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Original Article

Stimulation of Haemopoetic Activity in Bone Marrow and Deformation of Red Blood Cells in Albino Mice, *Mus musculus* Exposed to Radiations from GSM Base Stations

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ABSTRACT

The levels of radiofrequency radiations around two global systems for mobile communication (GSM) base stations located around a residential quarter and workplace complex were measured. The effects of the radiofrequency radiations on albino mice placed in exposure cages and located around the base stations over a six months period were investigated. The levels of radiofrequency (RF) radiations around the base stations were found to range between 383 mV/m to 730 mV/m compared to 59 mV/m in control stations. In the exposed mice, a pattern of pancytosis was observed and significant increases were observed in the Packed Cell Volume (PCV), White Cell Count (WBC), Platelet count (PLT) and Red Cell Count (RCC) throughout the period of exposure in both stations when compared with control values. No significant (p>0.05) differences were observed in these values when both stations were compared. After 90 days of exposure, marginal increases occurred in the mean cell volume of exposed mice at station 1 and 2 (53.1±3.6fl Vs 55.9±1.8fl and 57.9±0.25fl respectively), with the mean cell haemoglobin concentration showing significant reduction after 180 days of exposure at station 2 (27.9±2.4g/dl Vs 25.7±0.29g/dl; p<0.05). Marked anisopoikilocytosis and striking polychromasia were seen on peripheral films of exposed mice, with bone marrow showing increased cellularity. Exposure of the mice to radiofrequency radiations therefore resulted in cellular proliferation with subsequent stimulation of haemopoetic activity and probable increase in the utilisation of folate and iron resulting in increased mean cell volume (MCV) and reduction in mean cell haemoglobin concentration (MCHC). The fears of a possible biological effect of chronic human exposure to radiofrequency radiations may therefore be reasonable enough to justify the clamour for the reduction in the proliferation of GSM base stations across the country.

Keywords: Bone marrow, GSM Base Station, Haematological effects, Mobile phone, Radiofrequency radiation

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INTRODUCTION

Due to the growing use of mobile phones and the explosive growth in the multitude of base stations to meet required efficiency from the networks, there is currently an increasing concern about the effects of electromagnetic exposure in the microwave range and radiofrequency (RF) radiation on exposed organisms and humans (Markov and Kostarakis, 2007). Several studies have indicated that exposure of biological systems to low level RF radiation may result in adverse biological effects.

For instance, disturbance or alteration of the nervous systems has been reported as a result of exposure to RF radiation and the consequent behavioural changes that may occur have been identified as early indicators of RF-related stress (Abdel-Rassoul *et al*., 2007). Abdel-Rassoul *et al.* (2007) concluded that inhabitants living close to

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mobile phone base stations are at risk of developing neuropsychiatric problems and some changes in the performance of neurobehavioural functions either by facilitation or inhibition.

Otitoloju *et al*. (2009) also reported that exposure of mice to radiofrequency radiations (RF) from mobile phone (GSM) base stations caused 39.78% and 46.03% respectively in sperm head abnormalities compared to 2.13% in control group. Other studies have documented that RF exposure causes cell proliferation (Velizarov *et al.*, 1999), increased enzyme activity (Paulraj and Behari, 2002, Barteri *et al*., 2004), cell membrane permeability and ion homeostasis (Goltsov, 1999), changes in the functions of genes, activation of proteins, and the internal chemical communication within cells (Vahdettin, 2009). The trigger for these changes is unknown. It is only known that this phenomenon is not the result of excessive heating of tissue. In a study in Bulgaria, Kouzmanova *et al*. (1997) documented that the treatment of rats with 5.6mm electromagnetic waves (EMW) only showed an increase of marrow cellularity compared to the control group. The increase in marrow cellularity after exposure to 7.1mm EMW was observed on the seventh day following exposure thereby demonstrating the effect of increasing intensity of radiation on haemopoetic activity.

Changes in the concentrations of circulating white blood cells have been observed in a number of animal species exposed to microwave energy. The changes were not consistent within or between species and depend on exposure conditions and thermal changes in tissues. A number of mechanisms have been proposed to explain changes in cellular dynamics, including stimulation of synthesis at thermal levels, recirculation of sequestered cells, and increased hypothalamic – hypophysial - adrenal function following thermal stress. Such changes may be related to changes in immune function noted by a number of investigators (Abdel-Rassoul *et al*., 2007).

Conversely, some authors have also observed that the current exposure level to RF radiations may have no adverse effects on biological cells (Valberg *et al*., 2007). Base on this apparent controversy on the potential biological effects of radiofrequency radiations, there is therefore the need for more independent research on the subject to either strengthen or weaken this hypothesis and some of the positions already taken by various stakeholders. Furthermore, the increased concern by the public about the safety and potential health effects of the multitude of cellular transmitter antennas in different neighbourhood and the apprehension of the unknown make it necessary to provide answers to questions about safety of telecommunication base stations. At present, little is known about the effect of long term exposure experienced by people living near these mobile phone base stations (Bortkiewicz *et al.*, 2004; Abdel-Rassoul *et al.*, 2007).

Studies on the haematological effects of radiofrequency radiations will therefore serve as a useful general indicator of the potential of RF radiations to cause adverse effects on exposed organisms. This is because the blood is a pathophysiological reflector of the whole body and, therefore, blood parameters are important in diagnosing the structural and functional status of organisms exposed to toxicants (Adhikari *et al*., 2004). On the basis of the above, the objectives of this study were to determine the level of electromagnetic frequency radiation around GSM base stations located around residential and workplace quarters, study the effects of the RF radiation and other activities around the GSM base stations on the haemopoetic system (peripheral blood and bone marrow) of albino mice, *Mus musculus*.

MATERIALS AND METHODS

Study Area

Three locations were used for the study, station 1 was a GSM base station located around an office block complex, station 2 was GSM base station located around a residential quarter and the third site (control station) was located away (300m radius) from any GSM base station.

Animal Breeding and Maintenance

Albino mice, *Mus musculus,* which served as the bioassay organisms, were purchased from the Nigerian Institute of Medical Research (NIMR), Yaba. Mice were fed per day with 40g of mice feed in pellets purchased from NIMR. The mice were kept in cages (0.53m x 0.35m x 0.23m) for at least 14 days to acclimatise to laboratory conditions (290C ± 20C and Relative Humidity - 70% ± 4%) before commencement of bioassay. Body weight of the mice was recorded at day 0, 30, 60, 120, 180 and the mean weight was recorded.

Measurement of Radiofrequency Radiation from GSM Base Stations

The four - week old mice were divided into three groups containing 10 mice each (5 males and 5 females). Each group was placed in the exposure cages located below the GSM base stations at a distance of 1-50m from the base. Total exposures due to radiofrequency field strength from mobile phone base station 1, station 2 and control station, were measured with the aid of a wide spectrum Aeritalia Radiofrequency Field Strength measuring meter at distances of 0, 50, 100, 150, 200, 250 and 300m as previously described (Otitoloju *et al.*, 2010).

Biological Effects Studies

Haematological Studies

500µl of blood was collected from the heart using syringe of 2.5ml into EDTA anticoagulant bottle. Estimation of hemoglobin concentration, white cell count, platelet count, red cell count, lymphocyte, pack cell volume (PCV) and red cell indices were carried out using electronic coulter counter (ADVIA-TM 60).

Bone Marrow Studies

Bone marrow sections were obtained from dissected femurs of mice, *Mus musculus* and fixed in 10% formalin over a period of 24 hours. It was then decalcified using abundant volume of Ethylene Diamine Tetra acetic Acid (EDTA) in pH solution of 5.0.The decalcified samples were now transferred to phosphate buffer (pH 6.8), after 24 hours of fixation in 10% formalin. The tissues were then dehydrated in graded alcohol, cleared in xylene before embedding in paraffin wax. Serial sections of 2*µ*m thickness was cut in rotary microtome then passed through xylene followed by absolute alcohol and water. The sections were stained with haematoxylin and eosin, dehydrated in graded alcohol, mounted in xylene and covered with cover slip. The slides were left to dry on the hot plate for 24 hours before observing under the light microscope.

Ethics

The experiments involved the utilisation of whole and live mice. However, aiming for the protection and welfare of animals, studies were conducted in accordance with University of Lagos Ethics Committee guidelines for experiments with whole animals.

Statistical Analysis

To test the null hypothesis that there was no difference between means for the various treatments and control, results were appropriately subjected to analysis of variance (ANOVA). Further analysis of associations by chi-square was carried out where there was a significant difference at the 5% (P< 0.05) level of significance (taken as minimum requirement).

RESULTS

During the study period, the exposed mice were observed to feed normally when compared to control group. Each mice consumed an average of 30g per day of the mice feed. The exposed mice were also observed to give birth to an average of five (5) offspring per female after a twenty-one day gestation period.

Measurement of Radio-Frequency (RF) Level around GSM Base Stations and Control Sites

The results of the RF measurements are presented in Figure 1. The level of RF radiations around the base stations based on electric strength measurements were found to range from 382.7mV/m to 730.1mV/m. The highest level of RF radiation based on electric field strength was detected around the base station 1 located around residential quarter. The RF radiation around base station 1 was found to be higher than that around 2nd base station located within the vicinity of a workplace complex. The RF radiation around the GSM base stations were found to be significantly (P<0.05) higher than the RF radiations (59mV/m) in the control stations where no base stations were located within 300m radius.

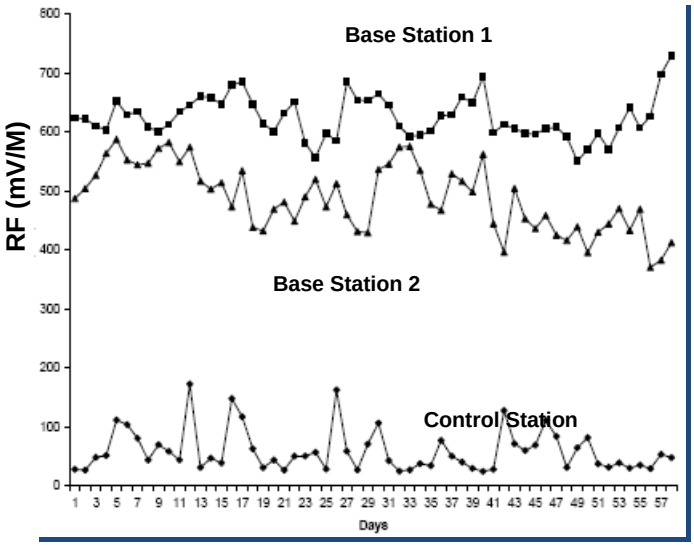

Figure 1: Level of Radiofrequency Radiations from GSM Base Stations at Different Locations

Base Station 1: Residential Quarters, Base Station 2: Workplace Complex

Table 1: The Values of Heamatological Parameters Measured at Various Exposure Periods

Length of exposure	Mean PCV (L/L)		Median RBCC ($x10^{12}/_L$)		Median WBC ($x10^9/_L$)		Median PLC ($x10^9/_L$)	
	90days	180 days	90days	180days	90days	180days	90days	180days
Control sample	41.1±1.6	46.2±2.4	7.85	9.19	7.83	4.95	516	231.5
Residential Complex (Station 1)	54.5±2.4	-	9.93	-	16.65	-	683	-
P-value	<0.05		<0.05		<0.05		<0.05	
Workplace Complex (Station 2)	52.5±3.6	55.55±2.38	9.92	10.38	15.03	8.03	1163	1216.5
P-value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Table 2: Red Cell Indices at Various Exposure Periods

Length of exposure	Mean MCV (Fl)		Mean MCH (Pg)		Mean MCHC (g/l)	
	90days	180 days	90days	180 days	90days	180 days
Control sample	53.1±3.6	55.7±3.3	15.56±0.35	15.5±0.42	27.6±2.4	**27.9±2.4**
Residential Complex (station 1)	55.9±1.8	-	15.63±0.56	-	28±0.78	**-**
P-value	0.118		*p*>0.05		*p*>0.05	
Workplace Complex (Station 2)	57.9±0.25	56.35±2.6	15.7±1.28	14.42±0.74	28.77±2.51	**25.7±0.29**
P-value	**0.043**	**0.407**	**>0.05**	**0.07**	**>0.05**	**0.05**

Haematological Studies

Cell Count after 90 days of Exposure

The mean Packed Cell Volume (PCV) of mice after 90 days of exposure to radio waves were 52.5 ± 3.6% and 54.5 ± 2.4% for station 1 and station 2, with a mean PCV of 41.1 ± 1.6% for control mice (p<0.05). The median Red Cell Count (RCC) for mice at station 1 and 2 were 9.93 x 1012/l and 9.92 x 1012/l respectively and the control mice had a median Red Cell Count of 7.85 x 1012/l (p<0.05). Median values of White Blood Cell Count (WBC) increased for both group of mice at station 1 and 2 (16.65 x 109/l and 15.03x109/l respectively) when compared with control values at 7.83 x 109/l (p<0.05). Platelet count (PLT) also increased progressively from a median value of 516x109/l in control mice to 683x109/l and 1163 x 109/l in mice located at station 1 and 2 respectively (p<0.05) (Table 1).

Cell Count after 180 days of Exposure

After 136 days of exposure to radio waves, there was a mass casualty of all the exposed mice at station 1. The mean Packed Cell Volume of the control mice and exposed mice at station 2 were 46.2 ± 2.4% and 55.6 ± 2.4% respectively (p=0.005). The median Red Cell count for control mice and mice at station 2 were 9.19 x 1012/l and 10.38 x 1012/l respectively (p=0.003). Median values of White Blood Cell count increased for mice at station 2 (8.03 x 109/l) when compared with control values at 4.95 x 109/l (p=0.009). Platelet count also increased progressively from a median value of 231.5 x 109/l in control mice to 1216.5 x 109/l in mice located at station 2 (p=0.000) (Table 1).

Red Cell Indices after 90 days Exposure

The mean cell volume (MCV), increased from 53.1±3.6fl in control mice to 55.9 ± 1.8fl (p=0.118) and 57.9 ± 0.25fl (p=0.043) for mice at station 1 and 2 respectively, mean cell haemoglobin (MCH) for control mice, and mice at station 1 and station 2 were 15.56 ± 0.35pg; 15.63 ± 0.55pg and 15.7 ± 1.28pg respectively (p>0.05), and their mean cell haemoglobin concentration (MCHC) were 27.6 ± 2.4g/dl; 28 ± 0.78g/dl and 28.77 ± 2.51g/dl respectively (p>0.05) (Table 2).

Table 3: Blood Morphology after Exposure to Radioactive Radiation

LOCATION	RED BLOOD CELLS	WHITE BLOOD CELLS
Control	Normocytic, Normochromic cells.	No atypical form of cells seen
Residential Complex (station 1)	Many target cells with deposits of haemoglobin at the parlor of the RBC (Heinz bodies) with polychromasia	No atypical form of cells seen
Workplace Complex (Station 2)	Moderate anisocytosis and Macrocytosis seen.	No atypical form of cells seen

Cell Indices after 90 days Exposure

The mean cell volume (MCV), increased from 53.1 ± 3.6fl in control mice to 55.9 ± 1.8fl (p=0.118) and 57.9 ± 0.25fl (p=0.043) for mice at station 1 and 2 respectively, mean cell haemoglobin (MCH) for control mice, and mice at station 1 and station 2 were 15.56 ± 0.35pg; 15.63 ± 0.55pg and 15.7 ± 1.28pg respectively (p>0.05), and their mean cell haemoglobin concentration (MCHC) were 27.6 ± 2.4g/dl; 28 ± 0.78g/dl and 28.77 ± 2.51g/dl respectively (p>0.05) (Table 2).

Red Cell Indices after 180 days of Exposure

The mean cell volume increased marginally from 55.7 ± 3.3fl in control mice to 56.3±2.6fl for mice at station 2 (p=0.407). MCH for control mice, and mice at station 2 were 15.5±0.42pg and 14.42 ± 0.74pg respectively (p=0.07), and their mean cell haemoglobin concentration were 27.9 ± 2.4g/dl and 25.7±0.29g/dl respectively (p=0.05) (Table 2).

Peripheral Film Findings

Marked anisopoikilocytosis was seen with increased target cell population, moderate macrocytosis and polychromasia in mice exposed to electromagnetic waves at 90 days and 180 days at both stations compared to the control (Table 3).

Bone Marrow Studies

The results of bone marrow sections are provided in Figure 2-4. The overall picture indicates bone marrow stimulation involving the trilinage of the marrow haemopoetic precursor cells (erythroid, myeloid and megakaroblasts). The underlining basis of this pathology could be a reflection of anaemia reaction to infective agents or myeloproliferative state.

DISCUSSION

In this study, the mean radiofrequency field strength was 730 mV/m and 383 mV/m around base stations 1 and 2 respectively. Compared to values approved by the International Commission on Non-Ionizing Radiation Protection (ICNIRP), these observed RF radiation levels are about 80-100 folds lower than the set guideline of (40,000 - 60,000mV/m) which is said to be considered safe to the general public.

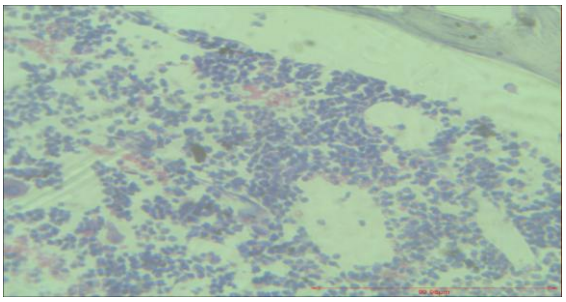

Figure 2: Control: Bone marrow histology showing precursor haemopoietic cells and few foci of fat cells.
The trabeculae are not remarkable

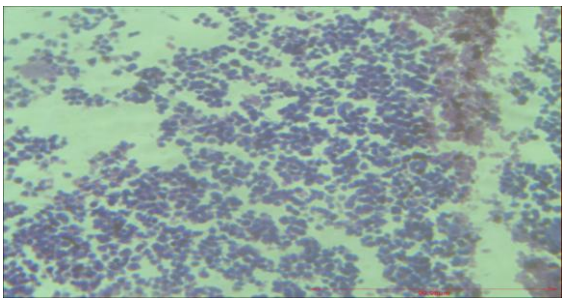

Figure 3: Bone Marrow Specimen of Rat from Station 1
It shows hypercellular marrow containing heterogeneous mixture of mature haemopoetic precursor cells.
Fat component is absent

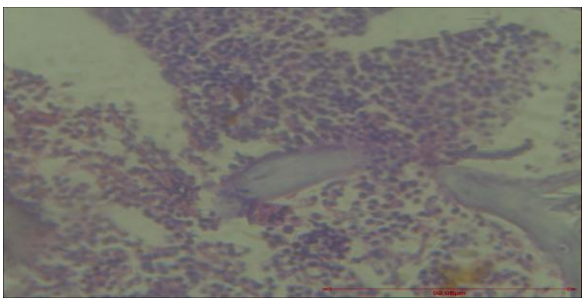

Figure 4: Bone Marrow Specimen of Rat from Station 2.
The marrow is hypercellular. All the three linage of haemopoetic precursor cells are increased in population.
Fat component is reduced.

Presently, since little is known about the effect of long term exposure on the people living near these mobile phone base stations (Bortkiewicz *et. al.*, 2004; Abdel-Rassoul *et al.*, 2007), a number of agencies in different countries have come up with widely varying RF safe limits or standards. For example, while the ICNIRP have set a limitation guideline of between 40,000-60,000mV/m, other countries such as France, Italy, Switzerland and Austria set guidelines of 200mV/m, 600mV/m, 400-500mV/m and 600mV/m respectively (ICNIRP 1998; Valberg *et al*., 2007). This wide variation in the set guidelines from different countries is a reflection of the state of information and risk perception of the potential health effect of RF radiations. Therefore, developing countries who have adopted the ICNIRP guidelines unreservedly need to reconsider this policy in line with current information which are emanating from the scientific community.

In this study, we also noted moderate disparities in the overall haematological parameters of the control mice population which may be attributed to a combination of normal physiological and environmental changes, occurring during the 180 days of incarceration, during which time conception and delivery had occurred among the mixed population of males and females. The mean Packed Cell Volume (PCV) of control mice (41.1 ± 1.6%) was significantly lower when compared with the PCV of mice located at station 1 and station 2 after 90 days of exposure to electromagnetic radiation (52.5 ± 3.6% and 54.5 ± 2.4% respectively, $p<0.05$).

After 180 days of exposure, a progressive increase was observed in PCV. The mean Red Cell count for mice at station 1 and 2 were also observed to have increased moderately after 90 days of exposure when compared to the mean Red Cell Count of 7.85 ± 0.8x1012 /l for the control mice ($p<0.05$), and even reaching a higher, statistically significant level at station 2 after 180 days of exposure (9.19 ± 0.30 x 1012/l Vs 10.38 ± 0.25 x 1012/l; $p=0.003$). These findings indicate an absolute increase in red cell mass rather than a dehydrative process as earlier reported by Villa *et al*. (1991). Likewise, there was an observed increase in the overall White Cell Count of mice in both station 1 and 2 (16.65 ± 3.22 x 109/l and 15.03 ± 2.43 x 109/l respectively) at 90 days and 8.03 ± 0.50 x 10/l at 180 days for station 2, when compared with those of the corresponding controls (7.83 ± 1.88 x 109/l; and 4.95 ± 1.62 x 109/l respectively; $p<0.05$). A progressive increase in lymphocyte percentage was observed in the mice in both station 1 and 2 after 90 days (81.4% and 87.6% Vs 74%; $p<0.05$), tending towards an absolute lymphocytosis. This finding was corroborated by Gagnon *et al*. (2003), on teratogenic effect of broadband electromagnetic field on neonatal mice (*Mus musculus*). Increase in the values of haematological parameters investigated in this study; strongly indicate an increase in haemopoetic process, possibly induced by electromagnetic effects on mice bone marrow stem cell proliferation and differentiation. This position was further strengthened by the observed increase in platelet count of the mice in both station 1 and 2 after 90 days of exposure, and at station 2 after 180 days of exposure (683 ± 102 x 109/l, 1163 ± 115x109/l and 1216.5 ± 77.3 x 109/l respectively; $p<0.05$) thus establishing a pattern of pancytosis.

Elevation of white blood cell (WBC) counts was observed in mice exposed to RF radiations when compared to control. This increase may be related to the induction of a protective mechanism in the exposed mice to the effect of the RF radiation and other activities around the GSM base stations. Bastide *et al.* (2001) reported from their study of mice exposed to GSM radiation, a 50% decrease in serum immunoglobulin and corticosterone levels. Hence, it can be deduced that exposure to radiofrequency waves induces stress in exposed animals and increase susceptibility to infections, which may lead to the synthesis of abnormal levels of white blood cell. Several studies including Pocock *et al*. (1989); Hoffman *et al*. (2004) and Jee *et al*. (2005) have identified the white blood cell count as an integrated indicator of inflammatory stimuli on both acute and chronic time frames. It is elevated acutely by infection and other stress or toxic exposures. A single measurement has been shown to predict risk for death and for specific diseases, including cancer and cardiovascular diseases. The elevation of white blood cell count is also said to indicate tissue destruction, disorders of white blood cells (leukaemia) and bone marrow failure. The significant elevation of the white blood cells was therefore a clear indication of a stress related effect in the exposed animals and should prompt further investigations on the immunological effect of RF radiations on exposed population.

Red cell indices showed marginal variations. There was a marginal increase in the mean cell volume (MCV) of the exposed mice and a corresponding decrease in the mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) in both stations. These might correspond to an increase utilisation of folate and iron required for the increased haematopoetic process in the mice. However clinical anaemia was not observed, contrary to the report of Manisha and Baile (2003) who observed anaemia in the exposed rat. Other abnormalities observed in the exposed mice were deformation of red blood cell morphology. The observed deformities in the red blood cells include increase in number of macrocytes, poikilocytes, polychromatic cells, target cells and presence of Heinz bodies in exposed mice compared to the control group. These deformities might have arisen from over stimulation of the bone marrow and oxidative stress.

This study therefore showed that there are marked differences in haematological parameters observed between exposed and control mice. These observed differences in the haematological parameters were found to be linked to the strength of the electromagnetic fields and the total duration of exposure. Furthermore, the study showed progressive stimulation of haemopoetic activity in the marrows of mice exposed to electromagnetic radiations which if it leads to uncontrolled haemopoesis might eventually lead to anaemia and bone marrow atrophy or fibrosis. These disease conditions may therefore become useful general indicators of RF- related stress in combination with other more specific symptoms.

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