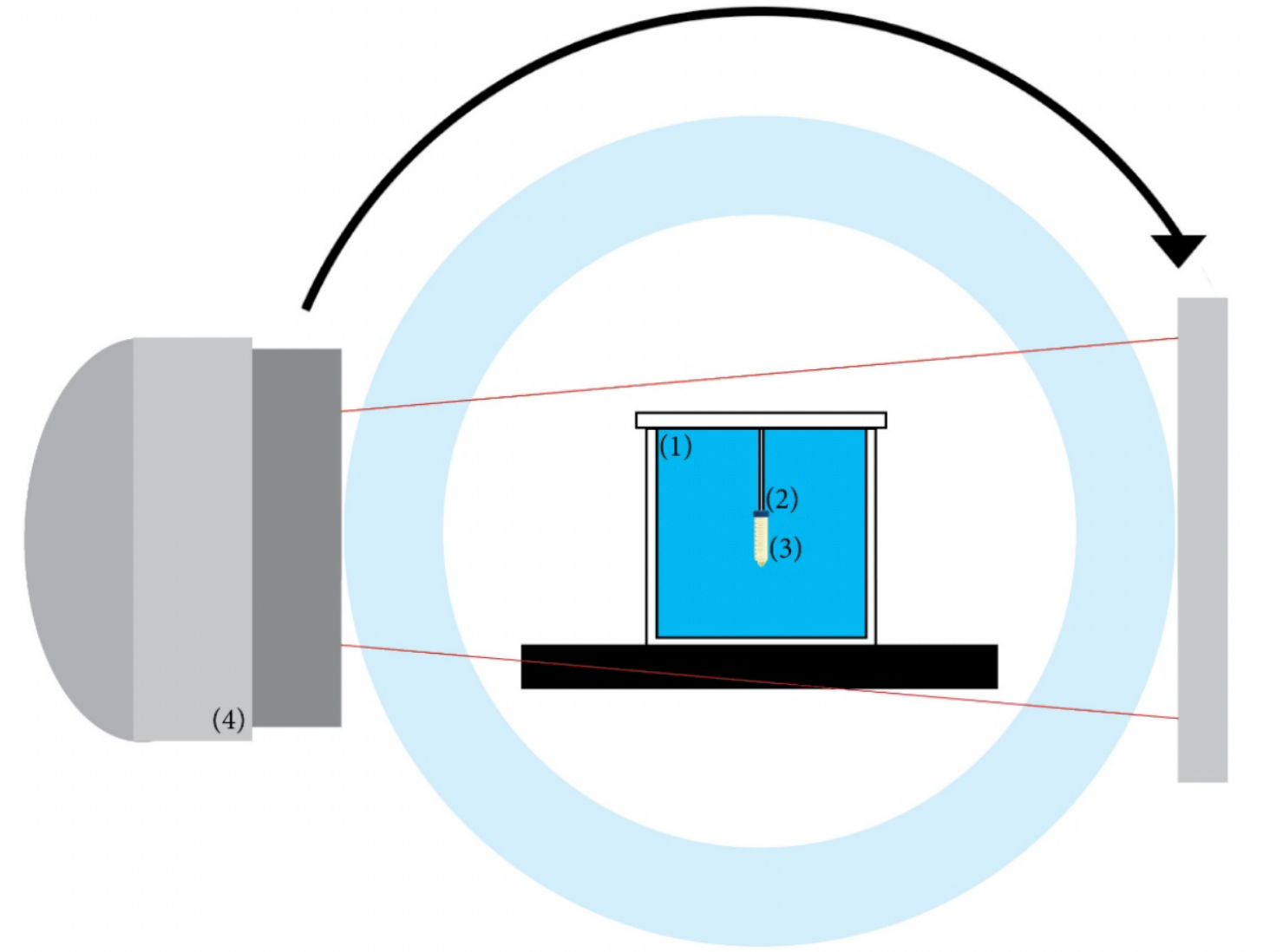

Figure 1

Schematic figure of set-up for irradiation. 1) The water tank is completely filled with water to maximise distribution of radiation without disruption by air; 2) The plasma tube is attached to the lid via an plastic extension for central positioning in the water tank; 3) Full plasma tube without air for optimal distribution of radiation; 4) The LINAC irradiates from multiple angles to ensure homogeneity of irradiation.

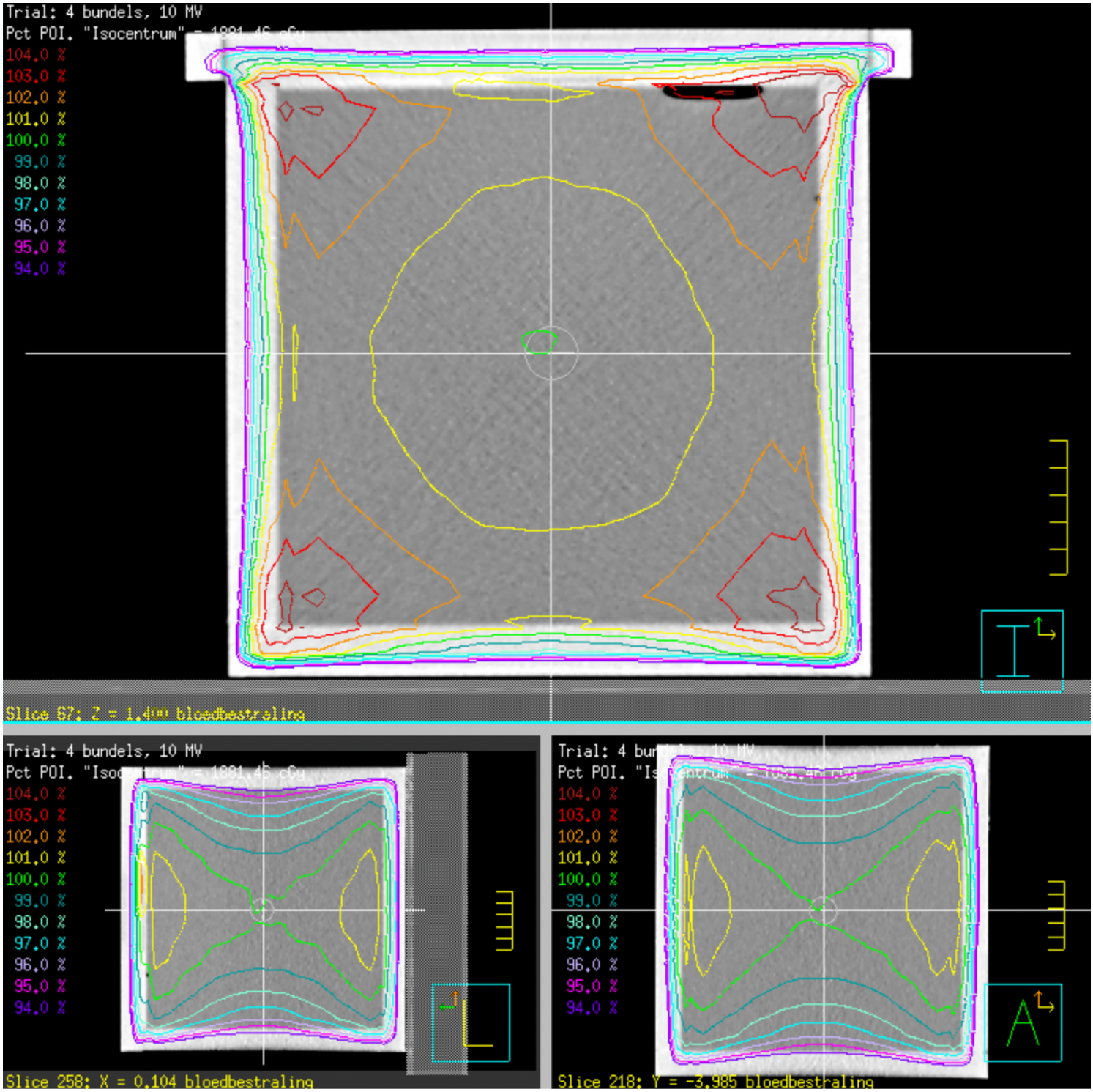

Figure 2

Axial(Top), sagittal (Bottom left) and coronal (bottom right) cross sections through the centre of water container. Iso-dose lines as percentages of central dose.