

## ORIGINAL ARTICLE

### Normal Tissue Radiosensitivity: Prediction on Deterministic or Stochastic Basis?

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#### ABSTRACT

For the same standardized physical radiation dose there is considerable variation, among different patients, of the magnitude of early and late normal tissue reactions. Technical and clinical factors account for about one third only of these variations. Genetic or epigenetic differences between patients account for the greater proportion of interpatient radiosensitivity differences.

Attempts have been made to correlate differences in the tissue radiosensitivity with the *in vitro* radiosensitivity of fibroblasts or lymphocytes as well as with other biological cellular processes related to cell death (mainly DNA repair and chromosomal aberrations). Apart from some genetic diseases such as ataxia telangiectasia, there were positive results in some studies that could not be reproduced in others. It is now realized that for normal tissue radiation-induced morbidity (a) cell kill is not the only factor, (b) interaction with a large number of gene products, such as IL-2, IL-6, TGF- $\beta$ , is involved, and (c) radiosensitivity differences can reflect genetic differences.

Polymorphism in a wide range of genes can now be demonstrated and analyzed as a source of variations in the radiation response. cDNA microarray procedures can also allow simultaneous measurement, in the same sample, of thousands of genes and thus avoiding basing the investigation on a restricted number of genes. Preliminary results could show retrospectively a correlation between late normal tissue reactions and expression of certain genes. Using cDNA microarray before radiotherapy to predict the occurrence of serious late reactions is still under consideration.

Theoretically, an efficient predictor procedure may serve in (a) detection of hypersensitive patients before submitting them to highly toxic radiotherapy regimes such as whole body irradiation, (b) detection of hypersensitive patients in clinical situations where radiotherapy can be effectively replaced by other modalities, and (c) selection of patients for elaborate radiotherapy techniques involving dose escalation to high levels.

#### 1- THE IMPORTANCE OF THE GENETIC FACTOR AS A DETERMINANT OF NORMAL TISSUE RADIOSENSITIVITY:

For the same standardized physical radiation dose there is considerable variation, among different patients, in the magnitude of early and late normal tissue reactions [1-4]. As a working hypothesis, factors responsible for such radiosensitivity variations can be classified as:

1- Stochastic (random) variations caused by the random nature of radiation-induced cell killing, random variations in dose distribution and delivery and random errors in the scoring system used.

2- Deterministic (patient-related) variations probably due to genetic and epigenetic differences between patients as regards clinical radiosensitivity [5]. A study on skin telangiectasia in breast cancer patients demonstrated that 80-90% of patient-to-patient variations may be accounted for by deterministic factors [6]. This study also demonstrated that factors such as age, menopausal status, smoking, hypothyroidism, cardiovascular disorders, diabetes, hypertension, endocrine treatment or chemotherapy contributed to only a part of the patient-to-patient difference as regards the risk of telangiectasia. This suggests that out of the patient factors, genetic or epigenetic factors are the main determinant of radiation-induced reactions, at least for telangiectasia.

### 1.1- Cellular Radiosensitivity in Some Genetic Syndromes:

The association between certain genetic syndromes and high cellular radiosensitivity is one of the strong indicators of the existence of intrinsic radiosensitivity variations. Patients with ataxia telangiectasia (AT) are known to be hypersensitive to radiation and are about 5 times more likely to develop cancer than the normal population. Cultures of fibroblasts, lymphoblasts and bone marrow progenitor cells derived from AT patients are 3-4 times as radiosensitive as cells derived from normal individuals [7]. About 1% of the population are heterozygous (carriers) for the AT mutated gene. When grown in culture, fibroblasts from AT heterozygotes have a  $D_0$  of 1.18 Gy compared to 1.45 Gy for normal fibroblasts. Since AT-heterozygotes account for about 4% of all breast cancer patients, ATM mutations were looked for in patients with breast cancer who expressed excessive normal tissue reactions. Using the standard ATM mutation detection technique, studies did not reveal a significantly increased occurrence of ATM mutations amongst hypersensitive individuals compared to control patients with no clinically detectable reactions. Similar findings could also be shown in patients with other forms of cancer.

There are other recessive diseases (e.g. Fanconi anaemia, Gardner's syndrome and Cockayne's syndrome) that combine an increased radiosensitivity with a high risk of cancer. Fibroblasts derived from patients with such syndromes show an increased radiosensitivity but this is much less remarkable than that of AT with overlap with the most radiosensitive "normal" subjects.

Patients with breast cancer who had pre-existing collagen vascular diseases (e.g. discoid lupus, systemic lupus erythematosus, progressive systemic sclerosis) and were subjected to breast conserving treatment were shown to suffer of severe early and late radiation reactions that might be life threatening. In the light of this limited experience, these rare collagen vascular diseases should be considered a contraindication to breast conserving treatment.

A study involving early and late radiation reactions in collagen vascular diseases (rheumatoid arthritis, scleroderma, systemic lupus erythematosus, dermatomyositis and polymyositis) could not demonstrate statistically signif-

icant enhancement of early or late reactions compared with controls. On the other hand, loss of function of either breast cancer susceptibility genes, BRCA1 or BRCA2, can lead to reduction of the efficiency or to loss of accuracy of the homologous recombination (HRR) DSB repair [8]. However, the possibility that BRCA mutations can contribute to the severity of normal tissue radiation reactions could not be shown.

### 2- FREQUENCY DISTRIBUTION OF RADIOSENSITIVITY OF NORMAL TISSUES:

Apart from the hypersensitivity associated with well recognised genetic syndromes, there is also a small group of apparently normal subjects without evidence of AT or other genetic diseases, who exhibit severe acute and late skin reactions and are referred to as "over-reactors", and constitute a minority of less than 1%. Some studies of these over-reactors could reveal evidence of increased radiosensitivity of their dermal fibroblasts or lymphocytes compared to normal volunteers but this did not reach the very high sensitivity level found in homologous AT patients.

After exclusion of the small proportion of over-reactors and those patients with known genetic diseases associated with hypersensitivity to radiation, there remain the vast majority of patients whose response covers what is called the "normal" range. This range represents the variation in normal tissue reactions seen during everyday radiotherapy practice involving the treatment of a large number of unselected patients without evidence of genetic syndromes. Within this "normal" range, the frequency distribution of the normal tissue response scores is approximately Gaussian and may then be described in terms of a mean value and a standard deviation ( $\sigma$ ) (Fig. 1). Over-reactors are expected to lie well out of the left side of the "normal" range. The most radiosensitive 5% segment of the normal range is referred to as the highly radiosensitive (HR) fraction. Though the values of over-reactors lie on the left of the HR values, yet the distinction may be statistically difficult.

### 3- RADIOSENSITIVITY PREDICTIVE ASSAY:

At present, the prescribed radiation dose is adjusted at the level associated with no more

than 5% risk of serious complications. This means that dose prescription is limited by the HR (5%) fraction. Theoretically, it would be of practical value to detect such hypersensitive patients and to base the choice of the prescribed dose of the remaining patients on the individually determined cellular radiosensitivity as discussed later. Detection of small radiosensitivity differences between target cells might prove to be worthwhile since a small difference is amplified by each radiation fraction during the course of multifraction radiotherapy. This motivated the search for a pre-treatment cellular radiosensitivity assay procedure that can forecast the severity of normal tissue reactions in the individual patient.

### 3.1- Requirements of a Radiosensitivity Assay Procedure:

In looking for an effective radiosensitivity assay procedure, the following should be taken into consideration:

1- Repeated in vitro measurements should show a larger interindividual than intraindividual variability at a very high level of statistical significance.

2- Two forms of intraindividual variability have to be explored: (a) Repeated radiosensitivity assays on one cell strain prepared from the same biopsy specimen can reveal intraindividual variability due to the assay procedure itself. This assay variability is referred to as the "assay noise" and may be reduced by repeated independent determinations. The predictive potential of the in vitro radiosensitivity assay may be improved when experiments are performed on cell cultures derived from the same biopsy or from 2 or more biopsies from the same patient. (b) Repeated radiosensitivity assays performed in repeated biopsies taken at different times from the same patient can reveal existence of heterogeneity of the cell population or, less likely, emergence of new subpopulations with different radiosensitivities.

3- A strong correlation between the measured radiosensitivity parameter and the severity of the normal tissue reaction should be shown. Such a demonstration should take into account the assay noise. The relative values of the assay noise and the variance due to actual biological variability determines the sensitivity and the specificity of the test. Sensitivity refers to the

proportion of patients expressing a severe reaction who have also a high radiosensitivity assay score. Specificity refers to the proportion of normally reacting patients whose predictive assay parameter value lies within the high range i.e. it refers to the frequency of false positive values. The predictive value of the test refers to the proportion of patients having an elevated assay and are also clinically hypersensitive (Table 1).

4- The demonstration that a certain radiosensitivity parameter is an independent risk factor requires conduction of a meticulous study where all other variables known to influence the severity of the reaction are adjusted for.

5- The in vitro radiosensitivity procedure should be relatively simple and short so that the results would be available within a relatively short time before a treatment decision is made.

### 3.2- Cell kill and/or DNA damage for Predictive Radiosensitivity Assay: Limitations:

#### 3.2.1- Fibroblast and lymphocytes as indicators of tissue radiosensitivity:

Fibroblasts [9-13] and lymphocytes [14-16] have been chosen for in vitro radiosensitivity assay on the assumption that their response can reflect the genetic constitution and response of other normal cell types.

In vitro colony formation has been the most commonly used end-point for expressing the radiosensitivity of fibroblasts or lymphocytes and its correlation with normal tissue reactions. Being a time consuming procedure other end-points have been adopted. These mainly include initial and residual DNA damage [17] and chromosomal damage expressed as either micronuclei formation in cells irradiated in the G1-phase or chromatid aberrations in cells irradiated in the G2-phase [18,19].

Attempts have been made to improve the resolution of the radiosensitivity assay procedures. Two examples are herewith outlined, (a) Fibroblasts have been shown to undergo low dose rate recovery (LDR) with consequent damage sparing. Over-reactors tend to have a much less LDR sparing relative to normal strains probably reflecting less capacity to recover. This is expected to accentuate the difference between the scores of over-reacting and normal

strains [20]; (b) When using residual DNA damage as an end-point it has been recommended to irradiate fibroblasts while in confluent culture. Cells in confluent culture have a small fraction (<2%) in the S-phase. This has the advantage of minimizing the interference with DNA migration by S-phase cells when using pulsed-field-gel-electrophoresis [21].

In contrast to patients with known genetic syndromes, normal subjects, or cancer patients who do not express extreme radiosensitivity, have much less impressive correlations between cellular radiosensitivity and the magnitude of normal tissue reactions. Patients suffering from genetically determined hypersensitivity diseases are thought to have a generalized radiosensitivity enhancement involving early and late tissue reactions. A different pattern seems to exist in patients not suffering from these hypersensitivity diseases. For example, there is no evidence for the existence of a correlation between the risk of telangiectasia and subcutaneous fibrosis. The susceptibility to fibrosis does not correlate with susceptibility to erythema or moist desquamation. On the cellular level, the radiosensitivity of fibroblasts differs from that of lymphocytes. These findings reduce the confidence in the possibility of taking the radiosensitivity of lymphocytes or fibroblasts as indicators of the radiosensitivity of other cell types [11]. This is particularly important as regards the relationship between early and late normal tissue reactions which (with exception of consequential late effects) may have different target cells.

A main criticism of lymphocyte or fibroblast assay procedures is that they are based on the target cell hypothesis with cell killing as the main determinant of clinical reactions [22]. This concept has been revised and radiation-induced tissue reactions are now considered to result from interactions within a complex network of different cellular and extracellular matrix components along with cytokines and growth factors [23]. Both functional derangements and inflammatory reactions can also contribute to early and late reactions. Furthermore DNA repair capacity and fidelity along with apoptosis induction and variations in the cell cycle along with fibrogenic agents and other radiobiological processes can be strong determinants of cell survival. These considerations stimulated attempts to develop predictors of clinical radi-

osensitivity on a genetic basis while simultaneously testing for a large number of genes.

### 3.2.2- DNA Damage/Repair and Intrinsic Radiosensitivity:

There is at present strong evidence that DSBs are precursor lesions for chromosome breaks which eventually develop into chromosomal exchange aberrations, some of which can lead to mitotic cell death. It is therefore logical to consider the question of whether DSB induction and/or repair correlate with intrinsic radiosensitivity as indicated by loss of reproductive activity. This correlation was looked for in relation to (a) the initial induction of DNA damage, (b) the speed at which repair takes place, (c) the residual damage detected after repair is completed and (d) repair fidelity. (Experimental data indicated that the relationship between the DSB level and cellular sensitivity is not a simple issue. In some cell types sensitivity appears to be related to initial strand break induction while in others repair defects seem more important [17, 24-26]. Fidelity of repair is an important factor since misrepair may lead to altered genetic constitution or cell death [27]. In a proportion of cell lines difference in radiosensitivity could be related to differences in chromatin condensation. In many other cell lines difference in radiosensitivity could not be related either to initial damage induction, repair, or chromatin structure. For such lines we have to look for other factors such as cell-cycle arrest, susceptibility to apoptosis or signal transduction

## 4- PREDICTION OF NORMAL TISSUE RADIOSENSITIVITY ON A GENETIC BASIS:

As mentioned above a large number of biological processes are involved in tissue radiosensitivity and each is controlled by a number of genes. Attempts are at present made to develop predictors of clinical radiosensitivity on a genetic basis. These attempts have been motivated by development of techniques that can simultaneously test for a large number of genes. Two principle techniques are herewith outlined: single nucleotide polymorphisms (SNPs) and cDNA microarray.

### 4.1- Single nucleotide polymorphisms (SNPs):

SNP is defined as "a single base alteration in genomic DNA, in which the least frequent allele has an abundance of 1% at least". SNPs

can alter the phenotype in a manner that depends on its location. Gene expression can be altered if the SNPs are located in a regulatory region. On the other hand, protein functions can be changed if the SNPs cause amino acid changes. Splicing of RNA can be affected by SNPs located in non-coding DNA regions. Through such mechanisms, SNPs can either influence the general clinical responses to irradiation or the effect may be limited to certain types of normal tissue reactions [28-29].

In a given study all selected polymorphisms have to be located in genes involved in the different pathways and processes known, or expected, to influence particular normal tissue reactions. Table (2) outlines, as an example, a pilot study investigating the influence of seven SNPs on normal tissue reactions, telangiectasia and subcutaneous fibrosis complicating post-operative radiotherapy in a group of breast cancer patients [28]. SNPs in TGFB1, SOD2 and XRCC1 genes were analyzed. The dose-response curves for subcutaneous fibrosis and telangiectasia were obtained in patients with different genotypes. The final analysis demonstrated a significant correlation between five of the seven SNPs and the risk of radiation-induced reactions. This gives support to the notion that clinical normal tissue radiosensitivity depends on the combined effect of variations in a number of genes. It is then likely that multiple genetic markers can have the ability to predict a normal tissue response to radiotherapy. One of the advantages of SNPs analysis is that the relevant DNA alterations can occur in all tissues including the readily accessible lymphocytes.

#### 4.2- cDNA Microarray:

The need for the determination of the activity of a large number of genes to account for a normal tissue response led to exploring the possibility of using cDNA microarray technology which allows the determination of thousands of genes in a single experiment [30].

Identification of post-radiation expression of certain genes can well provide a first step towards verifying the existence of a causal relationship between the expression of such genes and the normal tissue reactions. In pilot studies, cDNA microarray technique can be used to identify differentially expressed gene

transcripts in cells obtained from two groups of animals or patients who suffer of either normal (mild) or severe reactions. If genetic differences are identified and their association with radiation-induced tissue damage firmly verified, the next step would be to perform controlled clinical studies to test whether cDNA microarray technique can be applied before starting radiotherapy to provide a guide for modification of the treatment protocol in order to minimize adverse normal tissue reactions.

Using the microarray technique, a number of pilot animal and clinical studies have been performed to test the possibility of identifying complex genetic patterns associated with tissue hypersensitivity. The target for radiation-induced lung damage is a complex intercommunicating system including type II pneumocytes, alveolar macrophages, vascular endothelial cells and septal fibroblasts. These cells communicate with each other through cytokines, chemokines and growth factors which are capable of inducing both inflammatory and fibrogenic reactions. It is therefore expected that post-irradiation altered regulation of production of growth factors, cytokines and chemokines by inflammatory cells would contribute to lung fibrosis. A study has been performed to test whether chemokines and chemokine receptor families can predict the severity of radiation-induced lung fibrosis. Fibrosis-sensitive and fibrosis-resistant mouse strains were exposed to a dose of 12.5 Gy to the thorax [31]. At 26 weeks, postirradiation messages encoding the chemokines BLC, C10, IP-10, RANTES and their receptors were found to be elevated in the lung of fibrosis-sensitive mice. In contrast, messages encoding SDF-1a and Ccr1 only were elevated at 26 weeks post-radiation in fibrosis resistant mice. These results point out that monocyte/macrophage and lymphocyte are involved in the radiation-induced lung fibrosis in the mouse. There is also the possibility that they may prospectively provide genetic markers, for identification of mice susceptible to fibrosis. Testing for this possibility in man requires developing a safe procedure for sampling lung tissue. Using a cytokine microarray technique in a small scale, preliminary clinical study could also identify differentially expressed cytokine gene transcripts in skin fibroblasts of patients who expressed either negligible or severe damage to normal tissues.

cDNA microarray analysis has also been tested for the possibility of providing an insight into the pathogenesis of late radiation enteritis [32]. Gene profiles of six samples obtained from patients suffering from late radiation enteritis were determined and compared with six healthy bowel tissue samples taken from patients without evidence of late radiation enteritis. Genes coding for recruitment of lymphocytes and macrophages, and for tissue contraction and myofibroblast transdifferentiation were altered in radiation enteritis samples and this could be related to the occurrence of radiation enteritis. These results provide provisional evidence that late radiation enteritis is a dynamic process involving remodelling of the mucosa, mesenchyme and blood vessels. It is still to be seen whether such changes can be reproduced and have functional significance. If this is proved, controlled clinical studies can be designed to test whether the pre-irradiation genetic profile can predict radiation enteritis.

The microarray technique has been also used to identify genes that are differentially expressed during development of telangiectasia in the mouse kidney and rectum [33, 34]. The kidney showed upregulation of 31 genes at 10 weeks post-irradiation and 42 genes at 42 weeks, with downregulation of 9 and 4 genes, respectively. Some of these genes have proved involvement in the regulation of vascular development and angiogenesis remodelling as well as in inflammatory pathways. Changes in a number of other genes have not been previously found to be associated with the biological processes after irradiation of normal tissues. Similarly, the mouse rectum showed upregulation of 278 genes at 10 weeks and of 86 genes at 20 weeks after irradiation. These included genes known for their role in vascular development and remodelling. It is interesting to note that only proportions of upregulated and downregulated genes were common to both the rectum and kidney at the period of development of identifiable telangiectasia. The interpretation of the genetic findings in the rectum and kidney should take into account the changes in the cellular population after irradiation which result in accumulation of inflammatory cells and fibrotic tissues. This means that, at different postradiation intervals, the RNA used in the mDNA array procedure can well be derived from different cell types. This problem can be managed by per-

forming immunohistochemical studies parallel with the mDNA array study in order to link the observed genetic changes at a particular time with the particular cell type(s) present in the sampled tissue at that time. So far, microarray analysis can only serve in identifying new genes involved in late tissue injury. The potential application of the array procedure before irradiation to predict vascular tissue injury in the kidney, rectum and other tissues is still to be developed.

### 5- POTENTIAL USE OF PREDICTORS OF NORMAL TISSUE REACTIONS:

Theoretically, an ideal assay procedure for prediction of normal tissue responses can have three main applications:

*A- Detection of over-reactors:* If patients with very high radiosensitivity are detected they may then be treated with reduced doses. The possibility of effective dose reduction in such patients has been illustrated in an interesting case report on an 11-year-old AT child with medulloblastoma. The child bone marrow myeloid progenitor cells grown in culture proved to be three times as radiosensitive as normal controls. Based on this assay, the daily dose was reduced from 1.8 to 0.6 Gy with a total dose of 15 Gy instead of 45 Gy. Treatment was also restricted to the posterior cranial fossa instead of the conventional craniospinal irradiation. The acute skin reactions were of the same degree as that observed after a standard treatment. A complete remission was obtained and maintained for 9 months at least. This interesting case report suggests that in AT, and probably also in similar other genetic diseases, normal tissue hypersensitivity is a universal phenomenon involving all or most tissues, including tumour cells.

Apart from AT and similar genetic syndromes there is also the small group (<1%) of over-reactors whose high radiosensitivity level does not reach that of AT patients. Since hypersensitivity usually involves early and late reactions, severe early reactions can be readily recognized during the course of radiotherapy. Treatment can then be either stopped or modified. This seems to reduce the need for a pre-treatment predictive cellular radiosensitivity assay. Though the radiosensitivity scores of over-reactors lie to the left of the "normal" range (Fig. 1) the predictive power of a given

test depends on: (a) the separation between the median values of the “normal” and the over-reacting groups, (b) the spread of the values around the mean score i.e. the variation coefficients and the (c) the prevalence of over-reactors. Accordingly, the positive predictive value of available radiosensitivity assays is expected to be quite high for AT homozygous patients in view of the wide separation from normal values (Fig. 1). The positive predictive value of the currently available assays is estimated to be much lower in case of other genetic syndromes (heterozygous AT, Fanconi, Gardner and Cockayne’s syndromes) and to lie between 5 and 15%. This is attributed to the relatively narrow separation between their median scores and that of the “normal” group, the wide spread around the mean value, and the low prevalence within the population. For the same considerations the identification of the 1% over-reactors would have a low positive predictive value of about 5%.

*B- Detection of highly radiosensitive (HR) normal individuals:* The objective of such a detection is to exclude the most radiosensitive proportion of patients and refer them to another treatment modality, e.g. mastectomy instead of radiotherapy with breast conservation. If such discrimination proves possible, the remaining cases would be treated with escalated doses to an extent that keeps the complication rate at the previously accepted level (conventionally 5%). This aims at increasing the tumour control rate at the same toxic cost. Theoretically, increasing the proportion of excluded radiosensitive patients would allow more dose escalation to the remaining patients with the possibility of more enhancement of the tumour control probability. After exclusion of the most radiosensitive fraction, the magnitude of escalation of dose to the remaining patients while keeping the complication rate at the previous accepted level depends on (a) the proportion of the most radiosensitive population excluded; increasing this proportion increases the possibility of increasing the extent of dose escalation before the accepted complication rate is reached amongst the remaining patients, (b) the steepness of the dose-response curve of the normal tissue reaction concerned; the shallower the curve the greater the dose escalation permitted within the accepted complication rate limits, and (c) the accepted complication level, which is tradition-

ally set at 5%, may be increased under certain circumstances to 10-15% and thus allowing more dose escalation after exclusion of the HR fraction. The impact of this dose increase on the tumour control probability depends on the steepness of the tumour control probability (TCP) curve, the steeper the slope the greater the improvement in the tumour control probability. Generally, the slope of the dose-response curve of normal tissue reactions is high and this limits the extent of dose escalation. This together with the fact that the TCP curve is generally shallow, limit the therapeutic gain from identifying the HR fraction.

*C- Individualised dose prescription:* This is the most ambitious goal of predictive normal tissue radiosensitivity assay. Ideally, this would involve increasing the dose to the radioresistant patients and reducing the dose to the radiosensitive one in such a way that, for each patient, the predicted normal tissue complication probability would remain at an acceptable level e.g. the traditional 5% level. In this way, all doses are adjusted such that the expected probability of a normal tissue complication is the same for all subjects irrespective of their intrinsic radiosensitivity. One small scale study showed that if a 15% incidence of complications is accepted, individualization of dose prescription would require an average dose reduction of 13.1 Gy in one third of patients and an average dose increase of 7.7 Gy would have been possible in the remaining patients. This corresponds to about 10% increase in TCP. In another study the impact of individualization of dose prescription on the overall TCP has been estimated using a simulation sample and adopting certain mathematical models for the TCP and the dose-response for normal tissues. In the simulation sample, all patients receive the same dose before individualisation and this results in a 50% TCP. In that model, individualisation (with some patients receiving reduced and others increased doses) has been shown to result in only a small change in the TCP. This is attributed to the fact that the TCP curve is not linear with a shallower slope above the 50% TCP level than the slope below that level. Accordingly, at the 50% TCP level, the magnitude of increase in the TCP resulting from a given tumour dose increment would be smaller than the magnitude of TCP reduction resulting from the same degree of dose decrement. This led to the proposal of

different strategies in individualisation of dose prescription based on predictive radiosensitivity assay. If we accept a 10% incidence of serious complications, calculations pointed out the possibility of about 18% increase in delivered dose to the 40% most radioresistant patients with a significant improvement in local control and possibly also in survival.

These speculations assume that there is no correlation between radiosensitivity of tumours and normal tissues in the same patient. If such a correlation is shown, it would increase the effectiveness of dose prescription individualization. Patients with higher probability of complications would also have a better chance of tumour control at lower doses.

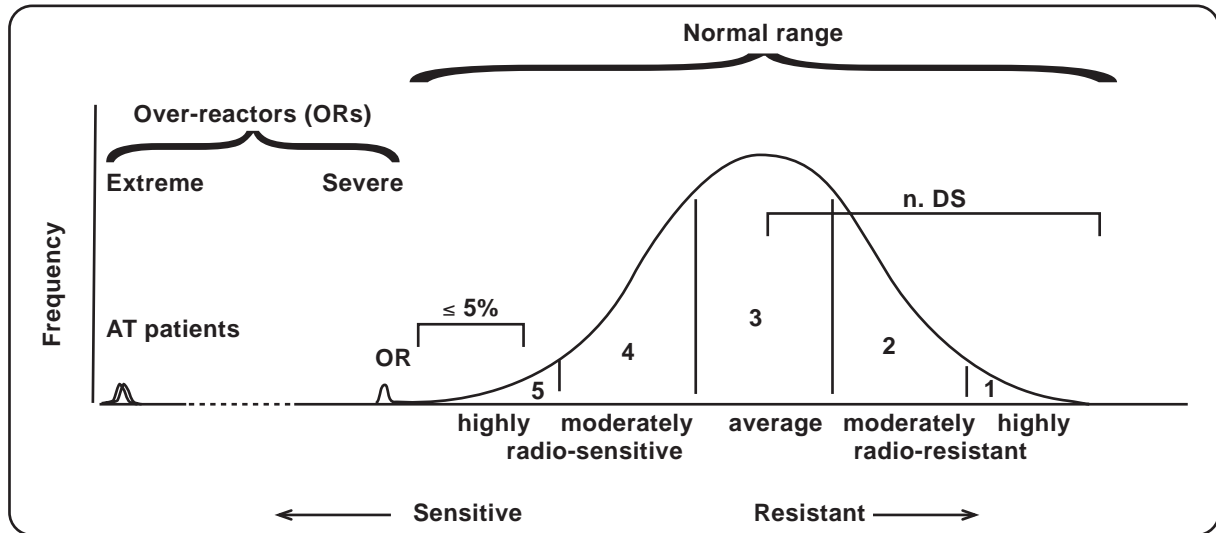


Fig. (1): Schematic representation of the frequency distribution of a normal tissue response in a group of patients receiving the same radiotherapy protocol. A Gaussian (normal) distribution (n.Ds) is assumed that can be described in terms of a mean value and a standard deviation. Patients are arbitrarily categorized into 5 radiosensitivity groups 1, 2, 3, 4 and 5 in ascending order of radiosensitivity. Group 5 (HR) represents the 5% most radiosensitive normal subjects. The AT patients lie on the extreme left while the over-reactors lie on the left of the HR group with the possibility of an overlap [Adapted from a report by the Steering Committee of The BioMed2 European Union Concerted Action Programme on Development of Predictive Tests of Normal Tissue Response to Radiation Therapy. *Int J Cancer (Pred Oncol)* 79: 606-613, 1998].

Table (1): Sensitivity, specificity and predictive value of a radiosensitivity assay.

	Number with a high radiosensitivity assay parameter	Number with a normal radiosensitivity assay parameter	
Number of hypersensitive patients	A	B	Sensitivity = $A / [A + B]$
Number of normally reacting patients	C	D	Specificity = $C / [C + D]$
Predictive value of the radiosensitivity assay procedure = $A / [A + C]$			

Table (2): A representative example of single nucleotide polymorphism (Modified from CN Andreassen, J Alsner J, M. Overgaard M, J.Overgaard).

Gene	Function of gene product	SNPs
TGFB1	Pro-fibrotic cytokine	Codon 10 Leu/Pro (T869n C )
TGFB1	Pro-fibrotic cytokine	Codon 25 Arg/Pro (G 915 C)
TGFB1	Pro-fibrotic cytokine	Promotor (C – 509 T)
SOD2	Scavenger of ROS	Codon 16 Val/Ala (T 47 C)
XRCC3	DNA repair (HR)	Codon 241 Thr/Met (C 18067 T)
XRCC1	DNA repair (BER)	Codon 399 Arg/Gln (G 28152 A)
APEX	DNA repair (BER)	Codon 148 Asp/Glu (T 2197 G)

ROS = reactive oxygen species, HR = homologous recombination, BER = base excision repair.

## REFERENCES

- 1- Tucker SL, Turesson I, Thames HD. Evidence for individual differences in the radiosensitivity of human skin. *Eur J Cancer*. 1992, 28A: 1783-91.
- 2- Turesson I. Individual variation and dose dependency in the progression rate of skin telangiectasia/. *Int J Radiat Oncol Biol Phys*. 1990, 19:1569-74.
- 3- Bentzen SM, Overgaard J. Patient-to-patient variability in the expression of radiation-normal tissue injury. *Semin Radiat Oncol*. 1994, 4: 68-80.
- 4- Begg AC. Prediction of radiation response. In Philips L, ed, *Textbook of Radiation Oncology*, 2<sup>nd</sup> edition, 2004, pp.61-75.
- 5- Bentzen SM, Hendry JH. Variability in the radiosensitivity of the normal cells and tissues. *Int J Radiol Biol*. 1999, 75: 513-7.
- 6- Safwat A, Bentzen SM, Turesson I, Hendry JH. Deterministic rather than stochastic factors explain most of the variation in the expression of skin telangiectasia after radiotherapy. *Int J Radiat Oncol Biol Phys*. 2002, 52: 198-204.
- 7- Jorgensen TJ, Shiloh Y. The ATM gene and radiobiology of ataxia-telangiectasia. *Int J Radiat Biol*. 1996, 69: 527-37.
- 8- Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TC, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature*. 2005, 434: 917-21.
- 9- Begg AC, Russell NS, Knaken H, Lebesque JV. Lack of correlation of human fibroblast radiosensitivity in vitro with early skin reactions in patients undergoing radiotherapy. *Int J Radiat Biol*. 1993, 64: 393-405.
- 10- Johansen J, Bentzen J, Overgaard J, Overgaard M. Evidence for a positive correlation between in vitro radiosensitivity of normal human skin fibroblasts and the occurrence of subcutaneous fibrosis after radiotherapy. *Int J Biol*. 1994, 66: 407-12.
- 11- Johansen J, Bentzen J, Overgaard J, Overgaard M. Relationship between in vitro radiosensitivity of skin fibroblasts and the expression of subcutaneous fibrosis, telangiectasia, and skin erythema after radiotherapy. *Radiother Oncol*. 1996, 40: 101-10.
- 12- Rudat V, Dietz A, Conradt C, Weber K-J, Flentje M. In vitro radiosensitivity of primary human fibroblasts, lack of correlation between radiation toxicity in patients with head and neck cancer. *Radiother Oncol*. 1997, 43: 183-8.
- 13- Wurm R, Burnet NG, Duggal N, Yarnold JR, Peacock JH. Cellular radiosensitivity and DNA damage in primary fibroblasts. *Int J Radiat Oncol Biol Phys*. 1994, 30: 625-33.
- 14- Barber JB, Burrill W, Spreadborough AR, Levine E, Warren C, Kiltie AE, et al. Relationship between in vitro chromosomal radiosensitivity of peripheral blood lymphocytes and the expression of normal tissue damage following radiotherapy for breast cancer. *Radiother Oncol*. 2000, 55: 179-86.
- 15- Lopez E, Guerrero R, Nunez MI, del Moral R, Villalobos M, Martinez-Galan J, et al. Early and late skin reactions to radiotherapy for breast cancer and their correlation with radiation-induced DNA damage in lymphocytes. *Breast Cancer Res*. 2005, 7: R690-8.
- 16- Nunez ML, Guerrero MR, Lopez E, et al. DNA damage and prediction of radiation response in lymphocytes and epidermal human skin cells. *Int J Cancer*. 1998, 76: 354-61.
- 17- El-Awady RA, Dikomey E, Dahm-Daphi J. Radiosensitivity of human tumour cells is correlated with induction but not with the repair of DNA double-strand breaks. *Br J Cancer*. 2003, 89: 593-601.
- 18- Russell NS, Arlett GF, Bartelink H, Begg AC. Use of fluorescence in situ hybridization to determine the relationship between chromosome aberrations and cell survival in eight human fibroblast strains. *Int J Radiat Biol*. 1995, 68: 175-96.
- 19- Nachtrab I, Oppitz U, Flentje M, Stopper H. Radiation-induced micronucleus formation in human skin fibroblasts of patients showing severe and normal tissue damage after radiotherapy. *Int J Radiat Biol*. 1996, 73: 279-87.
- 20- Burnet NG, Wurm R, Peacock JH. Low dose rate fibroblast radiosensitivity and the prediction of patient response to radiotherapy. *Int J Radiat Biol*. 1996, 70: 289-300.
- 21- Dahm-Daphi J, Dikomey E. Separation of DNA fragments induced by ionizing radiation using a graded-field electrophoresis. *Int J Radiat Biol*. 1995, 67: 161-8.
- 22- Moore JV, Hendry JH, Hunter RD. Dose-incidence curves for tumour control and normal tissues injury, in relation to the response of clonogenic cells. *Radiother Oncol*. 1983, 1: 142-57.
- 23- Barcellos-Hoff MH. How do tissues respond to damage at the cellular level? The role of cytokines in irradiated tissues. *Radiat Res*. 1998, 150 (Suppl.): S109-S120.
- 24- Dikomey E, Dahm-Daphi J, Brammer I, Martensen R, Kaina V. Correlation between cellular radiosensitivity and non-repaired double strand breaks studied in nine mammalian cell lines. *Int J Radiat Biol*. 1998, 73: 269-78.
- 25- El-Awady, RA, Mahmoud M, Saleh EM, El-Baky HA, Lotayef M, Dahm-Daphi J, et al. No correlation between radiosensitivity or double strand break capacity of normal fibroblasts and acute normal tissue reaction after radiotherapy of breast cancer. *Int J Radiat Biol*. 2005, 81: 501-8.
- 26- Zhou P-K P, Sproston ARM, Marples B, West CML, Margison GP, Hendry JH. The radiosensitivity of human fibroblast cell lines correlates with residual levels of DNA double-strand breaks. *Radiother Oncol*. 1998, 47: 271-6.
- 27- Löbrich M, Kühne K, Rothkamm K. Joining of correct and incorrect DNA double strand break ends in normal human and ataxia telangiectasia fibroblasts. *Genes. Chromosomes and Cancer*. 2000, 27: 59-68.

- 28- Andreassen CN, Abner J, Overgaard M, Overgaard J. Prediction of normal tissue reactions from polymorphisms of candidate gene. *Radiother Oncol.* 2003, 69: 127-135.
- 29- Quarmby S, Fakhoury H, Levine E, Barber J, Wylie J, Hajeer AH, et al. Association of transforming growth factor beta-1 single nucleotide polymorphisms with radiation-induced damage to normal tissues in breast cancer patients. *Int J Radiat Biol.* 2003, 79: 137-43.
- 30- West CM, McKay MJ, Holscher T, Baumann M, Stratford IJ, Bristow RG, et al. Molecular markers predicting radiotherapy response: report and recommendations from an International Atomic Energy Agency technical meeting. *Int J Radiat Oncol Biol Phys.* 2005, 62: 1264-73.
- 31- Johnston CJ, Williams JP, Okunieff P, Finkelstein J. Radiation-induced pulmonary fibrosis evaluation of chemokine and chemokine receptor families. *Radiat Res.* 2002, 157: 256-65.
- 32- Vozenin-Brotans MC, Milliat F, Linard F, Strup C, Francois A, Sabourin JC, et al. Gene expression profile in human late enteritis obtained by high-density cDNA hybridization. *Radiat Res.* 2004, 161: 299-311.
- 33- Kruse JJ, te Poele JA, Russell NS, Boersma LJ, Stewart FA. Microarray analysis to identify molecular mechanisms of radiation-induced microvascular damage. *Int J Radiat Oncol Biol Phys.* 2004, 58: 420-6.
- 34- Kruse JJ, te Poele JA, Velds A, Kerkhoven RM, Boersma LJ, Russell NS, et al. Identification of differentially expressed genes in mouse kidney after irradiation using microarray analysis. *Radiat Res.* 2004, 16 (1): 28-38.