Triage tools to cope with radiological or nuclear event

Abstract In the case of radiological or nuclear events (RN), radiation exposed individuals might require either early intensive therapy, early radionuclide decorporation treatment or psychological support. It is also important to identify individuals who got no exposure. The sorting of and allocation of treatment to patients (triage) in large scale RN scenarios is challenging. This manuscript describes established concepts to facilitate triage in RN situations. They are either based on exposure/dose estimation or on disease prediction. The contribution of these concepts for triage purposes is critically examined and some outlook on future developments and requirements are provided. An important example is the development of new generation tools to use in the field.
Keywords **Prodromi, ARS, gene expression, peripheral blood, qRT-PCR, TREX, NGS.**

Résumé **Outils de triage pour faire face à un accident radiologique ou nucléaire**
Dans le cas d'événements nucléaires ou radiologiques (NR), il peut être nécessaire d'engager un traitement précoce intensif, un traitement précoce de décorporation de radionucléides ou un soutien psychologique. Il est également important d'identifier les individus impliqués mais non exposés. Le triage et l'attribution de traitement dans ce contexte d'afflux de victimes sont difficiles. Dans ce manuscrit sont décrits les concepts établis pour faciliter le triage lors d'événements NR. Ces concepts sont basés sur une estimation de l'exposition/dose ou sur une prédiction de la maladie. Leur contribution à des fins de triage est examinée de façon critique. Enfin est abordée l'évolution des besoins et des procédures de diagnostic précoce des victimes, en particulier l'adaptation au terrain des outils de nouvelle génération.
Mots-clés **Prodrome, ARS, expression du gène, sang périphérique, qRT-PCR, TREX, NGS.**

Triage in RN scenarios, where do we stand?

Triage describes "…the sorting of and allocation of treatment to patients and especially battle and disaster victims according to a system of priorities designed to maximize the number of survivors" (*Webster Dictionary*).
In the case of radiological or nuclear events (RN), radiation exposed individuals might absorb high doses so that they suffer from what is called the "acute radiation syndrome" (ARS). This is a disease summarizing symptoms originating from different affected organ systems (mainly the hematopoietic, gastrointestinal and nervous systems). The pathomechanism is a massive cell death associated with an organ function deficit. For instance, the haematological acute radiation syndrome (HARS) is characterized by pancytopenia (global decrease of blood cell count: white cells, red cells and platelets) and a consecutive immune deficiency and haemorrhage. ARS patients require early diagnosis and treatment and under these prerequisites their chances to survive are high. The advances in intensive care medicine, transplantation and especially new therapies based on animal experiments did not only lead to a major increase of the lethal dose (LD50/60) from 4 to about 8 Gy [1], but also paved the way to innovative and highly sophisticated treatments like stem cell treatments for local injuries [2] or the use of cytokines to prevent or overcome the HARS [3]. The usage of the latter, with the granulocyte colony stimulating factor (G-CSF) filgastrim approved as first drug to treat the H-ARS by the FDA (Food and Drug Administration). The disease follows four phases, starting with prodromi (early symptom warning of the onset of a disease like nausea, vomiting or diarrhea), a latency period (lasting dose dependently over days or weeks), the manifestation of the disease and either the recovery or the patient's death. Hence, ARS is life threatening, but not an emergency such as the obstruction of the trachea. It also develops after high doses ($\geq$ 1 Gy) so that e.g. stem cells are dying. Hence, radiological scenarios, where radionuclides are released in the environment probably don't lead to an ARS (absorbed doses are not high enough due to the dispersion of the radionuclides), but nuclear events do.
Although ARS patients don't represent an emergency, for improvements in prognosis an early diagnosis, early medication, early hospitalization and early intensive care are mandatory. Predictions on the patient's clinical outcome can be either performed by doing an exposure estimation (dose-to-effect association), or by predicting the clinical outcome based on some clinical or biological parameter (bioindicator-to-effect prediction). Also, three areas of diagnosis have been established over time which are entitled "physical dosimetry", "biological dosimetry/biological effect prediction" and "clinical dosimetry/clinical effect prediction". These different approaches will be addressed in the following chapters.

Exposure/dose estimation *versus* clinical outcome (effect) prediction

"*All things are poison, and nothing is without poison, the dosage alone makes it so a thing is not a poison*" (Paracelsus). Clearly, the higher the dose, the greater the damage and the more aggravated the ARS. This association of the exposure with an effect even represents one of the most important Bradford Hill criteria established for causality check [2, 4]. However, estimating the absorbed dose becomes difficult when dealing with heterogeneous instead of homogeneous radiation exposures, as well as in the case of partial-body irradiation (PBI) *vs.* total-body irradiation (TBI). When dealing with stochastic effects (random mutations and other cell changes leading to cancerogenesis for example), the International Commission on Radiological Protection (ICRP) suggested tissue weighting factors so that by multiplication of the equivalent dose with

the locally irradiated tissue an effective dose could be calculated. Through the effective dose, local exposures can be compared with each other and made comparable with a whole-body exposure. Weighting factors such as that are missing for deterministic effects where cell death mechanisms predominate and not mutations. Hence, the conversion of a local exposure into a whole-body equivalent becomes challenging. Also, when dealing with different dose rates or different radiation exposure qualities (gamma, alpha, neutron), or combined internal (incorporation of radionuclide) or external radiation exposure, the determination of the whole exposure will be challenging and time consuming. For instance, the first dose estimates of the Fukushima accident from 11th March 2011 were published about one year later [5]. And even a single whole-body exposure of 1-5 Gy was of limited value for medical decision-making regarding, e.g., hospitalization for clinically relevant HARS and treatment decisions, thereby challenging an individual recommendation based solely on dose [6]. This recent work performed on Chernobyl clean-up worker is in particular interesting, because the 1-5 Gy dose band reflects the dose, where individuals might respond differently to the same radiation dose due to inter-individual differences in radiosensitivity. That is reflected by the LD50/60, estimated to be between 3-4 Gy, as well, because without therapy 50% (the more radiosensitive individuals) will die, but the other more radioresistant individuals will survive. Clearly, individual responses to radiation exposure cannot be deduced even when knowing the exposure. That is a limitation to bear in mind. Otherwise the recent work on the Chernobyl clean-up worker demonstrated that in most cases, no clinically relevant ARS severity at exposures $\leq$ 1 Gy are observed. But almost all individuals exposed to $\geq$ 5 Gy develop a high HARS severity degree. Hence, at this dose, all individuals respond in the same way, since cells are dying massively in each individual – the exposure overruns differences in radiosensitivity at this exposure height. In other words, exposure height has a value at the extremes, but is challenged by inter-individual radiosensitivity in the middle dose band ranging between 1-5 Gy (whole body exposure).

When consulting the doctor, he will ask for symptoms (clinical parameter or bioindicator of effect prediction) and from there the medical doctor will conclude about the disease. This very well accepted procedure in medicine became recently reinvented in radiobiology [7-8]. Radiobiology traditionally has a strong interphase to physics, but in particular nowadays it is missing a link to medicine, although with the ARS we are facing a disease and a medical problem. Hence, using e.g. prodromi such as vomiting or diarrhea for ARS (effect) prediction or other molecular changes occurring after exposure and leading finally to an effect (disease) along a so called "causal pathway" represents an approach alternative to the dose estimation (*figure 1*). It is more reflecting the "conventional" medical procedure and it avoids the challenges associated with the exposure estimation (individual differences in radiosensitivity), but it also opens other sources of limitations. Clearly, no method is perfect, but combining different approaches improves the prediction.

Physical dosimetry

Initial irradiation after a nuclear atomic bomb or by a hidden Cs-137 source can be measured with a dosimeter. The dosimeter doesn't discriminate partial from whole body exposure, which is important for a meaningful effect prediction. However, additional questions explaining details of the exposure situation (e.g. shielding by houses) will help to define the exposure situation. If irradiation took place in the absence of dosimeters, it is still possible to perform physical dosimetry on some biological tissues that can capture radicals, like bone and teeth enamel using electron paramagnetic resonance (EPR) techniques. Those are nowadays forward deployable and can be used *in vivo* (teeth don't have to be extracted for this technique anymore) [9-11].

The Institute of Radiobiology (Germany) has a mobile medical task force (TF) which provides medical expertise in a R/N scenario. It comprises dosimeters and mobile gamma spectroscopy for identification of released radionuclides as well (*figure 2*). Radionuclide identification and decontamination of patients can be performed by the medical TF by decontamination measures and an immediate start of a radionuclide decorporation therapy using chelating agents and others. Hence, physical dosimetry supports a triage (initial irradiation) under certain prerequisites (wearing dosimeters during the event). With regard to radionuclide identification and consecutive early treatment decisions (radionuclide decorporation therapy) and guidance of limited resources (e.g. chelating agents are not available in the high amounts required), it surely adds to the triage in this context.

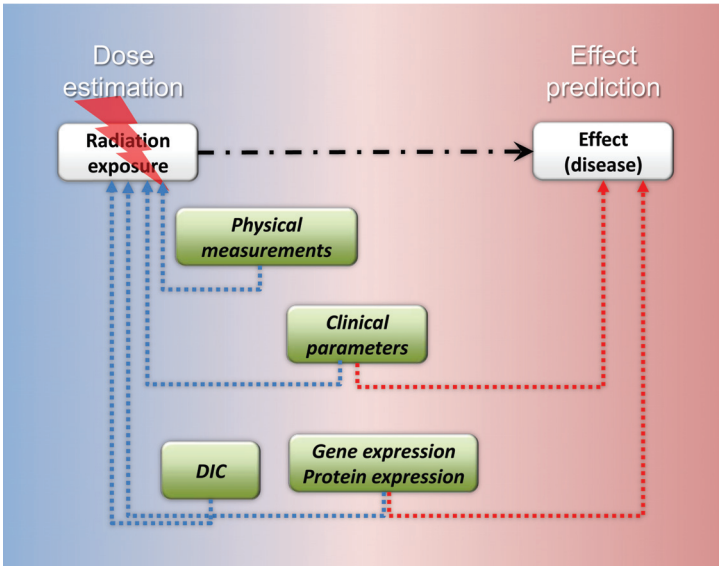

Figure 1 - Two approaches facilitate the triage under RN conditions, namely dose estimation and effect prediction. Radiation exposure along a causal pathway (black arrow) leads to certain effects such as the acute radiation syndrome (ARS). Physical measurements, clinical parameter (e.g. vomiting or diarrhea) or biological changes such as DIC (dicentric chromosomal aberrations) or gene and protein expression changes can be used for both, dose estimation (blue arrows) and/or effect prediction (red arrows).

Biological dosimetry and effect prediction

Following the scenario outlined above, with no dosimeter measurements available after exposure, quantifying radiation-induced biological changes can come into play. Ionizing radiation induces DNA damage in a dose-dependent manner and the incorrect repair of these DNA damages can generate chromosomal aberrations. Therefore, the rate of chromosomal aberrations (observable by cytogenetic techniques) correlates with exposure dose. Cytogenetic changes such as the induction of unstable (e.g. dicentric chromosomal aberrations, DCA) or stable chromosomal aberrations (translocations) are

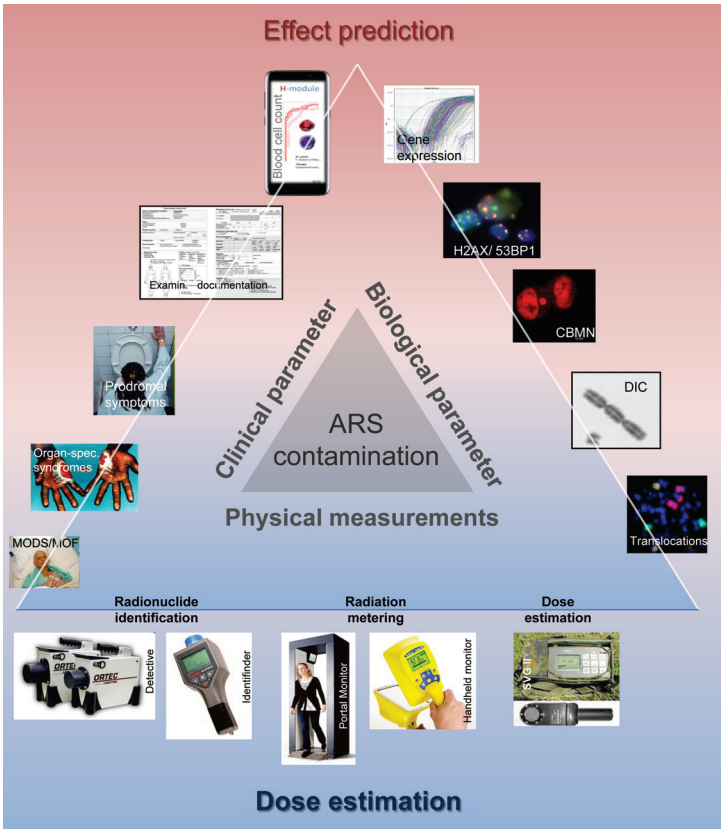

Figure 2 - Use of different assays and instruments for dose estimation or effect prediction of the acute radiation syndrome (ARS) and the detection of radionuclide decontamination by the German Medical RN Task Force. These assays or instruments do fall in the three categories clinical or biological parameter and physical measurements.

well established assays for biodosimetry [12-13]. The DCA represents the gold-standard in biodosimetry (*figure 2*). It provides hints for a whole or partial body exposure [14-15]. Still, these techniques are too slow to produce results to be used in the first triage. This is caused by the biological manipulations required to do the measurements on peripheral blood lymphocytes: the structure of dicentric chromosomes is easier to distinguish in the metaphase stage of dividing cells and it takes at least two days in optimized culture conditions to obtain a sufficient amount of lymphocytes in metaphase. Furthermore, the microscope analysis that follows can be time-consuming and require highly trained experts.

Currently, radiation-induced gene expression changes measured in the peripheral blood are being considered to be used for triage. Different scientific groups identified either promising single genes (e.g. FDXR, DDB2, P21) [16] or complex gene signatures (comprising dozens or hundreds of genes) which can be applied for biodosimetry purposes, but also for the effect prediction of clinical outcomes (e.g. WNT3, POU2AF1) [17]. Those genes are deregulated even within hours after exposure (unpublished own results) and the quantification after blood draw takes only a few hours. The potential for early diagnosis (a prerequisite for triage) of this approach has been shown in several independent studies and by different groups [18-19]. Recently, a study was finished in order to examine the high-throughput potential of gene expression measurements for biodosimetry purposes as well as the prediction of clinical outcomes such as the requirement for hospitalization or the prediction of the HARS severity (manuscript in preparation). The authors conclude that with a low number of genes (FDXR, DDB2 and POU2AF1), clinically relevant decisions regarding hospitalization and the identification of the HARS severity category could be accomplished with an overall agreement between 90-97% for 1,000 samples within 30 hours. However, this required an experienced team of six technicians and a senior scientist as well as special laboratory equipment in high quantity (e.g. seven Qiasymphony robots for RNA isolation and three MiSeq NGS machines to run targeted next generation sequencing). Employing a well-established workflow and routine are prerequisites that need to be considered as well.

Currently, different laboratories worldwide are working on a "point of care" (POC, to be used on the field) diagnosis based on radiation-induced gene expression changes and employing e.g. microfluidics. Running the appropriate tests in hospitals is another prerequisite for a triage. Sending samples to few specialized laboratories in the world appears challenging when considering the chaos and the likely breakdown of the infrastructure in the context of RN scenarios.

Hence, in particular molecular biological driven approaches such as early radiation-induced gene expression changes measured in the peripheral blood do have the potential to facilitate the triage, but e.g. an established POC represents a prerequisite.

Clinical dosimetry and clinical effect prediction

Clinical signs and symptoms are evaluated for decades to be used as early diagnostic tools for clinical dosimetry. The "intermediate dose program" in particular reflects the radiation-induced onset and intensity of prodromi such as vomiting or diarrhea with increasing dose and is used for dosimetry purposes [20] (*figure 2*). Prodromi occurs within the first 24-48 h after irradiation. That makes them an ideal tool for triage purposes. However, these are unspecific symptoms and very common for many other less harmful diseases which has to be considered.

These prodromi symptoms are also used in order to predict the later occurring ARS [20]. In combination with simple and omnipresent blood cell count laboratory test including sequential diagnostics they gave guidance for the treating physicians in many significant accidents in the past [21]. The METREPOL (MEdical TREatment ProtocOLs) document and the related concept developed by Fliedner is a nucleus of the clinical effect prediction. METREPOL for instance categorizes HARS into five classifications of severity based on blood cell count changes in the weeks after exposure: no HARS (H0), low (H1), medium (H2), severe (H3) and fatal (H4) HARS. Only H2-4 HARS severity degrees require early hospitalization and early onset of intensive therapy. Surveillance for H1 severity HARS is recommended, because lower radiation exposures where cells survive increase the risk for chronic diseases such as cancer or non-cancer diseases (e.g. cardiovascular disease). METREPOL also considers other prodromi associated to the dermatological (e.g. erythema), gastro-intestinal (e.g. diarrhea) and neurovascular syndrome (e.g. vomiting).

Several software tools were developed in order to take advantage of prodromi as well as early changes in blood cell counts for biodosimetry purposes or effect prediction. These applications clearly represent a step towards triage, because of their immediate availability at the POC and the fast use (enter the clinical parameter into the software and receive results immediately). Two of them are BAT and WinFRAT [22]. These programs provide either a dose estimate only (BAT)

or deduce clinically relevant information from the dose estimate (WinFRAT). Another software currently in development is the H-module App (a beta version will be released in early 2019). Contrary to BAT or WinFRAT, the H-module uses changes in blood cell counts (BCC, lymphocytes, granulocytes and, if available, thrombocytes) only as input data and from there it deduces clinically relevant recommendations on hospitalization requirement and provides therapeutic options as well as specialized infrastructure (e.g. ICU).
A three-day workshop will be organized at the French Armed Forces Biomedical Research Institute (IRBA, Brétigny/Paris, 9-11 October 2019) focusing on an early and high-throughput ARS diagnostic using these software tools. This practical workshop ends with an exercise using 191 case histories. After the workshop, all these materials will be given to the participants for teaching purposes (teach the teacher).

Future requirements

A triage of individuals exposed to RN scenarios can be facilitated by taking advantage of different tools combined. These can be based on physical measurements (e.g. mobile gamma spectroscopy for internal contamination and decorporation therapy), biological measurements (e.g. changes in gene expression) or the use of early clinical signs and symptoms after irradiation comprising erythema, vomiting or diarrhea and changes in blood cell counts. Early diagnosis and early and innovative treatment capabilities do not only save thousands of lifes in a mass casualty radiation scenario but also minimize late effects e.g. by applying highly effective radionuclide decorporation treatment.
Emergency medical doctors are not used to triage in a RN scenario. A training and in particular the awareness and an introduction into already existing software tools to deal with this kind of exposure is absolutely required.
A triage without treatment options due to limited amounts of stockpiled drugs is meaningless. Clearly, Nations have to work on stockpiling strategies, because ARS countermeasures or radionuclide decontamination therapy requires thousands of units for treatment.
Existing promising molecular biology driven approaches for early and high-throughput diagnostic have to be developed to a POC to make them early available at the place where they are required.
The scientific and medical society should further follow the two approaches of dose estimation and effect prediction which together facilitate a triage.

[1] Anno G.H., Young R.W., Bloom R.M., Mercier J.R., Dose response relationships for acute ionizing-radiation lethality, *Health Phys.*, **2003**, *84*, p. 565.
[2] Hill A.B., The environment and disease: association or causation?, *J. R. Soc. Med.*, **1965**, p. 295.
[3] Farese A.M. *et al.*, Filgrastim improves survival in lethally irradiated nonhuman primates, *Radiat. Res.*, **2013**, *179*, p. 89.
[4] Fedak K.M., Bernal A., Capshaw Z.A., Gross S., Applying the Bradford Hill criteria in the 21st century: how data integration has changed causal inference in molecular epidemiology, *Emerg. Themes Epidemiol.*, **2015**, *12*, doi:10.1186/s12982-015-0037-4.
[5] World Health Organization (WHO), *Preliminary dose estimation from the nuclear accident after the 2011 Great East Japan Earthquake and Tsunami*, **2012**.
[6] Port M. *et al.*, Correlation of radiation dose estimates by DIC with the METREPOL hematological classes of disease severity, *Radiat. Res.*, **2018**, *189*, p. 449.
[7] Port M., Abend M., Clinical triage of radiation casualties: the hematological module of the Bundeswehr Institute of Radiobiology, *Radiat. Prot. Dosimetry*, **2018**, *182*, p. 90.
[8] Dörr H. *et al.*, Using clinical signs and symptoms for medical management of radiation casualties: 2015 NATO exercise, *Radiat. Res.*, **2017**, *187*, p. 273.
[9] Brady J.M., Aarestad N.O., Swartz H.M., In vivo dosimetry by electron spin resonance spectroscopy, *Health Phys.*, **1968**, *15*, p. 43.
[10] Demidenko E., Williams B.B., Sucheta A., Dong R., Swartz H.M., Radiation dose reconstruction from L-band in vivo EPR spectroscopy of intact teeth: comparison of methods, *Radiat. Meas.*, **2007**, *42*, p. 1089.
[11] Chumak V., Sholom S., Pasalskaya L., Application of high precision EPR dosimetry with teeth for reconstruction of doses to Chernobyl populations, *Radiat. Prot. Dosimetry*, **1999**, *84*, p. 515.
[12] Beinke C. *et al.*, Laboratory intercomparison of the dicentric chromosome analysis assay, *Radiat. Res.*, **2013**, *180*, p. 129.
[13] Lloyd D.C., Dolphin G.W., Radiation-induced chromosome damage in human lymphocytes, *Br. J. Ind. Med.*, **1977**, *34*, p. 261.
[14] Darroudi F. *et al.*, Detection of total- and partial-body irradiation in a monkey model: a comparative study of chromosomal aberration, micronucleus and premature chromosome condensation assays, *Int. J. Radiat. Biol.*, **1998**, *74*, p. 207.
[15] Liniecki J., Bajerska A., Wyszyńska K., Dose-response relationships for chromosome aberrations in peripheral blood lymphocytes after whole- and partial-body irradiations. II. Decline of aberration-carrying cells in blood with time post-exposure, *Mutat. Res. - Fundam. Mol. Mech. Mutagen.*, **1983**, *110*, p. 103.
[16] O'Brien G. *et al.*, FDXR is a biomarker of radiation exposure in vivo, *Sci. Rep.*, **2018**, *8*, art. 684, doi:10.1038/s41598-017-19043-w.
[17] Port M. *et al.*, First generation gene expression signature for early prediction of late occurring hematological acute radiation syndrome in baboon, *Radiat. Res.*, **2016**, *186*, p. 39.
[18] Badie C. *et al.*, Laboratory intercomparison of gene expression assays, *Radiat. Res.*, **2013**, *180*, p. 138.
[19] Abend M. *et al.*, Examining radiationi *in vivo* and *in vitro* gene expression changes of the peripheral blood in different laboratories for biodosimetry purposes: first RENEB gene expression study, *Radiat. Res.*, **2016**, *185*, p. 109.
[20] *Medical Management of Radiation Accidents – Manual of the Acute Radiation Syndrome*, T.M. Fliedner, I. Friesecke, K. Beyrer (eds), The British Institute of Radiology, **2001**; Shimazawa R., Ikeda M., Medical management of the acurate radiation syndrome, *Ann. Intern. Med.*, **2011**, *155*, p. 135.
[21] Azizova T.V. *et al.*, Predictability of acute radiation injury severity, *Health Phys.*, **2008**, *94*, p. 255.
[22] Sandgren D.J. *et al.*, Biodosimetry assessment tool (BAT) software-dose prediction algorithms, *Health Phys.*, **2010**, *99(Suppl. 5)*, p. 171.

Matthias PORT[1], **Marco VALENTE**[2], **Patrick OSTHEIM**[1], **Matthäus MAJEWSKI**[1], **Julian HAUPT**[1] and **Michael ABEND**[1]*.

[1] Bundeswehr Institute of Radiobiology, Munich, (Germany).
[2] Institut de Recherche Biomédicale des Armées, Brétigny-sur-Orge (France).
* e-mail: michaelabend@bundeswehr.org