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Strategy and tactics for stimulation of haemopoiesis in patients developing the acute radiation syndrome

Keywords: Acute radiation syndrome, hematopoiesis, diagnostic indicators, therapeutic principles

In this strategic paper, the scientific basis for the clinical management of persons is reviewed who were accidentally whole-body exposed to ionizing radiation. The most relevant organ system to evaluate is the hematopoiesis with important diagnostic indicators and as a target for a "base line" therapy. A grading code is presented that will help the medical doctor to recognize the severity of effect and guide in therapeutic approaches.

1. Introduction

In the context of the "Joint IAEA-WHO Technical Committee Meeting on Follow-up of Delayed Health Consequences of Acute Accidental Radiation Exposure: Lessons to be Learned", which was held from October 1-3, 2001 in WHO Headquarters, the objectives were to review delayed health consequences of acute exposure to radiation which occurred as a result of a number of recent radiation accidents and to analyze lessons to be learned in their diagnosis and treatment.

This presentation is essentially based on the outcome of a concerted research action of the European Communities entitled "Medical Treatment Protocols for Radiation Accident Victims as a Basis for a Computerized Guidance System" (METREPOL) which was conducted as a joint effort between research establishments in Paris, Oxford, Rotterdam, Munich and Ulm. The results of this concerted action have been published by the British Institute of Radiology in 2001 with the following title: "Medical Management of Radiation Accidents: Manual on the Acute Radiation Syndrome" [1]. In this manual, experience was used from more than 800 case histories of persons that were subjected to whole body radiation exposure during radiation accidents. A total of 70 acci-

dents in 14 countries provided the material for the manual.

These case histories form the core of an international database system entitled SEARCH (System for Evaluation and Archiving of Radiation Accidents based on Case Histories) [2].

The Manual on the Management of the Acute Radiation Syndrome comes clearly to the conclusion that the hemopoietic cell renewal systems are of paramount importance in the clinical management of the acute radiation syndrome for two reasons.

First of all, the hemopoietic tissue is distributed throughout the skeleton characterized by an enormous hemopoietic cell turnover as well as by a high radiation sensitivity. Furthermore, it is regulated to act as one organ by humoral factors and by a continuous monitoring of stem cell content in the bone marrow units by a migration stream of circulating hematopoietic stem cells. Therefore, the hemopoietic blood cell response patterns after total body exposure to ionizing radiation reflect in a very precise way the extent of damage to the entire organism and are able to predict the probable clinical course of the patient and allows preparation for the different treatment options. Thus, it is obvious that the hemopoietic cell renewal system is of high diagnostic significance in the management of the acute radiation syndrome.

Secondly, the hemopoietic tissue is also of crucial importance for the principle survival chance of an exposed person. If hemopoietic regeneration can be achieved, then other organ radiation impairments may well have a chance to be successfully treated. Due to modern treatment options such as stem cell transplantation as well as growth factor therapy, the vulnerability of the hemopoietic system is not any longer the limiting factor in radiation accident survival of victims. The critical question that remains is whether the subsequent multiorgan damage can be overcome by appropriate therapeutic methods.

Therefore, this review will, first of all, discuss the structure and function of the hemopoietic cell renewal systems and their relevance for the acute radiation syndrome. It will, secondly, address the question of the strategic approaches to assess the severity of radiation injury to hematopoiesis. And thirdly, it is its purpose to briefly summarize the approaches to treat hematological consequences of whole body radiation exposure.

2. Structure and function of hemopoietic cell renewal systems

In clinical radiation accident management, it is of paramount importance to recall the principle structure and function of the hemopoietic system. The blood cell forming bone marrow is distributed throughout the skeleton. More than 200 bones are, in principle, endowed in their marrow cavity to produce hemopoietic cells. This widely distributed bone marrow has an overall size of about 2,600 g of which about 1,500 g are active in producing the blood cells that the human being needs in order to maintain its

integrity in changing environments. It has been calculated that the bone marrow contains 126 x 10^{10} cells and that the marrow cell production can be assessed to be 18.8 x 10^{7} per kg body weight/hour. The blood cell forming bone marrow is not a "cell culture" that is "accidentally" localized within the bone cavity. Rather, one should recall that the blood cell forming bone marrow is a highly specialized organ with a specific neurovascular structure. There are nutrient capillaries and a complex sinusoidal system operating in an encapsuled situation not permitting growth volume changes of the organ unit as such. Furthermore, it is important to stress that the blood cell formation in the bone marrow is regulated by unmyelinated and myelinated nerve fibres as well as by stimulating and inhibiting humoral factors. This situation has recently been reviewed [3].

Of particular importance for radiation accident management is the knowledge about the function of the different hemopoietic cell renewal systems.

The granulocytic cell renewal system produces every day 120 x 10^{9} blood granulocytes. An equal number of cells is lost from the blood per day. The life-expectancy of granulocytes has been measured to be about one day. This means that every day the entire granulocyte pool is turned over once. This fact is of paramount importance for radiation accident management as will be highlighted below.

Every day, 200 x 10^{9} erythrocytes are being produced in the bone marrow and are lost from the blood. The life-span, however, of erythrocytes is in the order of 120 days. Therefore, after acute radiation exposure, it takes weeks rather than days before the red cell count becomes critical unless there is thrombocytopenic bleeding influencing the number of red cells in the peripheral blood.

The number of platelets produced per day is in the order of 150 x 10^{9}. An equal number is removed every day from the blood. One has to consider the life-expectancy of blood platelets to be in the order of 10 days. Therefore, if there is a complete destruction of the megakaryocytic system, one would expect that the blood platelets decrease to critical "thrombopenic" levels within the first 10 days after radiation exposure.

As far as lymphocytes is concerned, one has to recall that these cells are very heterogeneous regarding their function. In addition, the life-span of lymphocytes is very different depending on the functional role of a particular lymphocyte. Nevertheless, one can calculate that this very heterogeneous group of cells has a turnover of 20 x 10^{9} per day. The response of lymphocytes after total body radiation exposure is often used as a first indicator of severity of exposure. However, lymphocytes (unlike the other blood cells) are "recirculating" cells. They enter the blood stream via the lymphatics and are emigrating out of the blood stream back into the lymphatic tissue (4). Thus, after total body or a significant partial body exposure (upper body), this process of

recirculation of lymphocytes is interrupted almost instantaneously. Therefore, the early response of lymphocytes is first of all caused by the impairment of recirculation. In addition, lymphocytes, unlike the other blood cells, are cells with a potential of further replication, proliferation and differentiation. Thus, they are also radiation-sensitive. This radiation sensitivity can be tested in cell culture studies using phytohemagglutin as a mean to induce cell proliferation [5].

Among the mononuclear cells in the peripheral blood (summarized as "lymphocytes") are cells of a completely different quality: hemopoietic stem- as well as progenitor cells. The fact that hemopoietic stem cells are part of the blood leukocyte population was first discovered by *Goodman* and *Hodgson* (1962) [6]. The research group in Ulm studied systematically the physiology and pathophysiology of circulating stem- and progenitor cells (7, 8, 9, 10). Today, it can be assumed that CD34+ cells, CFU-mix, GM-CFU and BFU as well as other progenitor cells are part of the normal "adult" blood stem cell population with a turnover between about 2-50 x 10^9 per day [7, 11].

Since the blood stem- and progenitor cells as well as the stem- and progenitor cells in the hemopoietic bone marrow are very radiation-sensitive, it goes without saying that after exposure to whole-body irradiation the number of stem- and progenitor cells in the bone marrow as well as in the peripheral blood are decreased almost momentarily. The D_0 of stem- and progenitor cells in human beings has been reviewed by *Nothdurft* [12]. In human beings, the D_0 values are between 0.6 and 1.6 Gy. The *in-vitro* sensitivity of GEMM-CFC was determined to be 0.57 Gy while the D_0 for GM-CFC was 0.86 Gy and for BFU-E 1.13 Gy.

Similar sensitivities were found for hemopoietic stem cells in several mammalian experimental animals [10]. These data mean – for all practical purposes – that a total body radiation exposure in the LD50 range reduces the stem cell pool dramatically to less than 10% of normal almost instantaneously.

In summary, hematopoiesis is of crucial significance for radiation accident management for the following reasons: First of all, it is distributed through the entire skeleton and therefore, homogeneity or inhomogeneity play a major role in the development of hemopoietic failure and in any recovery process. Secondly, blood cell concentrations (granulocytes, lymphocytes, platelets and red cells) are maintained through continuous replacement of old cells by means of new cells, and therefore reflect type and extent of radiation damage. Thirdly, cell production in active sites of hematopoiesis is continuously controlled and maintained by blood stem cell traffic and by humoral factors determining largely the capacity to overcome hemopoietic failure after whole body radiation exposure.

As far as the significance of the hemopoietic system for radiation accident management is concerned, one

should recall that in radiation accidents rarely, if ever, a homogenous whole body exposure will occur. Therefore, it is extremely difficult to assess any "exposure dose" to the widely distributed hemopoietic stem cell pool. For instance, if the upper body has received the major radiation exposure, then it is most likely that in the lower part of the body there are bone marrow sites with less radiation exposure and therefore with a higher probability of autochthonous regeneration. These relatively less exposed sites may produce new stem cells that are migrating through the blood stream to repopulate more heavily exposed bone marrow sites. Therefore, any inhomogeneity of whole body radiation exposure increases the chance of an autologous hemopoietic recovery.

3. Pathophysiological principles for the response of hemopoiesis to whole body radiation exposure

If one is applying the pathophysiological principles of the response of hematopoiesis to whole body radiation exposure to a concrete patient, the following observations can be made:

In *Fig. 1a*, one can see the blood cell changes in the first 4 weeks after essentially lethal whole body radiation exposure. The data shown are taken from the *Tokaimura* accident review [13].

It is of great interest to note that the leukocytes (WBC) of Patient A show a significant initial granulocytosis with values up to about 30,000 per mm^3 within 24 hours. For about 4 days the leukocyte count remains above 5,000 (normal range). Thereafter, one sees a precipitous drop of leukocytes to reach minimal levels after 5-6 days. Thereafter, for another 10 days, there is hardly any leukocyte countable in the peripheral blood. Beyond day 15 one sees a leukocyte recovery which is due to the successful engraftment of peripheral blood stem cells. The peripheral blood stem cell transplantation was performed on day 5 and 6.

As far as the lymphocytes is concerned, they drop to essentially zero within 2 days after exposure. However, already within 24 hours one can see that there are hardly any lymphocytes left in the blood. A recovery is not recognizable essentially within 20 days.

As far as platelets is concerned, one can recognize a progressive decline within 5 days with a slight shoulder between day 5 and day 10. The course of platelets is of course influenced by platelet transfusions that had to be given in order to combat thrombocytopenic bleeding.

In order to recognize the significance of hematopoietic cell renewal for the diagnosis and prognosis of the acute radiation syndrome, it is of paramount importance to understand why the blood cell changes occur the way they do. This can be derived schematically from *Fig. 1b*.

The first key element to understand the behavior of granulocyte changes in the peripheral blood is to recognize that the transit time of the maturing-

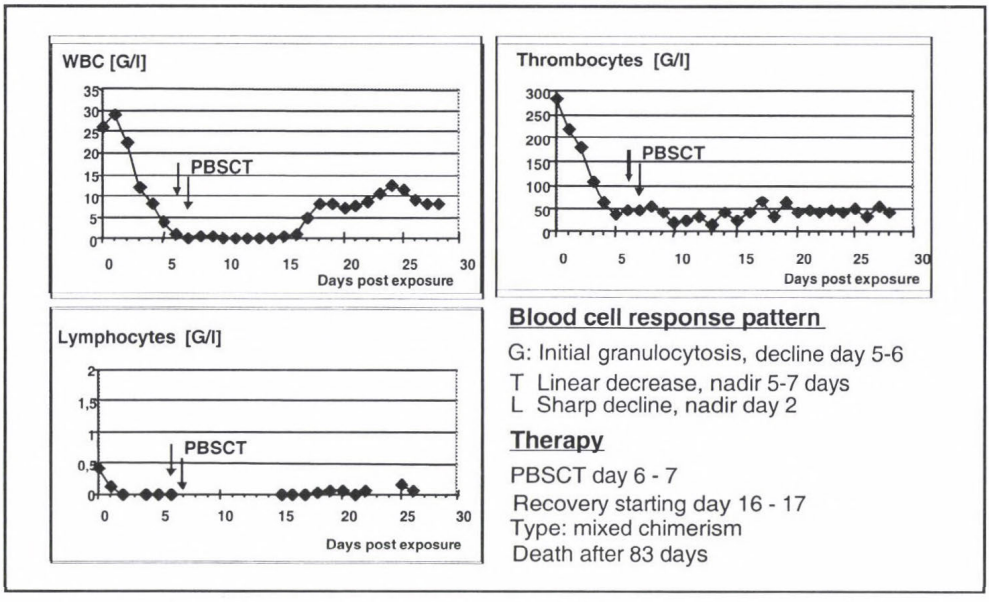

Fig. 1a: *Clinical course of Patient A (Tokaimura accident in 1999)*

only pool ($t_b + t_c$) of granulocytic cells is about 4 days [14]. If one assumes a situation - "Case 1" - whereby the dividing maturing pool of granulocytic precursor cells (t_a) is completely eradicated by ionizing radiation, then one would expect that the maturing-only cells mature to become neutrophilic granulocytes and are released into the peripheral blood. Since these maturing-only cells ($t_b + t_c$) are relatively radioresistant, one would expect that a normal granulocyte concentration is maintained for up to about 4 days. If the dividing maturing compartment (ta) (transit time about 6 days) is completely eradicated by ionizing radiation, then one would expect a precipitous drop of granulocytes in the peripheral blood between days 4 and 6 with a granulocyte disappearance halftime of about 7 hours. Thus, a severe granulocytopenia by day 5-6 would indicate that the dividing maturing pool of granulocyte precursors was essentially destroyed by radiation. The stem cell pool (St. + P. Cells) - which is even more radiosensitive than the cells in the dividing maturing pool - is obviously eradicated also and would not be able to restore the granulocytic cell renewal system.

If one would assume - "Case 2" - that the ionizing radiation would not affect the dividing maturing pool (which is an unrealistic assumption), but would only affect the stem cell pool (St. + P. Cells), then the entire behavior of the blood response curve would be different. Under these circumstances (only stem cell eradication), one would expect a continuing granulocyte production for about 10 days (4 + 6 days) before then the system fails. Under these (theoretical) considerations, one would have a pre-

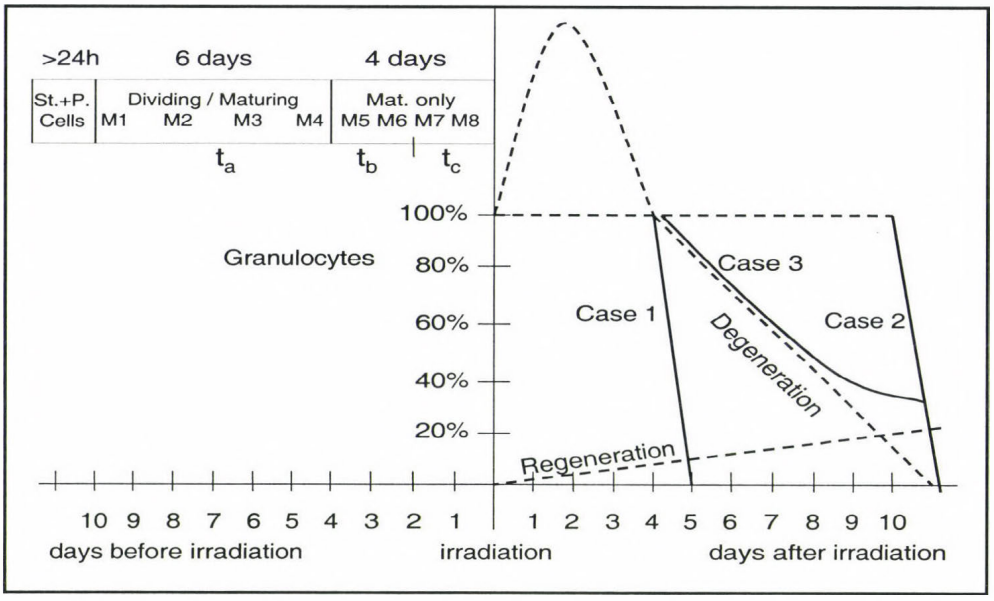

Fig. 1b: *Schematic representation of the pathophysiological mechanisms resulting in "early" granulocyte changes after acute whole body radiation exposure (for explanation see text)*

cipitous drop of cells between day 10 and 12.

Thus, the granulocyte pattern in Patient A (Fig. 1a) may well be interpreted to mean that, first of all, the entire stem cell pool as well as the dividing maturing pool of granulocytes has been eradicated indicating an essentially "irreversible damage" to the blood cell replication and production. Otherwise, the pattern of granulocytes would be different as seen in cases of essentially reversible damage of the hemopoietic system. This granulocyte response is schematically depicted as "Case 3" in Fig. 1b. In this scheme, it is assumed that the cells in compartment tb and tc continue to mature and to be released into the blood for about 4 days. Thereafter, there is a phase of "degeneration" due to the fact that the pool of "dividing-maturing" cells (M1, M2, M3 and M4) is partially damaged so that there are cells that enter the pool t_b and t_c and are released into the blood. Thus, there is a granulocyte decrease between days 5 and 10 but the shallower slope indicates the continuation of some proliferation and maturation in t_a. At the same time, there will be a regeneration of cells in the pool of stem- and progenitor cells (St. + P. Cells) somewhere in the marrow of an irradiated person, resulting in a recruitment of cells in the t_a pool, resulting in newly regeneration granulocytes ("regeneration"). Therefore, the granulocyte pattern of "Case 3" is compatible with an onset of an "abortive rise" and the resulting slope of granulocyte concentration is indicative of a potentially "reversible damage" to the system (see later hematological grading H3).

Secondly, it is of interest to interpret the early granulocytosis. It is known from experimental studies [15] that there is a bone marrow stress syndrome resulting in a severe and immediate granulocytosis. Unlike the platelet system and the red cell system, there is in granulocytopoiesis a relatively large reserve of mature granulocytes in the bone marrow as well as in the so-called marginal blood pool that are mobilized under "stress conditions" [15]. This mobilizable granulocyte pool is released into the peripheral blood as a "stress reaction". The more severe the stress is, the higher is the granulocytosis in the peripheral blood.

As far as the interpretation of the thrombocyte curve is concerned, it appears sufficient to state here that a biomathematical model of the megakaryocyte-platelet curve indicates how such a precipitous drop of platelets can occur (16). Very similar to the argumentation of the pathophysiology of the granulocyte changes one has to state that if the platelets disappear from the peripheral blood even earlier than 10 days, one has to consider that the entire megakaryocyte plus the stem cell system is destroyed by ionizing radiation and that there is even a thrombocytopenia caused by premature removal of platelets from the peripheral blood (reduction of life-span of platelets as in ITP (idiopathic thrombocytopenic purpura)). Such a premature precipitous drop of platelets together with the typical granulocyte pattern of most severe granulocytopenia within 5 days is indicative of an irreversible damage of the hemopoietic stem cell pool. This severe damage is confirmed also by the lymphocyte depression due to severe impairment of lymphocyte recirculation as well as lymphocyte radiation sensitivity. Thus, the early blood changes observed (within 6 days after irradiation) in granulocytes, platelets and lymphocytes can be taken together as a response pattern are indicative of an irreversible hemopoietic damage which only can be overcome by stem cell transplantation.

In summary, if there is within 24 hours an extensive granulocytosis combined with an extensive lymphopenia and an early "downhill course" of thrombocytes, then the likelihood of a severe, if not irreversible damage of the stem cell pool can be assumed.

The experienced hematologist will of course perform one or several bone marrow aspirations to obtain a bone marrow particle smear for cytological evaluation. Such a bone marrow particle smear would indicate within 24 hours after exposure severe cytological abnormalities, such as nuclear edema, increased karyorhexis and karyolysis and, beginning after 24 hours, mitotically connected abnormalities (clumpymetaphases, binucleated cells, cytoplasmic bridges etc.) [17].

One of the lessons learned from reviewing radiation accident victim case reports is that within 24 hours the experienced hematologist can state with high probability whether

one must expect an essentially irreversible damage of hemopoiesis or whether there is a chance for survival leading to the proposal of using stimulatory factors to induce hemopoietic recovery.

It goes without saying that the time and space for this paper does not allow a detailed description of the pathophysiological mechanisms resulting in the hematological changes that can be observed after total body radiation exposure and the reader is referred to appropriate references [1].

4. Strategic approaches to assess the severity of radiation injury to hematopoiesis

In 1965, *Bond, Fliedner* and *Archambeau* [18] suggested on the basis of reviewing the experimental radiobiological literature that radiation-induced lethality can be best explained as a consequence of a perturbance of cellular kinetics. The authors showed very clearly that all signs and symptoms of the acute radiation syndrome both in animals and in man are the consequence of the interaction of ionizing radiation with the different most critical cell renewal systems of the body, such as hemopoiesis, gastrointestinal, mucous membranes and other cell renewal systems, such as the skin.

The enormous wealth of data collected in the international SEARCH database system confirm the reproducibility of clinical signs and laboratory symptoms in accidentally whole body exposed human beings. This lead to the conclusion that a sufficient number of biological indicators of effect and repair are available very early after radiation exposure to assess the severity of effect, to predict the most likely outcome and to decide on the principle modes of treatment of the acute radiation syndrome and especially of the effects on hemopoiesis.

To examine this proposal, a concerted action entitled "METREPOL" (Medical Treatment Protocols for Radiation Accident Victims as a Basis for a Computerized Guidance System) [1] was started by the European Commission's Nuclear Fission Safety Programme to find a consensus regarding the indicators to be used in radiation accident management.

Fig. 2 shows the METREPOL approach for the triage phase of radiation accident management. If there is a suspicion of an accidental total or significant partial body exposure, the diagnostic process consisting of establishing the case history, the physical examination, the laboratory tests and additional diagnostic measures will result in an organ-specific grading of the severity of radiation effect. This will allow the establishment of a grading code resulting in a response category (RC) for a given time point after the acute radiation exposure. The logic behind this approach was taken from clinical medicine: in oncology it is well established to use a grading code (TNM-classification) to describe the severity of a cancer lesion in order to plan the appropriate therapeutic measures. What is of im-

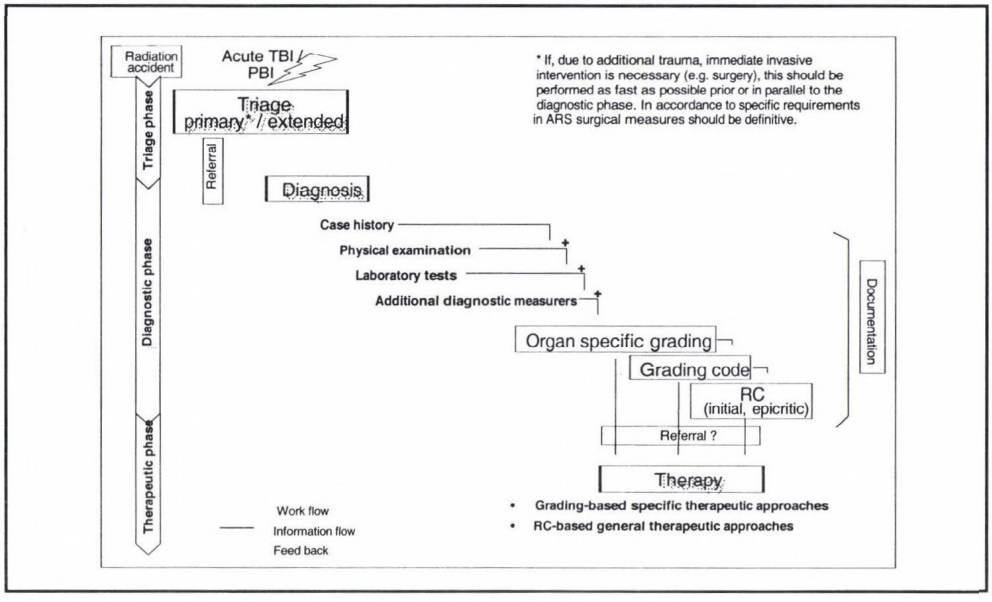

Fig. 2: *Medical radiation accident management - the METREPOL approach*

portance to a medical doctor is to assess the 4 most important organ systems regarding the severity of damage.

The most important organ systems are the neurovascular system, the hemopoietic system, the cutaneous system and the gastrointestinal system. For each organ system, 4 degrees of damage were identified. For each degree of severity the question has to be answered whether an autochthonous recovery is possible or not or whether specific therapeutic measures might be indicated if an autochthonous recovery is not possible or most unlikely *(Fig. 3)*.

For each organ system several indicators are recommended to be used to classify a patient according to a degree of severity code relevant to the question of spontaneous recovery or not. The diagnostic approach taken in this triage process would require the systematic evaluation of 25 indicators as a function of time after acute exposure *(Fig. 4)*. As far as the hemopoietic system is concerned, this grading approach relies on the response pattern of granulocytes, thrombocytes and lymphocytes as a function of time as well as on the question of the manifestation of infection and/or blood loss.

Using this approach recognizing not only the hemopoietic system but the neurovascular system, the cutaneous system and the gastrointestinal system as well *(Fig. 4)*, it is possible to determine the grading code and, on that basis, the response category for a given time after exposure (see *Fig. 5*).

As far as hemopoiesis is concerned,

Organ system	Grading number and severity of damage **1** **Mild damage**	Grading number and severity of damage **2** **Moderate damage**	Grading number and severity of damage **3** **Severe damage**	Grading number and severity of damage **4** **Serious/fatal damage**
N	Recovery certain	Recovery with possible deficit	Recovery with severe deficit	Recovery most unlikely
H	**Autologous recovery certain**	**Autologous recovery likely**	**Autologous recovery possible**	**Autologous recovery most unlikely**
C	Recovery certain	Recovery without deficit likely	Recovery with deficit likely	Recovery most unlikely or with serious deficit
G	Recovery certain	Recovery with possible deficit	Recovery may be possible	Recovery most unlikely

Fig. 3: *Grading code in the METREPOL approach*

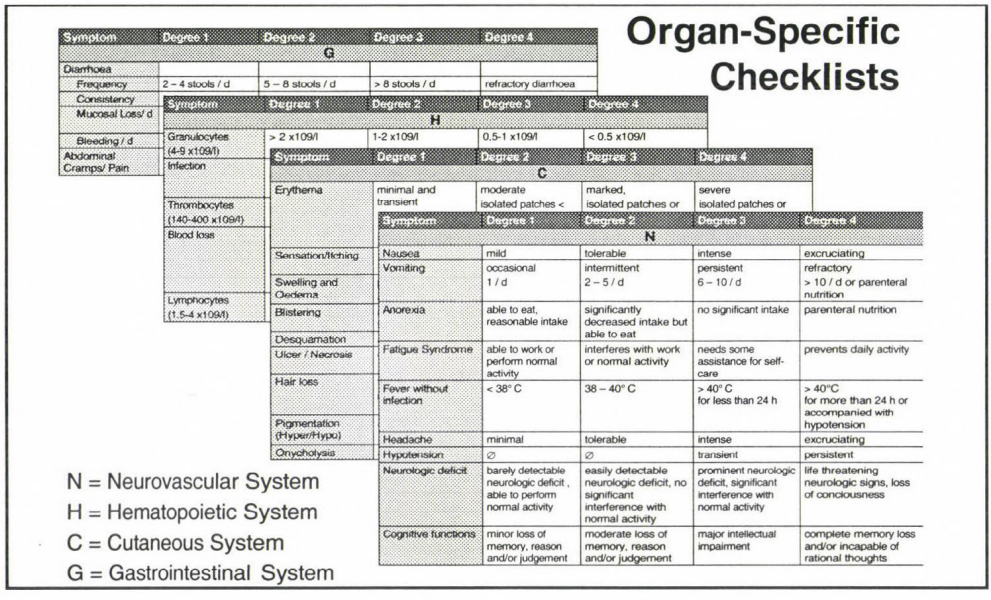

Fig. 4: *Organ-specific checklist in the METREPOL approach*

the systematic assessment of signs and symptoms allows within 24 hours the first prediction of the possible clinical course and the therapeutic measures to be taken. The appropriate response category for a given patient is firmly established for the hemopoietic organs within 5-6 days. In the example given in *Fig. 5*, one can see that for a patient with a moder-

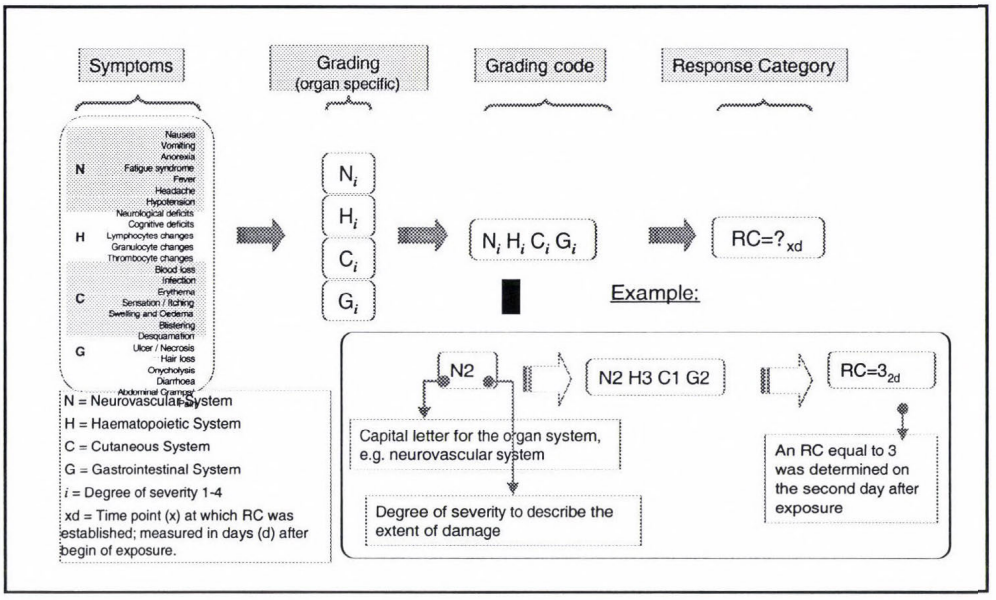

Fig. 5: *The response category (RC) concept*

ately damaged neurovascular system (N2), with a grading code H3 for hemopoiesis (meaning that autologous recovery is possible), with hardly any evidence of a cutaneous syndrome and with some evidence of a gastrointestinal damage, such a grading leads to a RC3 on the second day after exposure.

In *Fig. 6*, the early blood cell pattern characteristic of an irreversible damage to the hemopoietic system, is shown for several radiation accidents. Such a pattern for a grading code H4 includes progressive decrease of granulocytes towards day 5 and day 6 to severely granulopenic levels, a progressive decline of platelet counts in the peripheral blood towards day 10 and a severe decline of lymphocytes within 1-2 days. This pattern is compatible with the assumption of an essentially irreversible damage of the hemopoietic stem cell pool throughout the blood cell forming bone marrow.

In contrast, the blood cell changes in patients assigned to the grading code H3 can be characterized as follows: the granulocyte do not show the excessive granulocytosis during the first 3 days as it is seen for grading code H4. There is some decline of granulocytes, but on day 5 and day 6 there is still a measurable granulocyte level in the peripheral blood and the nadir of granulocytopenia is only seen between days 20-25 (for a pathophysiological explanation see Fig. 1b).

As far as platelets is concerned, it can clearly be shown (*Fig. 7*) that there is an initial shoulder for the platelets during the first 10 days. A severe thrombocytopenia develops only be-

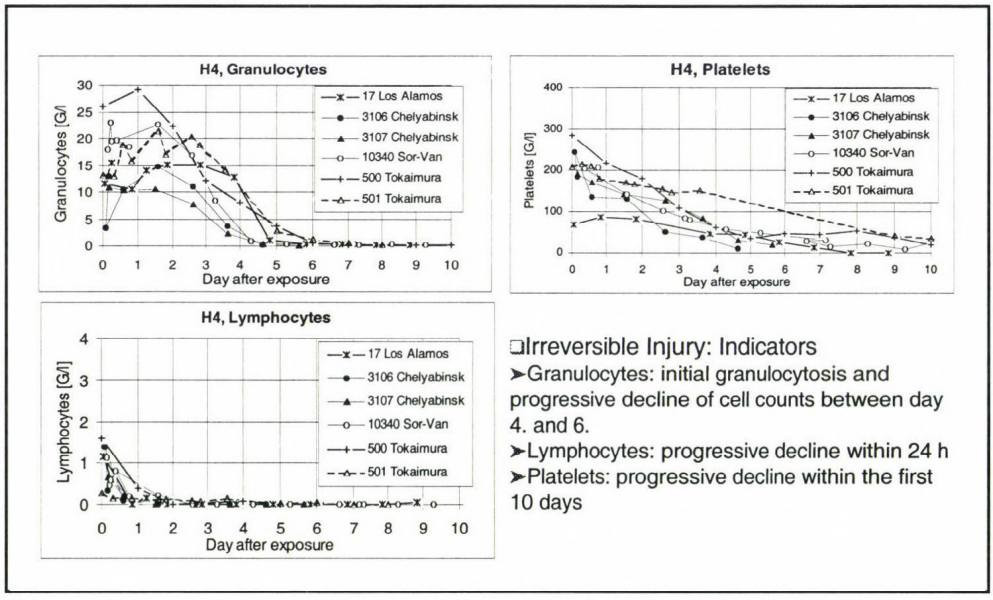

Fig. 6: *Examples of irreversible damage (grading code H4) in different accidents*

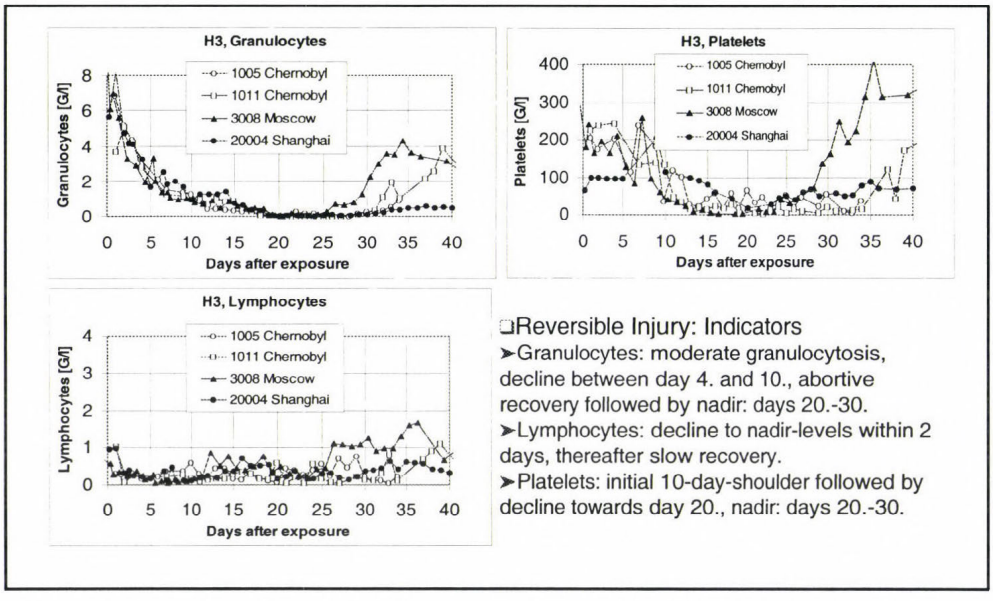

Fig. 7: *Examples of reversible damage (grading code H3) in different accidents*

tween days 20-30. The platelet numbers in the graph are higher than they would be, if no platelet transfusions were given. A slow recovery occurs beyond day 25.

The pathophysiological basis for this type if behavior of granulocytes and platelets is given in *Fig. 8*. In the above mentioned monograph on Mammalian Radiation Lethality: A

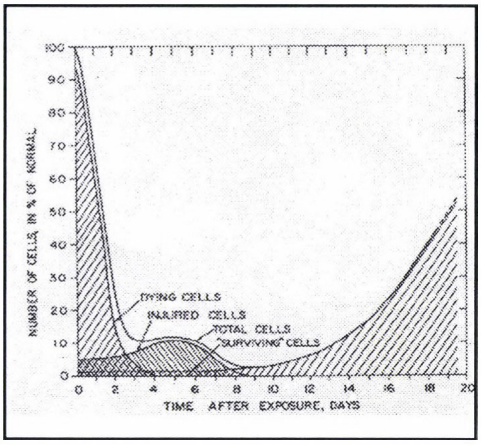

Fig. 8: *"Injured cell hypothesis" in radiation pathophysiology: Schematic representation of the abortive rise, oriented about one plausible explanation for the phenomenon. "Dying cells" are grossly damaged and disappear rapidly from the system. "Injured" cells proliferate for a time, but these cells and all progeny die after a few divisions. Surviving cells are those capable of proliferation indefinitely.*

Disturbance of Cellular Kinetics [18] it was postulated that this type of granulocyte and platelet response after ionizing whole body radiation can be explained by the so-called "injured cell hypothesis" (*Fig. 8*). In this hypothesis, it is assumed that acute radiation exposure results in a almost instantaneous death of more than 95% of hemopoietic stem cells. The final recovery after acute radiation commences from the intact or completely repaired hemopoietic stem cells (time parameters on the abscissa are derived from rodent experiments). The hypothesis further postulates that the radiation exposure results in the production of "injured stem cells". These are cells that are injured and repaired to such an extent that they can undergo a limited number of cell replications before their clone dies out. If such assumptions are made, all biomathematical models indicate that there would be an "abortive rise". This is the reason for the particular pattern of granulocytes assigned to a characteristic for a grading code H3 and also the shoulder seen in the platelets. If one is analyzing the bone marrow in these human beings, one can find between days 5 and 10 evidence of some hemopoietic recovery and a significant number of mitotically connected abnormalities indicating the attempt of the bone marrow to recover from the inflicted radiation damage.

In *Fig. 9* granulocyte values and platelet values are plotted for 21 *Chernobyl victims* who were accidentally exposed during the night of April 26, 1986* [19]. These patients in essence would be assigned initially to the severity grade H3. These cell plots indicate very clearly the early abortive rise between the first 15 days after radiation exposure, the nadir of granulocytes and of platelet counts between days 20 and 30. In all these patients there was a spontaneous recovery of hemopoiesis and during the first year there seems to be a slight overshoot of numbers and then a slow progressive return of counts into the normal range.

This METREPOL approach to classify patients after acute radiation exposure into "severity groups" that are prognostically meaningful regarding

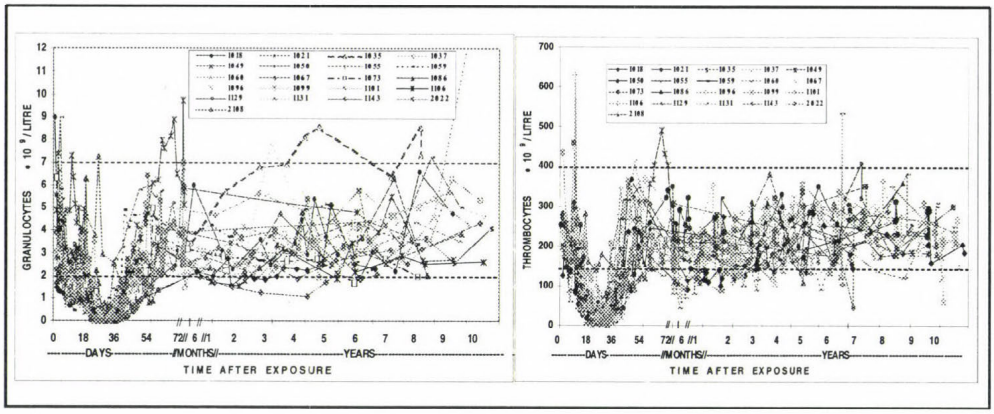

Fig. 9: *Patterns of granulocytes and thrombocytes of Chernobyl patients (grading code H3) in the course of time*

especially the therapeutic measures to be taken, allows a number of conclusions.

The assessment of blood cell changes (granulocytes, lymphocytes and platelets) allow the medical doctor to determine the severity of damage and hence the grading (H4, H3, H2 and H1) for the hemopoietic tissue within a maximum of 5-6 days after exposure.

Such a hematological grading allows one to answer the following question: Is there a high probability of a reversible or an irreversible damage to the hemopoietic tissue distributed throughout the skeleton?

The hemopoietic grading should be considered as an integral part of the entire triage process and allows the medical doctor to determine the response category comprising the severity of radiation-induced damage to the most critical organ systems (neurovascular, cutaneous, gastrointestinal and hemopoiesis). The systematic assessment of 25 indicators of effect that can be easily determined during the first few days after radiation exposure of the critical organ systems are sufficient for a patient grading and, if necessary, performing the appropriate therapeutic measures.

The METREPOL approach is not relying on any "physical dosimetry". It acts on indicators of effect and repair without necessarily disregarding indicators of exposure (physical dosimetry). The justification for such an approach is that in most, if not all, radiation accidents there is a high probability of an inhomogeneous exposure. However, the hematological damage depends very closely on the extent of radiation effects of the hemopoietic stem cell compartment, which is distributed throughout the body and interconnected by blood stem cell traffic. Therefore, any inhomogeneity of exposure acts in favor of the recovery potential of the patient and may allow a spontaneous recovery of hemopoiesis in spite of the ex-

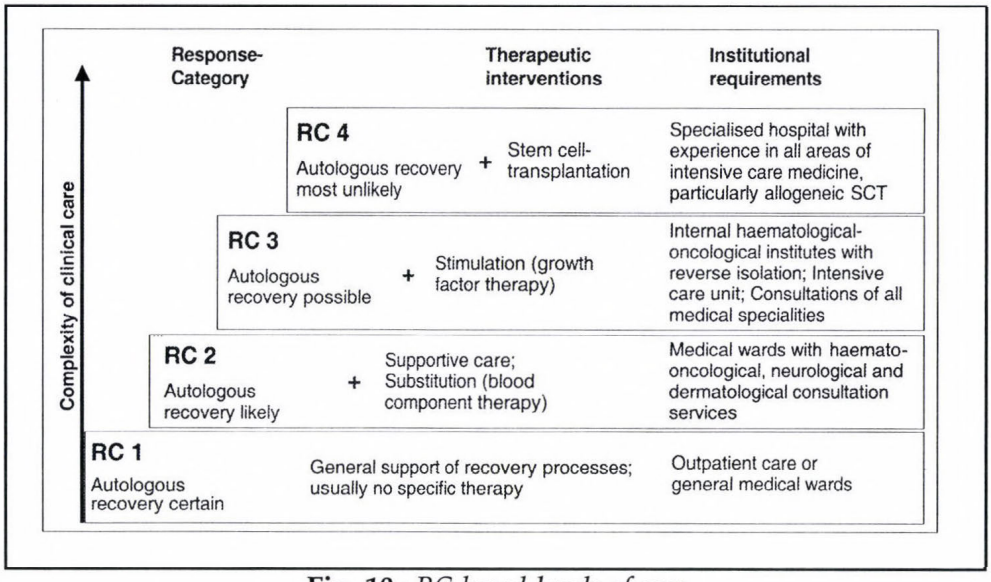

Fig. 10.: *RC-based levels of care*

tent of damage on other organ systems, such as the skin. The details of this approach and the scientific reasoning can be found in the appropriate reference [1].

5. Approaches to treat hematological consequences of whole body radiation exposure

The details of recent approaches to treat radiation accident victims suffering from an acute radiation syndrome are comprehensively covered in the METREPOL manual [1]. Therefore, it is sufficient to give in this review the principles used to propose therapeutic options.

In Fig. 10 one can derive the logic of the approach. Patients suffering from the RC1 will experience an autologous recovery. Therefore, the only therapy necessary will be general support of recovery processes in affected organs, but no specific therapy is usually needed. Of importance is that patients classified on the basis of organ-specific grading codes into the category RC1 can be taken care of on an out-patient basis. They do not need hospitalization. However, what is important is to register all signs and symptoms in the course of disease and to establish beyond doubt that they have had an exposure to ionizing radiation (for instance, by chromosomal analysis). This is important, not only for clinical reasons, but also for forensic reasons and later juridical claims.

Patients classified into RC2 will also experience an autologous hematological recovery. The examples for patients in RC2 derived from the database system SEARCH indicate that sometimes supportive care is needed and in case of a manifestation of in-

fection during the treatment phase or evidence of a slight blood loss (nose bleeding, petechiae, erythrocytes in the urine) some medical doctor may feel obliged to give a blood transfusion or a platelet transfusion or to administer antibiotics. For these patients, medical wards are sufficient, if there is appropriate hematological-oncological, neurological or dermatological consultation services.

From the viewpoint of hematology, the patients assigned to RC3 and to RC4 require the most intensive attention. In the case of RC3 an autologous recovery is possible, if all modern tools and techniques of therapy of an extensive but nevertheless transient hemopoietic failure can be applied and used. Therefore, these patients should be admitted to hematological-oncological services, which are used to treat cancer patients with high-dose chemotherapy. The respective medical services will usually have available protected environment beds which allow an essentially "gnotobiotic" treatment. These patients are vulnerable to their own endogenous microbial flora during the time of hemopoietic failure. These patients may also need the services of an intensive care unit. They will be the patients that benefit most from growth factor therapy in order to enhance hemopoietic recovery.

If the clinical judgement shows that a patient belongs to RC4, it is clear that a spontaneous hemopoietic recovery from the hematopoietic stem cell pool is most unlikely. In this case, the first and most important approach is to select a stem cell source. Since in most cases autologous stem cells are not available (except in the situation where the patient has an identical non-irradiated twin), one has to look for an appropriate histocompatible donor. The stem cells can be taken from the bone marrow, but it may well be that enough can be removed from the peripheral blood, such as was done in *Tokaimura* in 1999. There may also be a chance to use cord blood cells (as in *Tokaimura* in 1999), however, one must be aware of the fact that for an adult a cord blood donation might not give enough stem cells. It may well be that one has to use a non-identical donor and this would require to initiate all the steps that are necessary as in any allogeneic stem cell transplantation. This means also that this type of treatment should and can be done in hospitals that are used to perform stem cell transplantation in oncological patients.

The specific therapeutic approaches for the different hematological grades of severity of effect can be summarized as follows: Treatment options grading H1 (autologous recovery certain): Firstly, it is recommended to use a general support approach of the recovery processes. Usually no specific hematological therapy is required. Therefore, out-patient care may be sufficient. Regular examinations to confirm the initial grading code as well as the response category is recommended. Secondly, it can be expected that in these patients the platelet levels do not drop below 50 x 10^9/l but slight platelet count depression may be observed between

days 15 and 30. Thirdly, the manifestation of bacterial infection is usually not seen, since the blood granulocyte level is maintained at a sufficient level of more than 2 x 10^9/l. Should, however, infection occur, it is advised to determine the antibiotic sensitivity of the microbial flora in order to give the best possible antibiotic treatment.

Therapeutic options grading H2 (autologous recovery likely): In these patients, a general support for overcoming the consequences of radiation exposure is advisable. The principle treatment strategy is to bridge the days of hemopoietic failure determined by the nadir of granulocytes and platelets between about days 15 and 30. In these cases substitution therapy may be necessary: platelet transfusions should be given to maintain a platelets concentration, if possible, of more than 20 x 10^9/l. The time during which platelet support may be needed in this category may be 5-10 days. As indicated later for H3, one transfusion requires 4 blood donors. Thus, in H2, the number of volunteers for 1 patient may be a minimum of 20. Stimulation therapy using growth factors may be indicated. The period of granulocytopenia without growth factor therapy may be up to 10 days. Since growth factors may also affect the relative differentiation of stem cells into the various hemopoietic cell lineages, one should restrict the administration of growth factors to patients in whom the time of a hemopoietic failure is too long. Further supportive therapy using antibiotics, fungostatic or antiviral drugs should be administered on the basis of observed signs and symptoms.

Treatment options grading H3: These patients are severely sick and require a lot of general support. However, the principle of hemopoietic treatment is to bridge the days of hemopoietic failure, since the stem cell pool may well recover spontaneously. On the basis of this assumption, substantiated by findings within the first 10 days after exposure, the therapeutic option consists of "substitution therapy". Platelet transfusions may need to be given to maintain platelet concentrations, if possible of more than 20 x 10^9/l. One should be aware of the fact that this type of platelet support may require a lot of donors. Patients in H3 may experience a thrombocytopenic period of 20 days. Therefore (if platelets are given every other day), 40 blood donors are needed. If a hospital has to treat only 10 patients of this category, a total number of 400 blood donors may become necessary. In these patients, it might be advisable to stimulate granulocyte recovery and to decrease the risk of bacterial infection by using growth factor therapy (G-CSF and GM-CSF) to shorten the period of granulocytopenia. Thrombopoietin (TPO) may be useful in combination with G-CSF. However, more clinical research needs to be done to validate the usefulness of TPO in the treatment of the acute radiation syndrome. Furthermore, it is of importance to reduce the risk of bacterial infections, either from endogenous or from exogenous sources. Therefore, it is recommended to induce a "gnotobiotic state" and maintain it in a germfree environment. This can be done by the

administration of non-resorbable antibiotics and the maintenance of the patient in a protective environment. Systemic antibiotics should be used whenever there is clinical evidence for the development of bacterial or other infections. Furthermore, antifungal and antiviral therapy should be used as indicated.

Treatment options grading H4 (autologous recovery most unlikely): Patients classified in this response category are of course very severely ill. As far as the hemopoietic system is concerned, they pattern recognition of the hemopoietic blood cell changes in the first 3-6 days clearly indicates that there is no chance for an autochthonous recovery of the stem cell pool within a reasonable time period. Therefore, all preparatory steps for a stem cell transplantation are essential. Of particular importance is the early blood sampling to try to use whatever lymphocytes are left to determine the histocompatibility code. This would then foster the search for a suitable stem cell donor. As a rule, it will be an allogeneic donor, either to donate bone marrow or to donate blood stem cells. If it turns out to be impossible to find a suitable bone marrow or blood stem cell donor, then cord blood cells is a real alternative. In any event, the success or failure of stem cell transplantation treatment relies on the administration of a sufficient stem cell number. Clinical evidence indicates that one needs from the bone marrow 3×10^6 CD34+ cells/kg body weight. If one is collecting stem cells from the peripheral blood (after appropriate mobilization), then $2\text{-}4 \times 10^6$ CD34+ cells should be in the transfusate/kg body weight. As far as cord blood stem cells is concerned, one should have 0.3×10^8 total nucleated cells/kg body weight. One should, however, be aware, that it takes at least 10 days to expect the first newly formed granulocytes in the peripheral blood. Therefore, one should monitor the likelihood of hemopoietic recovery in the first 10 days after exposure by bone marrow examination. It is also necessary to make sure that the blood platelet concentration remains higher than $10\text{-}20 \times 10^9$/l. The time period during which platelets may be needed is difficult to predict: it depends on the speed of platelet recovery. Since there may be competition between granulocytic and thrombopoietic stem cells in the stem cell pool, one should consider whether growth factor therapy would potentially influence the balance between granulopoietic and thrombopoietic cell differentiation. It is of interest to know that in some patients that have received stem cell support and simultaneously growth factor therapy, the platelets had a difficulty to recover as one would have expected it.

6. Concluding remarks

It should be stressed that in this strategic paper only the hematological aspects of the acute radiation syndrome were considered in some detail. In the real accident situation, however, all other critical systems are involved to a larger and smaller extent: the neurovascular system, the cutaneous system or the gastrointestinal system. The acute radiation syndrome is a multiorgan syndrome requiring, for

treatment, all tools of clinical medicine. Therefore, for the grading codes RC3 and RC4 only the well established hospitals with most, if not all medical services are suited to take care of radiation induced disorders. The METREPOL approach as laid down in the Manual of the Acute Radiation Syndrome [1] recognizes the complexity of managing patients with the acute radiation syndrome. In this paper, only the surface of possibilities and limitations of treating the acute radiation syndrome could be touched. Therefore, the reader is requested to consult the original monograph.

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A vérképzőrendszer állapotának stratégiai és taktikai befolyása akut sugárszindrómában

A sugárkárosodás súlyosságának megítélésében négy szervrendszer: az idegrendszer, a vérképzőrendszer, a bőr és az emésztőrendszer károsodása játszik főszerepet. Ezekből kiemelhető a vérképzőrendszer, amelynek sejtválasza, a csontvellői elemek megjelenése és változása a keringésben diagnosztikai értékű, a csontvelő regenerációjának elősegítése (transzplantáció, növekedési faktor kezelés, stb.) megnöveli a túlélési esélyeket.

Arra a következtetésre jutottak (több munkacsoport összehangolt vizsgálatai), hogy ez elv alapján több biológiai indikátor áll rendelkezésünkre, amelyeknek összevetése módot nyújt a sugárkárosodás tényének és mértékének korai meghatározására és elsősorban a vérképzőrendszeren keresztül, a kezelés elinditására.

Ez az összefoglaló rendszer a METREPOL (Medical Treatment Protocols for Radiation Accident Victims as a Basis for a Computerized Guidance System), ennek hematológiai összetevőit ismertetik. A szerzők felhívják az olvasó figyelmét arra, hogy a közlemény egy alapmunka részbeni ismertetése (l. irodalom: Fliedner).

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