Acta Oncologica Vol. 34, No. 3, pp. 379–384, 1995

TUMOR OXYGENATION IN ANEMIC RATS: EFFECTS OF ERYTHROPOIETIN TREATMENT VERSUS RED BLOOD CELL TRANSFUSION

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Anemia was induced in rats by the development of a hemorrhagic ascites. These animals also bore solid tumors (DS-sarcomas) on the hind foot dorsum. The effects of two methods for anemia correction on oxygenation in the solid tumors were compared in this study. Anemia was corrected either chronically by erythropoietin administration (1000 IU/kg) over 14 days (EPO) or acutely by transfusion with red blood cells (TR). Non-anemic and untreated anemic animals served as controls. Tumor oxygenation was determined in anesthetized animals using polarographic needle electrodes and pO_2 histography. The reduction in hematocrit and hemoglobin content found in anemic animals could successfully be corrected either by EPO or by TR. Anemia resulted in a worsening of tumor oxygenation which could partially be reversed by EPO or TR in small tumors (<1.4 ml). In larger tumors (≥ 1.4 ml), neither method of anemia correction resulted in significant changes in tumor oxygenation.

Microenvironmental abnormalities found in experimental rodent and human malignancies can have a profound effect on tumor response to various non-surgical modalities (1–3). For example, the presence of low pO_2 levels can protect cells from ionizing radiation and some chemotherapeutic agents, and there is much evidence to suggest that the presence of hypoxic cells in human tumors may be a major reason for the failure of conventional radiation therapy in the local control of malignancies (4–6). This problem may be augmented in situations where the oxygen-carrying capacity of the blood is decreased. One such situation, often occurring in tumor patients, is progressive anemia. Its etiology is multifactorial and not always associated with acute blood loss. Numerous studies have investigated the relationship between anemia and response to radiotherapy (for a recent review see (7)) and strongly suggest that low hemoglobin levels are a marker for poor prognosis in patients undergoing radiotherapy. Consequently, homologous blood transfusion is frequently given to severely anemic cancer patients prior to radiotherapy. An alternative to transfusion is the use of erythropoietin (EPO)—a glycoprotein hormone regulating the differentiation and maturation of red blood cells—which has recently become available for clinical use. To date, however, no studies are available in which the effects of EPO and blood transfusion (TR) on tumor oxygenation in anemic tumor patients or animals have been compared. In the present study, an animal model of tumor anemia was established and the effects of these two methods of anemia correction on tumor oxygenation were determined in order to ascertain which of these approaches might have implications for improving tumor oxygenation and subsequently radiotherapy outcome in anemic tumor patients.

Received 7 October 1994.
Accepted 18 November 1994.
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Paper presented in part at the Tumour Microenvironment Workshop in Granada, Spain, September 23–25, 1994.

Material and Methods

Animals. Male Sprague Dawley rats (Charles River Wiga, Sulzfeld, Germany; body weight 200–300 g), housed

 ISSN 0284-186X

under specific pathogen free conditions were used in this study. They received a standard diet and water ad libitum.

Tumors in control animals. Solid tumors were induced by injection of DS-sarcoma ascites cells (0.4 ml; approx. 10^4 cells/μl) subcutaneously (s.c.) into the hind foot dorsum (for more details see (8)). The tumors investigated appeared as flat spherical segments. They replaced the subcutis and corium completely, and invariably reached the avascular epidermis.

Tumors in anemic animals. Solid tumors were induced on the hind foot dorsum as described above for control animals. Additionally, approximately 10^8 DS-sarcoma cells were injected i.p. (following commencement of rhEPO or PBS treatment) to induce anemia which results primarily from the development of a hemorrhagic ascites.

Erythropoietin (EPO). Recombinant human erythropoietin (Recormon®, Boehringer-Mannheim, purity >98%) was dissolved in buffered saline and administered (1 000 IU/kg) three times per week, over 14 days, to animals by s.c. injection. Control animals received an equivalent volume of phosphate-buffered saline (PBS). Studies in rats have shown that there is no significant production of antibodies against EPO over this treatment period (G. Bode, personal communication).

Red blood cell transfusion (TR). TR was carried out 1 h before the commencement of oxygenation measurements. Blood obtained from donor rats was washed with phosphate-buffered saline and centrifuged so that a suspension of packed red blood cells was obtained (hematocrit ~70%). On average, 0.01 ml/g body weight needed to be transfused so that a hematocrit of ~43% could be attained.

Measurements. Rats were anesthetized with sodium pentobarbital (40 mg/kg i.p., Nembutal®, Ceva, Paris, France). Polyethylene catheters were surgically placed into the thoracic aorta via the left common carotid artery and connected to a Statham pressure transducer (type P 23 ID, Gould, Oxnard, CA, USA) for mean arterial blood pressure measurement and into the left external jugular vein (for subsequent anesthetic administration and TR). Throughout all experiments, animals lay supine on a heated operating pad and the rectal temperature maintained at 37.5°C. Animals breathed room air spontaneously. At the end of a 30 min stabilization period, blood samples were taken and the hematocrit and hemoglobin concentration investigated. The arterial blood gas status was assessed using a pH/blood gas analyzer (type 178, Ciba Corning, Fernwald, Germany). The oxyhemoglobin saturation of the arterial blood (sO_2) was obtained nomographically (9).

Tumor oxygen tensions were determined using polarographic needle electrodes (recessed gold in glass electrode; shaft diameter 250 µm; diameter of the cathode 12 µm) with stainless steel shafts (of the hypodermic needle type) and pO_2 histography (model KIMOC-6650, Eppendorf, Hamburg, Germany; for more details see (10)). A midline incision was made in the skin covering the lower abdomen and the silver/silver chloride reference electrode was placed between the skin and the underlying musculature. Calibration was performed in saline solutions equilibrated with either air or pure N_2 immediately before and after pO_2 measurements. A small incision was made into the skin overlying the tumor and the O_2-sensitive electrode advanced to a depth of approximately 1 mm. The electrode was then automatically advanced through the tissue in preset steps of 0.4 mm. These steps consisted of a rapid forward step of 0.7 mm immediately followed by a back-

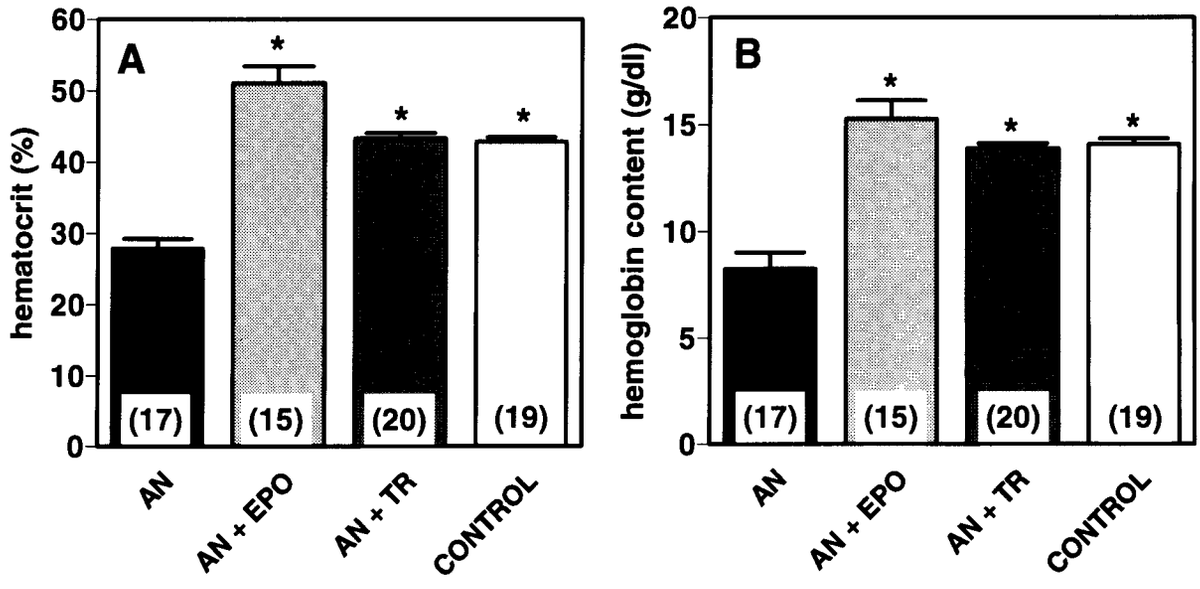

Fig. 1. Hematocrit (A) and hemoglobin content (B) in anemic (AN), EPO-treated anemic (AN + EPO), TR-treated anemic (AN + TR) and control animals. Values indicate means ± SEM with the number of observations in brackets. * $p < 0.001$

Table

Mean arterial blood pressure (MABP), arterial blood gases, pH and oxygen saturation (sO_2) in control, anemic (AN), erythropoietin-treated anemic (AN + EPO) and transfusion-treated anemic (AN + TR) animals. Values are means ± SEM, with n indicating the number of observations

	n	MABP (mmHg)	pO_2 (mmHg)	pCO_2 (mmHg)	pH	sO_2 (%)
CONTROL	19	146 ± 3	76 ± 1	44 ± 2	7.44 ± 0.01	95 ± 1
AN	17	117 ± 3	71 ± 2	36 ± 2	7.42 ± 0.01	93 ± 1
AN + TR	20	123 ± 2	76 ± 1	31 ± 1	7.44 ± 0.02	96 ± 1
AN + EPO	15	128 ± 5	77 ± 2	41 ± 1	7.39 ± 0.01	96 ± 1

ward step of 0.3 mm in order to minimize compression artifacts. The O_2 probe was automatically removed from the tissue at the end of each track measurement. Four or five parallel electrode tracks were evaluated per tumor and a minimum of 70 pO_2 readings obtained for each tumor. Using a computing system, data were pooled a) for individual tumors in the case of analysis of median pO_2 and the hypoxic fraction and b) for each treatment group for the construction of cumulative frequency distributions.

Statistical analysis. Results are expressed as means ± SEM with the numbers of experiments indicated within brackets. The significance of the differences between the various groups was assessed using the Wilcoxon test for unpaired samples. Results were considered as being significant if p-values were less than 5% ($p < 0.05$).

Results

Hematocrit and hemoglobin content of blood from the four treatment groups are shown in Fig. 1A and B. The tumor anemia induced in the present study was seen to result in a significant decrease in these parameters as compared to control animals ($p < 0.001$). The administration of EPO or TR to anemic animals resulted in significant increases in the hematocrit and hemoglobin content ($p < 0.001$). In anemic animals receiving EPO, the hematocrit increased by 13 % (v/v) after 7 days and by another 10 % (v/v) in the second week of treatment.

Mean arterial blood pressure, arterial blood gas and pH status, together with the oxygen saturation are shown in the table. The arterial blood pressure and pCO_2 in all anemic animals was significantly lower than in control animals ($p < 0.01$), but this effect was seen to be at least partially reversed in animals receiving EPO treatment. Arterial blood pO_2 was lower in anemic untreated animals, an effect which could be eliminated by either TR or EPO treatment ($p < 0.05$).

Following measurements of tumor oxygenation, cumulative frequency graphs for tumors of two size ranges (i.e., < 1.4 ml and ≥ 1.4 ml) were constructed and are presented

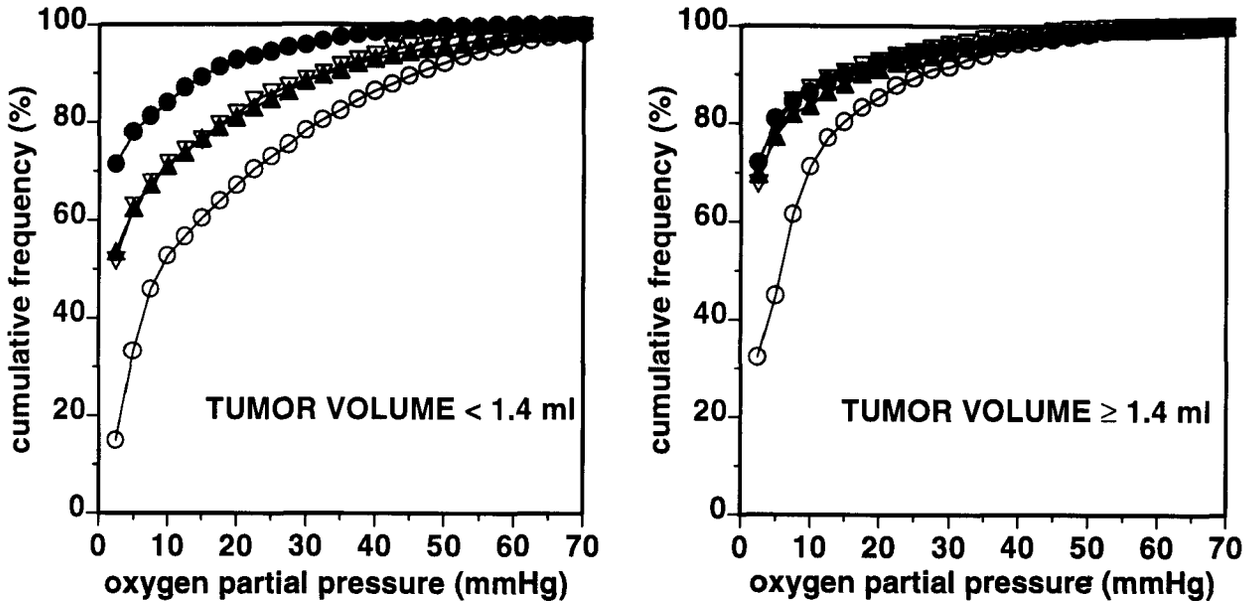

Fig. 2. Cumulative frequency distributions of oxygen partial pressures measured in tumors of two size ranges (left panel: < 1.4 ml; right panel: ≥ 1.4 ml) in control ○–○, anemic ●–●, EPO-treated anemic ▲–▲ and TR-treated anemic △–△ animals. Each curve indicates values obtained from a minimum of 8 tumors.

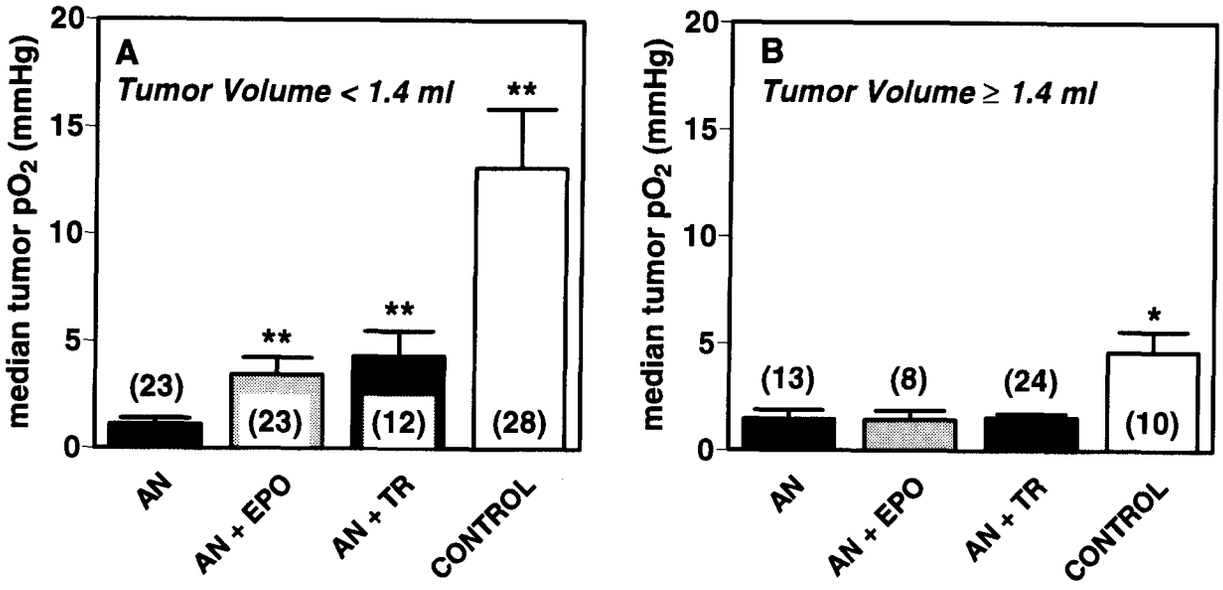

Fig. 3. Median pO_2 in tumors in anemic (AN), EPO-treated anemic (AN + EPO), TR-treated anemic (AN + TR) and control animals. Values indicate means ± SEM with the number of observations in brackets. *p < 0.01, **p < 0.001

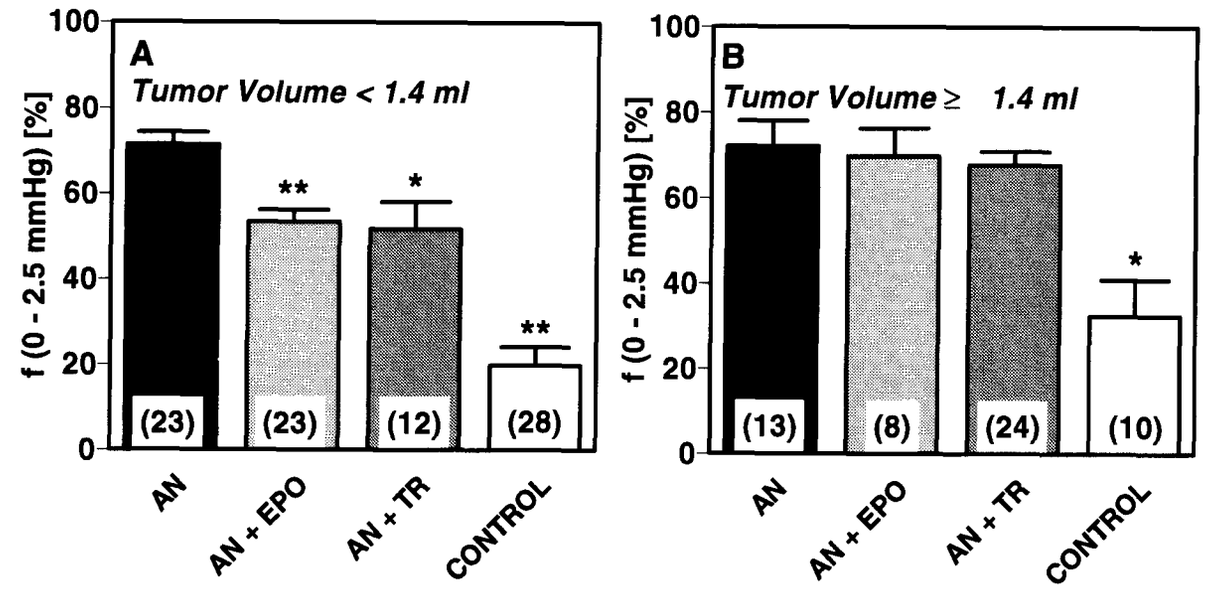

Fig. 4. Hypoxic fraction (0–2.5 mmHg) in tumors in anemic (AN), EPO-treated anemic (AN + EPO), TR-treated anemic (AN + TR) and control animals. Values indicate means ± SEM with the number of observations in brackets. *p < 0.05, **p < 0.01

in Fig. 2A and B. In the smaller tumor volume range (Fig. 2A), anemia resulted in a worsening of tumor oxygenation as compared to the oxygenation found in tumors of control, non-anemic animals. Correction of anemia with either EPO or TR was found to result in similar improvements in tumor oxygenation although full recovery to levels found in control animals was not achieved. In the larger tumors (Fig. 2B) anemia was again found to cause a worsening of tumor oxygenation although here the correction of anemia with either treatment modality brought about no significant improvement in tumor oxygenation. Similar effects could be seen if the median pO_2 was considered (Fig. 3A and B). Again, anemia resulted in a significant reduction in the median pO_2 in tumors of the smaller volume range. This reduction could be partially reversed by either EPO treatment or TR. In the larger tumors, the median pO_2 in control tumors was considerably lower than in the smaller tumors and was further decreased by anemia. Here, however, no increase in tumor median pO_2 could be achieved through anemia correction. When the hypoxic fraction

was considered (that is the fraction of pO_2 measurements ranging from 0 to 2.5 mmHg) a similar trend was again seen (Fig. 4A and B). Anemia led to a much larger hypoxic fraction which could be significantly decreased by EPO treatment or TR in the smaller tumors, although a return to control levels could not be achieved. In the larger tumors neither anemia correction method brought about significant reductions in the hypoxic fraction.

Discussion

Progressive anemia is a common clinical problem for cancer patients, with the response of tumors to standard radiotherapy in these patients being often less satisfactory than in subjects with normal hemoglobin levels (7). Several studies, in which this problem has been addressed, have shown increased radiosensitivity when radiation was given soon after blood transfusion (11–14). Studies on radiosensitivity following EPO treatment are, however, still scarce. In one study by Joiner et al. (15), EPO was found to have no influence on tumor radiosensitivity although only a mild anemia (hematocrit ~39%) at the time of radiotherapy was induced with this experimental model. The present study is, to our knowledge, the first in which a direct comparison of EPO treatment and TR has been carried out. The animal model used allowed the induction of a moderate tumor anemia (hematocrit ~29%) at the time of oxygenation measurements. This anemia could, in terms of both the hematocrit and hemoglobin content of blood, be corrected either chronically by the administration of EPO or acutely by TR.

Despite an improved release of oxygen to the tissue from erythrocytes in anemic animals (resulting from a right shift of the HbO_2 dissociation curve due to the anemia), the tumor oxygenation in these animals was significantly lower than in non-anemic animals, as indicated by significant decreases in the median pO_2 and increases in the hypoxic fraction. This is in agreement with the work of McCormack et al. (16) who suggested that tumors grown in anemic mice have a higher hypoxic fraction than those grown in control mice. Correction of anemia with either EPO treatment or TR resulted in an improvement in tumor oxygenation in smaller tumors suggesting that either treatment modality may sensitize tumors to standard radiotherapy and/or oxygen-dependent chemotherapy. In larger tumors, however, neither method of anemia correction resulted in improvements in tumor oxygenation.

Of special interest is the fact that in those tumors where improvement of tumor oxygenation occurred, a return to oxygenation levels found in size-matched tumors of non-anemic, control animals could not be achieved, despite the reestablishment of hematocrit levels. One factor which may play a role in this phenomenon is the slower growth rate of tumors in anemic animals. In the present study, in order to obtain comparable tumor volumes, tumors in control animals grew for 7 days, whereas those in anemic animals (with or without EPO treatment) needed 10 days in order to reach the target volume range. This phenomenon of slower tumor growth during anemia has previously been reported in mouse (16) and rat (17) experimental models.

By comparing EPO treatment and TR, a comparison of chronic and acute anemia correction has been possible in the present study. Hirst (13) discussed the possibility of tumor adaptation to the anemic state since he found that the reduction in the hypoxic cell fraction in tumors of anemic mice was only transient and indicated that the duration of any benefit presumably depends on how quickly a tumor adapts. In the present study, however, no tumor adaptation was evident since tumor oxygenation could be equally improved by either chronic or acute correction of anemia. These results would suggest that both forms of anemia correction would be equally effective in increasing tumor radiosensitivity in anemic tumor patients where the success of radiotherapy might be limited by tumor hypoxia. However, one factor which could however not be addressed in this study is a possible suppression of the immune system following TR which may compromise tumor cure (18, 19) and thus make EPO treatment more favorable for anemia correction in tumor patients.

ACKNOWLEDGEMENTS

The recombinant human erythropoietin used in this study was kindly donated by Boehringer-Mannheim. DS-sarcoma was provided by Dr. H. Löhrke from the German Cancer Research Center in Heidelberg. The authors wish to thank Simone Dahms-Prätorius and Anisa Kosan for technical assistance in carrying out this study. Financial support was granted by Stiftung Rheinland-Pfalz für Innovation (No. 836-386261/49).

REFERENCES

1. Vaupel P. Oxygenation of human tumors. Strahlenther Onkol 1990; 166: 377–86.
2. Vaupel P. Pathophysiological mechanisms of hyperthermia in cancer therapy. In: Gautherie M., ed. Biological basis of oncologic thermotherapy. Berlin, Heidelberg, New York: Springer, 1990: 73–134.
3. Vaupel PW. Oxygenation of solid tumors. In: Teicher BA, ed. Drug resistance in oncology. New York: Marcel Dekker, 1993: 53–85.
4. Bush RS. Current status of treatment of localized disease and future aspects. Int J Radiat Oncol Biol Phys 1984; 10: 1165–74.
5. Bush RS, Jenkin RDT, Allt WEC, et al. Definitive evidence for hypoxic cells influencing cure in cancer therapy. Br J Cancer 1978; 37 (Suppl. III): 302–6.
6. Dische S, Anderson PJ, Sealy R, Watson ER. Carcinoma of the cervix - Anaemia, radiotherapy and hyperbaric oxygen. Br J Radiol 1983; 56: 251–5.

7. Levine EA, Vijayakumar S. Blood transfusion in patients receiving radical radiotherapy: A reappraisal. Onkologie 1993; 16: 79–87.
8. Vaupel P, Otte J, Manz R. Oxygenation of malignant tumors after localized microwave hyperthermia. Radiat Environment Biophysics 1982; 20: 289–300.
9. Krüger W, Mayer W-K, Schaefer C, Stohrer M, Vaupel P. Acute changes of systemic parameters in tumour-bearing rats, and of tumour glucose, lactate and ATP levels upon local hyperthermia and/or hyperglycaemia. J Cancer Res Clin Oncol 1991; 117: 409–15.
10. Vaupel P, Schlenger K, Knoop C, Höckel M. Oxygenation of human tumors: Evaluation of tissue oxygen distribution in breast cancers by computerized HO_2 tension measurements. Cancer Res 1991; 51: 3316–22.
11. Hewitt HB, Blake E. Effect of induced host anaemia on the viability and radiosensitivity of murine malignant cells in vivo. Br J Cancer 1971; 25: 323–36.
12. Hill RP, Bush RS, Yeung P. The effect of anaemia on the fraction of hypoxic cells in an experimental tumour. Br J Radiol 1971; 44: 299–304.
13. Hirst DG. Anaemia: a problem or an opportunity in radiotherapy. Int J Radiat Oncol Biol Phys 1986; 12: 2009–17.
14. Hirst DG, Hazlehurst JL, Brown JM. The effect of alterations in haematocrit on tumour sensitivity to X-rays. Int J Radiat Biol 1984; 46: 345–54.
15. Joiner B, Hirst VK, McKeown SR, McAleer JJA, Hirst DG. The effect of recombinant human erythropoietin treatment on tumour radiosensitivity and cancer-associated anaemia in the mouse. Br J Cancer 1993; 68: 720–6.
16. McCormack M, Nias AHW, Smith E. Chronic anaemia, hyperbaric oxygen and tumor radiosensitivity. Br J Cancer 1990; 63: 752–9.
17. Kelleher DK, Baussmann E, Friedrich E, Vaupel P. The effect of erythropoietin on tumor oxygenation in normal and anemic rats. In: Vaupel P, Zander R, Bruley RF, eds. Oxygen transport to tissue XV. New York: Plenum Press, 1994: 517–24.
18. Blumberg N, Agrawal MM, Chuang C. Relation between recurrence of cancer of the colon and blood transfusion. Br Med J 1985; 290: 1037–9.
19. Burrows L, Tartter P. Effects of blood transfusions on colonic malignancy recurrence rate. Lancet 1982; 2: 662.