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Low hemoglobin levels: influence on tumor biology and radiotherapy treatment outcome

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Abstract

Anemia promotes intratumoral hypoxia, and in turn, hypoxia may adversely impact treatment outcomes by reducing the effectiveness of radiation therapy and by promoting molecular and cellular changes that favor malignant progression and formation of metastases. Recent experimental and clinical studies have been aimed at further exploring the mechanisms by which anemia and hypoxia exert their negative influence in order to identify the more effective interventions to improve clinical prognosis and outcomes. In a recent study of patients with squamous cell carcinoma of the oral cavity and oropharynx, pretreatment hemoglobin (Hb) level and epoetin alfa therapy were independent prognostic factors for response to radiochemotherapy and locoregional tumor control ($P < 0.01$). Patients with pretreatment Hb levels $\geqslant 14.5$ g/dl had significantly ($P \leqslant 0.001$–$P < 0.05$) higher complete response, 2-year locoregional control, and 2-year survival rates than patients with Hb levels < 14.5 g/dl who had not received epoetin alfa. Further, the response, locoregional control, and survival rates of epoetin alfa–treated patients with a pretreatment Hb < 14.5 g/dl were significantly higher than those of patients with pretreatment Hb levels < 14.5 g/dl not given epoetin alfa, and were equivalent to those of patients with a pretreatment Hb level $\geqslant 14.5$ g/dl. These observations and those of several other studies suggest that stabilization of normal Hb levels and correction of treatment-related anemia may contribute to improved therapeutic outcomes in cancer patients. Controlled prospective clinical trials in larger numbers of cancer patients are clearly warranted.

Keywords: Radiotherapy; Anemia; Intratumoral hypoxia; Epoetin alfa; Angiogenesis

1. Introduction

The presence of hypoxia in solid tumors is a clinical concern as it can negatively impact disease prognosis and therapeutic outcome in patients with these malignancies [1,2]. Notably, hypoxia can reduce the effectiveness of radiation therapy and some O_2-dependent cytotoxic agents. Additionally, hypoxia can promote changes in the genome and proteome of tumor cells that ultimately favor malignant progression and formation of metastases [1,3].

Clinically relevant hypoxia is detected in approximately 50% of all solid tumors irrespective of their size and histologic features. Tumor hypoxia results from an imbalance between cellular oxygen (O_2) consumption and O_2 supply to the cells [4]. The causes of tumor hypoxia are multifactorial and can include inadequate blood flow to the tumor due to structural and functional abnormalities of the tumor microvasculature (perfusion-limited or 'acute' hypoxia), or due to increased diffusion distances subsequent to tumor expansion, which lead to inadequate supply of O_2 and other nutrients for cells distant from the nutritive vessels (diffusion-limited or 'chronic' hypoxia). Tumor hypoxia can also be caused by decreased O_2 transport capacity of the blood as a result of anemia, a frequent complication in cancer patients, as well as methemoglobin formation or carbon monoxide poisoning [4].

2. Relationship of anemia to tumor hypoxia

Results of several experimental and clinical studies suggest a relationship between declining hemoglobin (Hb) levels and decreasing tumor oxygenation. In a study that used a rat model of tumor-associated anemia,

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tumor oxygenation was poorer in anemic animals than in normal animals, a condition that could be partially reversed by administration of recombinant human erythropoietin (rHuEPO, epoetin alfa) [5,6]. Also, in a study in mice with radiation-induced chronic renal failure, a strong correlation ($r = 0.57$; $P < 0.001$) was detected between increased hematocrit and increases in tumor oxygenation [7,8].

In the clinical setting, an association between oxygenation and Hb level seems probable, but is not yet completely clear, as variations have been found among different tumors [9]. A recent clinical study measured tissue oxygen pressure (pO_2) in squamous cell carcinomas and normal sternocleidomastoid muscles from 133 patients with head and neck cancer [10]. Patients were grouped according to whether they had normal Hb levels ($\geqslant 12$ g/dl in women and $\geqslant 13$ g/dl in men), mild anemia (Hb $\geqslant 11$ g/dl but < 12 or 13 g/dl), or severe anemia (Hb < 11 g/dl). In the normal tissue, the average pretreatment median pO_2 was 38 mm Hg (range 21–68 mm Hg) and decreasing Hb levels did not cause significant impairment of oxygenation in this tissue. In comparison, the tumors were more poorly oxygenated, having an average median pO_2 of 12 mm Hg (range 0–58 mm Hg). Although pO_2 in the tumors did not differ significantly between patients with mild anemia and nonanemic patients, tumor oxygenation in patients with moderate or severe anemia was significantly reduced relative to the other two patient groups ($P < 0.0001$) (Fig. 1), and nearly all tumors of patients with moderate or severe anemia were hypoxic. Multivariate analysis showed an Hb level < 11 g/dl to be the strongest predictor of tumor hypoxia. These findings suggested that tumors are less able than normal tissue to compensate for anemia, and are therefore more prone to be hypoxic. Also, a direct correlation has been found between Hb level and median pO_2 in breast cancer patients [11,12]. Correlations between Hb level and median pO_2 or the fraction of hypoxic pO_2 values have not generally been found in cancer of the uterine cervix [13,14]. However, a study has demonstrated significantly ($P = 0.05$) lower pO_2 values in cervical cancer patients with severe anemia (Hb $\leqslant 11$ g/dl) than in those without anemia (Hb > 14 g/dl) [15]. Further, in a study that examined radiation-induced changes in pO_2 in cervical cancer patients, a baseline Hb level < 13 g/dl, compared with a level $\geqslant 13$ g/dl, was associated with significantly poorer oxygenation (median pO_2 12.4 mm Hg versus 28.1 mm Hg; $P = 0.003$) and a significantly higher rate of treatment failure after 1 year (56% versus 22%; $P = 0.046$) [16]. Together, these findings clearly prove a relationship between lower Hb levels, increased hypoxia, and a negative impact of anemia/hypoxia on treatment outcome.

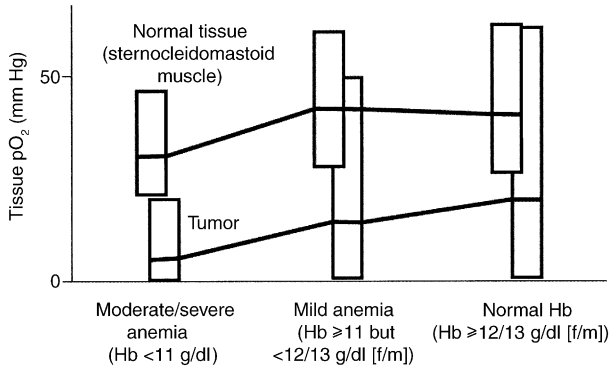

Fig. 1. Oxygenation of normal tissue (sternocleidomastoid muscle) or head and neck tumors. Patients were grouped according to their hemoglobin (Hb) levels. The histograms depict the range of pO_2 values in normal tissues and tumors, and the line shows the median values. Reprinted with permission [10].

3. Impact of hypoxia on tumor biology and treatment resistance

3.1. Impact on tumor biology

Hypoxia can influence tumor cells by acting as a stressor that impairs growth or causes cell death (apoptosis or necrosis). Or it can influence tumor cells by driving processes that ultimately further malignant progression and increased resistance of the tumor to radiation therapy and some forms of chemotherapy [9,17]. These effects variously reflect hypoxia-induced changes in the proteome and genome of the tumor cells.

Hypoxia-induced changes of the proteome may lead to growth impairment or death through molecular mechanisms that result in cellular quiescence, differentiation, and apoptosis [18]. Alternatively, hypoxia-induced proteome changes may activate anerobic metabolism, angiogenesis, change of cell contacts, and other processes that promote survival and propagation by enabling the tumor cells to adapt to or escape from their hostile environment. For example, hypoxia leads to upregulation of glycolytic enzymes and the glucose transporters GLUT1 and GLUT2; of growth factors including vascular endothelial growth factor (VEGF), erythropoietin and endothelial growth factor; and of enzymes and other proteins involved in tumor invasiveness, such as urokinase-type plasminogen activator. Additionally, hypoxia may lead to downregulation of cell-surface integrins, which facilitates tumor cell detachment.

Importantly, the phenotypic results of the hypoxia-induced proteome changes appear to be determined by the genome of the tumor cells, in addition to environmental factors [18]. The existence of a tumor cell population with genomic alterations (e.g. decreased

capacity for cell-cycle arrest, loss of apoptotic potential, increased angiogenic potential) that allow their survival in a hypoxic environment furthers tumor hypoxia. Sustained hypoxia, in turn, increases genomic instability, genomic heterogeneity, and selection for cell variants better adapted to a hypoxic environment, with eventual consequences for malignant progression and treatment resistance.

3.2. Genomic instability

As indicated above, hypoxia favors genomic instability. This is evidenced by the observation that hypoxia induces fragile chromosomal sites, leading to gene amplification and genome rearrangements [19]. Fragile sites have long been recognized as a chromosomal expression of genomic instability. Also, the impact of hypoxia on genetic stability was demonstrated in a classic study reported in 1996 [20]. In this study, cells from a tumorigenic cell line were grown concurrently either in culture or as tumors in nude mice. Oxygen tension measurements showed that all developing tumors in the mice contained numerous hypoxic areas. The frequency of mutation in the cells that arose within the mouse tumors was 5 times higher than that in the cultured cells ($P < 0.0001$). Distinctive mutation patterns were also observed, with tumor-grown cells displaying more deletions and transversions than the cells grown in culture. Of particular interest, exposure of the cultured cells to hypoxic conditions resulted in an increase in mutation frequency and a mutation pattern similar to that observed in the tumor-grown cells. These findings support the hypothesis that the genetic instability of malignant tumors may be due at least partially to specific mutagenic properties of the hypoxic environment [21].

3.3. Genome changes, clonal selection and malignant progression

The genome of tumor cells is less stable than that of normal cells, a condition reflected by a high rate of point mutations, gene amplifications, deletions, and chromosomal rearrangements [22]. Hypoxia, with or without reoxygenation, promotes genomic instability, and thus may increase the number of mutations (genetic variants).

Point mutations may be generated in tumor cells exposed to hypoxia and reoxygenation via several mechanisms including diminished DNA repair, errors in DNA replication, metabolic damage of DNA bases, or any combination of these factors [20,23]. Also, as demonstrated in the study of fragile sites [19], hypoxia followed by reoxygenation can lead to gene amplification. Both amplification and chromosomal rearrangements can be caused by DNA strand breakage or diminished repair of DNA strand breaks [22]. Hypoxia-induced genome changes may, in turn, promote metastatic disease by inactivating metastasis suppressor genes or enhancing expression of genes involved in the metastatic process, e.g. genes encoding for angiogenesis.

In addition to increasing the number of genetic variants, hypoxia concomitantly exerts a strong selection pressure on tumor cells [4,17,18,24]. Thus, any tumor cells with proteomic or genomic adaptations that enhance survival under hypoxic conditions (e.g. decreased apoptosis potential, increased angiogenesis potential) will have growth advantages over nonadapted cells. These adapted cells expand through clonal selection, i.e. their progeny proliferate at a rate exceeding that of the progeny of the non adapted cells, and eventually become the dominant tumor sub population. The adapted cells also tend to have favorable traits related to invasiveness, metastatic capability and aggressiveness which, in the clinical setting, manifest as increased local recurrence, metastasis and increased resistance to treatment. The expansion of cell clones with favorable adaptive changes can further aggravate tumor hypoxia, thereby establishing a vicious circle of hypoxia, malignant progression and treatment resistance that underlies advanced (and usually incurable) disease.

3.4. Resistance to radiation therapy

Hypoxia directly decreases the effectiveness of radiation therapy, presumably as a result of the low intratumoral concentrations of molecular O_2. The precise mechanism involved remains unclear. According to one hypothesis, the presence of O_2 increases the breakage of DNA strands via formation of O_2-derived free radicals, which occurs primarily after interaction of ionizing radiation with intracellular water [1,9,25,26]. Thus, in the absence of adequate O_2, radiation-induced DNA damage and subsequent cell death are less likely to occur. Alternatively, O_2 may 'fix' (make permanent or unrepairable) the DNA breakage caused by free radicals, again resulting in cell death. However, under hypoxic conditions, the damage can be repaired, thus negating the cytotoxic effects of radiation therapy.

The impact of hypoxia on the effectiveness of a cancer treatment is reflected by the treatment's oxygen enhancement ratio (OER). In 1951, Hollaender *et al.* reported that the radiation dose required to eradicate *Escherichia coli* under anoxic conditions was three times that required under normoxic conditions [27]. This finding led to the development of the OER for photons (X-rays), neutrons, and heavy ions, the OER being defined as the ratio of the radiation dose needed to achieve the same cell survival fraction under hypoxic conditions as under normoxic conditions [26]. Because of the oxygen enhancement effect, the oxygen enhancement ratio for photon therapy is about 3, indicating that

3-fold higher doses of radiation are needed to kill hypoxic cells compared with normoxic ones. However, even with the higher doses, some cells survive and can re-establish tumors at the site of the original malignancy or in distant areas [28]. With fractionated radiotherapy, the situation is more complex, since cells can be reoxygenated between treatments. With reoxygenation, radiosensitivity is restored, thereby increasing the responsiveness of the tumor to subsequent radiation therapy. However, reoxygenation does not occur consistently across all cells, and some cells may reoxygenate less rapidly and extensively. These cells are thus still relatively resistant to radiation, and form a pool of cells that continue to proliferate, thereby limiting the effectiveness of radiation therapy [28].

In addition to directly enhancing resistance to radiation therapy, hypoxia may indirectly accomplish this via proteome and genome changes, and clonal selection. For example, hypoxia-induced proteome changes may lead to elevated levels of DNA repair enzymes, while genome changes may result in loss of apoptosis potential and emergence of resistant clonal variants. Table 1 summarizes a number of direct and indirect effects of hypoxia that result in acquired treatment resistance to radiation therapy (a) or chemotherapy (b).

3.5. *Resistance to chemotherapy*

As in the case of radiation therapy, the effectiveness of some chemotherapeutic agents can be reduced directly by decreased generation of free radicals in a hypoxic cellular environment, which limits DNA damage by such agents (e.g. bleomycin and anthracyclines). Indirectly, poor and fluctuating blood flow due to structural or functional abnormalities of the tumor blood vessels, as well as the limited diffusion distance through tumor tissue, can result in reduced and erratic distribution of chemotherapeutic agents, with subsequently reduced effectiveness [29,30]. Also, some agents, e.g. cyclophosphamide, carboplatin and doxorubicin, have been shown to be oxygen-dependent under experimental conditions [28,31–33]. One proposed explanatory mechanism is increased production of nucleophilic substances such as glutathione, which can compete with the target DNA for alkylation (as reflected in the acquired resistance to alkylating agents); another is increased activity of DNA repair enzymes, which can diminish the efficacy of alkylating agents and platinum compounds (Table 1b) [9].

4. The role of VEGF in angiogenesis

Angiogenesis is a complex process in which new blood vessels develop from existing vasculature (neovascularisation), thereby providing a conduit for blood flow in expanding cell populations, including those of tumor tissue. Angiogenesis is a major prognostic factor for the promotion of malignant disease and the formation of metastases [34,35].

The angiogenic process is regulated by several cytokines. The most important of these is VEGF, and the most important stimulus for secretion of this factor is tumor hypoxia or ischemia. Elevated levels of VEGF appear to reflect increased angiogenic activity and are associated with poor prognosis in a variety of malignancies [36–38]. Nevertheless, the impact of anemia on increased levels of angiogenic cytokines in patients with metastatic disease has not been investigated.

In a recent study, serum levels of VEGF were determined in 56 patients with previously untreated, locoregionally confined gynecologic, head and neck, gastrointestinal, lung or prostate carcinomas [39]. VEGF levels were significantly higher in 26 patients with Hb levels <13 g/dl compared with the 30 patients with normal Hb levels $\geqslant 13$ g/dl (805 versus 438 pg/ml, $P=0.016$) (Fig. 2). All patients without anemia had VEGF levels within the normal range. Other variables, including patient age and tumor stage, size, site, grade and histology, were unrelated to VEGF levels. These findings are consistent with the hypothesis that low Hb levels promote intratumoral hypoxia, which in turn triggers the release of VEGF. This hypothesis infers that anemia may have the potential to stimulate angiogenesis by causing intratumoral hypoxia; however, further investigation is needed to determine if this hypothesis has any therapeutic significance.

5. Improving prognosis by treating anemia

The prognostic significance of anemia in patients with solid tumors is illustrated by the results of a recent retrospective study conducted at seven Canadian institutions [40]. The outcomes of 605 patients with

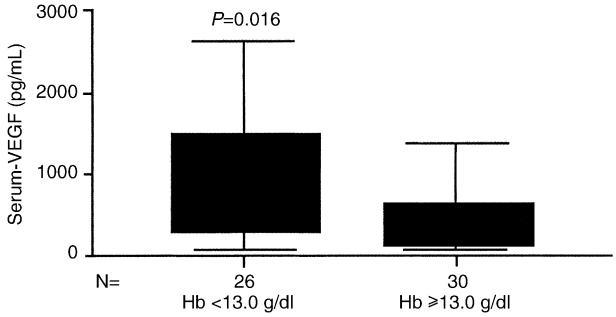

Fig. 2. Serum levels of VEGF in 56 consecutive patients with previously untreated, locoregionally confined gynecologic, head and neck, gastrointestinal, lung, or prostate carcinomas. Patients are grouped according to their hemoglobin (Hb) levels. VEGF, vascular endothelial growth factor. Reprinted with permission [39].

Table 1
Selected mechanisms for hypoxia-related tumor resistance to (a) radiotherapy (adapted from Refs. [4,9,29] and (b) chemotherapy (adapted from Refs. [4,9,28,29])

(a) Radiotherapy
Direct effects
- Through reduced 'fixation' of DNA damage (X- and γ-rays)

Indirect effects (related to proteome changes[a])
- Slowing of proliferation kinetics
- G_1-/S-phase arrest; increase in number of cells in G_0 phase
- Elevated levels of glutathione and related thiols
- Elevated levels of DNA repair enzymes and of resistance-related proteins

Indirect effects (related to genome changes[b] and clonal selection)
- Loss of apoptosis and differentiation
- Clonal heterogeneity
- Proliferation of resistant clonal variants
- Increase in number of cells with aggressive phenotype

Secondary indirect effects (related to intensified glycolysis and extracellular acidosis)
- Cell-cycle effects
- Activation of repair processes

(b) Chemotherapy
Direct effects
- Through decreased generation of free radicals, and thus, less DNA damage (bleomycin, anthracyclines)

Indirect effects (related to proteome changes)
- Slowing of proliferation kinetics; increase in number of cells in G_0 phase
- G_1-/S-phase arrest (e.g. vinca alkaloids, methotrexate)
- Elevated levels of glutathione and DNA repair enzymes (alkylating agents, bleomycin, platinum compounds)

Indirect effects (related to genome changes and clonal selection)
- Loss of apoptosis and differentiation
- Clonal heterogeneity
- Proliferation of resistant clonal variants
- Increase in number of cells with aggressive phenotype

Secondary indirect effects (related to intensified glycolysis with extracellular acidosis)
- Transport of drugs across the cell membrane
- Intracellular drug accumulation
  - Weak acids ↑ (e.g. melphalan, mitomycin C)
  - Weak bases ↓ (e.g. anthracyclines, bleomycin)
- Drug activity
  - ↑ Cyclophosphamide, cisplatin, melphalan
  - ↓ Vinblastine, doxorubicin, bleomycin
- Activation of prodrugs

Secondary indirect effects (related to chaotic angiogenesis and impact of microcirculation on intratumor pharmacokinetics)
- Impaired and uneven drug delivery
- Arteriovenous shunt perfusion
- Large diffusion distances

[a] Resulting from modulation of gene expression, posttranscriptional effects, and/or posttranslational effects.
[b] Through point mutations, gene amplification, and/or chromosomal rearrangements.

cervical cancer who received radical radiation therapy at doses $\geqslant 35$ Gy were reviewed. Patients receiving definitive surgery, neoadjuvant chemotherapy, or epoetin alfa were excluded. Patients were grouped according to whether they had low (<12 g/dl) or normal ($\geqslant 12$ g/dl) Hb levels at the beginning and end of radiation therapy. Patients with normal Hb levels at both determinations had a 5-year survival rate of 74%, whereas those with low Hb levels at both had a survival rate of 51% ($P < 0.002$). A group of 25 patients with low Hb at the start of radiation therapy received transfusions to correct their anemia and had normal Hb levels by the end of therapy. In this small group, the 5-year survival rate was 70%, which did not differ significantly from that of patients who started and remained at normal Hb levels. In the subgroup of 82 patients who presented with normal Hb levels ($\geqslant 12$ g/dl) but whose levels fell below 12 g/dl during radiation therapy, the 5-year survival rate was significantly reduced to 50% ($P = 0.0118$). Overall, the difference in survival between patients with low and normal Hb levels at the end of radiation therapy remained significant even after adjustment for other

prognostic factors, such as tumor stage and histology ($P < 0.0002$). The overall tumor recurrence rate was significantly lower for patients whose Hb levels remained within the normal range during radiation therapy compared with those whose levels fell below 12 g/dl (33% versus 58%, $P = 0.001$). These patients also had significantly lower pelvic failure rates ($P < 0.0001$). This finding is consistent with the impact of anemia on tumor hypoxia, inasmuch as these patients had received radiation as a local treatment. Patients with normal Hb levels ($\geqslant 12$ g/dl) additionally had significantly lower rates of distant metastases ($P < 0.0006$), a finding that was independent of the pelvic failure rate.

In another retrospective study, which included 847 patients with squamous cell carcinoma of the supraglottic larynx, Hb levels after radiotherapy were at least as important as overall treatment time as a prognostic factor for outcome [41]. After a minimum follow-up of 3 years, Hb levels were strongly correlated with the probability of local tumor control. Hemoglobin levels at the start of radiotherapy did not correlate with treatment outcome, but any decrease in Hb levels during therapy was a strong prognostic factor for treatment failure.

Although these studies were not designed prospectively, the results suggest that Hb levels during and at the end of radiation therapy have prognostic significance with respect to tumor recurrence and survival. Even if the benefit of treating anemia were actually only half as large as suggested by the retrospective analyses of patients with cervical cancer, it would still be greater than the impact of adjuvant chemotherapy on survival of breast cancer patients, which is well recognized as a standard of care.

6. Effect of epoetin alfa on treatment outcomes

The optimal strategy for treating anemia as a means to improve outcomes after radiation therapy or radiochemotherapy remains to be defined in controlled clinical trials. Epoetin alfa is a recombinant human glycoprotein with an amino acid sequence identical to isolated natural erythropoietin [42]. In cancer patients with anemia, epoetin alfa is effective in reducing transfusion requirements, maintaining adequate Hb levels, managing fatigue, and improving overall quality of life [43–47].

The influence of epoetin alfa therapy and Hb level on therapeutic response, locoregional tumor control, and overall survival was retrospectively assessed in 191 patients who received neoadjuvant chemoradiotherapy and surgery for squamous cell carcinoma of the oral cavity and oropharynx at the General Hospital Vienna, Austria, between November 1989 and October 1998 [42]. The patients were treated with mitomycin C (15 mg/m^2 on day 1), 5-fluorouracil (750 mg/m^2/day, days 1–5), and radiotherapy (50 Gy in 25 fractions during weeks 1–5), followed by resection of the primary tumor and regional cervical lymphatics at week 10 or 11. Beginning in May 1996, patients with a low Hb level before or during chemotherapy received epoetin alfa 10 000 IU/kg subcutaneously three times a week until the week of surgery. The frequency of epoetin alfa administration was increased to six times weekly in patients whose Hb did not rise by at least 0.3 g/dl during the first treatment week. For data analysis, the patients were divided into four groups according to whether their pretreatment Hb level was < 14.5 or $\geqslant 14.5$ g/dl and whether or not they had received epoetin alfa during neoadjuvant radiochemotherapy. Results of multivariate analysis indicated that the use of epoetin alfa and pretreatment Hb level were independent prognostic factors for response to chemoradiotherapy and locoregional tumor control ($P < 0.01$). Patients with pretreatment Hb levels $\geqslant 14.5$ g/dl had significantly ($P \leqslant 0.001$–$P < 0.05$) higher complete response, 2-year locoregional control, and 2-year survival rates than patients with Hb levels < 14.5 g/dl who had not received epoetin alfa. Moreover, epoetin alfa-treated patients with a pretreatment Hb level < 14.5 g/dl had response, locoregional control, and survival rates that were significantly ($P \leqslant 0.001$) higher than those for patients with pretreatment Hb levels < 14.5 g/dl not given epoetin alfa and that were equivalent to those of patients with a pretreatment Hb level > 14.5 g/dl ($P \geqslant 0.30$) (Table 2, Fig. 3). These findings confirmed those of other studies that suggested that low Hb levels are a negative prognostic factor for response to cancer therapy. These findings also demonstrated that the negative influence of low Hb levels can be reversed by epoetin alfa administration during neoadjuvant chemoradiotherapy.

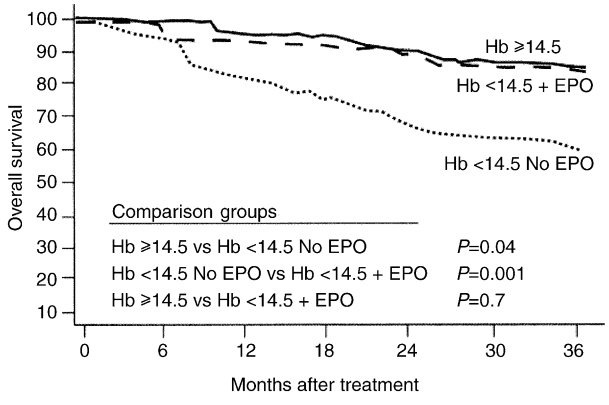

Fig. 3. Overall survival of patients with oral squamous cell carcinoma following radiochemotherapy. In this retrospective study, complete response, survival, and locoregional control were compared between 4 groups of patients categorized by Hb level < 14.5 g/dl or $\geqslant 14.5$ g/dl, and whether or not they had received epoetin alfa therapy. Reprinted with permission [47].

Table 2
Complete response, 2-year actuarial locoregional tumor control, and 2-year actuarial overall survival rates by pretreatment hemoglobin level and epoetin alfa therapy

Outcome	Group 1 Pretx Hb $\geqslant$14.5 g/dl No epoetin alfa	Group 2 Pretx Hb $<$14.5 g/dl No epoetin alfa	Group 3 Pretx Hb $<$14.5 g/dl Epoetin alfa	Group 4 Pretx Hb $\geqslant$14.5 g/dl Epoetin alfa
Complete response	28/43 (65%)*	15/87 (17%)	35/57 (61%)*	3/4 (75%)
2-year locoregional control	38/43 (88%)**	63/87 (72%)	54/57 (95%)*	4/4 (100%)
2-year survival	35/43 (81%)**	52/87 (60%)	50/57 (88%)*	2/4 (50%)

Pretx Hb, pretreatment haemoglobin level.*$P \leqslant 0.001$ versus Group 2; **$P < 0.05$ versus Group 2 [48].

The effect of epoetin alfa on treatment outcomes was also evaluated in a Greek study that included 385 patients with pelvic malignancies who were undergoing definitive radiation therapy [48,49]. Patients were randomly assigned to receive epoetin alfa subcutaneously five times per week with iron supplements or iron supplements alone (control group). Patients received radiation therapy at a dose of 2 Gy daily, with the total dose determined by the site and stage of disease. At baseline, the two treatment arms were well matched with respect to age, Hb levels, and site and stage of disease. The group that received epoetin alfa had significantly higher mean Hb levels during radiation therapy compared with the control group (12.9±2.6 g/dl versus 10.6±2.5 g/dl; $P = 0.0001$). The 4-year disease-free survival rate was 85.3% in patients treated with epoetin alfa versus 67.2% in control patients ($P = 0.0008$) [49].

7. Conclusion

It is well recognized that both anemia and tumor hypoxia have prognostic significance in patients with solid tumors and that hypoxia is an important stimulus for angiogenesis. Results of recent studies suggest that anemia promotes tumor hypoxia, partly explaining the unfavorable prognostic significance of anemia. Moreover, anemia was associated with increased serum VEGF levels, suggesting that it may also contribute to angiogenesis. In patients receiving radiation therapy or radiochemotherapy, Hb level at the end of treatment had prognostic significance in terms of survival and tumor recurrence. The Hb level at the end of treatment appears to be at least as important as the pretreatment level, if not more important, in predicting outcome. Accordingly, stabilization of normal Hb levels and prevention of treatment-related anemia is likely to be desirable. The results of preliminary studies show that epoetin alfa was effective in increasing Hb levels in patients receiving radiation therapy or radiochemotherapy. However, more importantly, these studies suggest that treatment of anemia with epoetin alfa may increase local disease control and improve overall survival.

Controlled clinical trials involving larger numbers of cancer patients are clearly warranted. The strategy used in these studies should be to assess the value of maintaining Hb levels within a normal range during radiation therapy and radiochemotherapy and of achieving a normal range by the end of such treatment. Control of anemia should be achieved with epoetin alfa but, if this goal is not reached, then additional transfusions should be considered. On the basis of available evidence, it is likely that most patients presenting with normal Hb levels or mild-to-moderate anemia will respond to epoetin alfa and will not require transfusions. However, it is still unclear whether patients presenting with severe anemia will have sufficient increases in Hb over the 6- to 8-week course of radiation therapy to reach normal Hb levels by the end of treatment.

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