Improved Recurrence-Free Survival with ARCON for Anemic Patients with Laryngeal Cancer

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Abstract

Purpose: Anemia is associated with poor tumor control. It was previously observed that accelerated radiotherapy combined with carbogen breathing and nicotinamide (ARCON) can correct this adverse outcome in patients with head and neck cancer. The purpose of this study was to validate this observation based on data from a randomized trial.

Experimental Design: Of 345 patients with cT2-4 laryngeal cancer, 174 were randomly assigned to accelerated radiotherapy and 171 to ARCON. Hemoglobin levels, measured before treatment, were defined as low when <7.5 mmol/L for women and <8.5 mmol/L for men. The hypoxia marker pimonidazole was used to assess the oxygenation status in tumor biopsies. Data were analyzed 2 years after inclusion of the last patient.

Results: Pretreatment hemoglobin levels were available and below normal in 27 of 173 (16%) accelerated radiotherapy and 27 of 167 (16%) ARCON patients. In patients with normal pretreatment, hemoglobin levels treatment with ARCON had no significant effect on 5-year loco-regional control (LRC, 79% versus 75%; P = 0.44) and disease-free survival (DFS, 75% vs. 70%; P = 0.46) compared with accelerated radiotherapy. However, in patients with low pretreatment, hemoglobin levels ARCON significantly improved 5-year LRC (79% vs. 53%; P = 0.03) and DFS (68% vs. 45%; P = 0.04). In multivariate analysis including other prognostic factors, pretreatment hemoglobin remained prognostic for LRC and DFS in the accelerated radiotherapy treatment arm. No correlation between pretreatment hemoglobin levels and pimonidazole uptake was observed.

Conclusion: Results from the randomized phase III trial support previous observations that ARCON has the potential to correct the poor outcome of cancer patients with anemia (ClinicalTrials.gov number, NCT00147732).

Introduction

Up to 40% of patients with solid tumors undergoing radiotherapy have anemia at presentation (1). A wealth of data indicates that low pretreatment hemoglobin levels are a strong prognostic indicator of poor disease control and survival (2–4). To overcome the adverse impact of low pretreatment hemoglobin levels, correction strategies have been applied, such as administering erythropoietin and packed cell transfusions. However, randomized trials failed to demonstrate the effectiveness of these approaches in patients with head and neck or breast cancer, and erythropoietin was even counterproductive in some studies (3, 5–7).

Disappearance of the adverse impact of anemia was observed in a nonrandomized phase II trial when accelerated radiotherapy was combined with carbogen breathing and nicotinamide (ARCON; ref. 8). This strategy counteracts tumor cell repopulation and hypoxic radioresistance (9). Carbogen breathing (98% O2 + 2% CO2) is used to...
Anemia is a strong prognostic indicator of poor disease control and survival in patients presenting with head and neck cancer. Randomized trials failed to demonstrate outcome improvement with transfusions, and erythropoietin was even counterproductive. In contrast, elimination of the adverse impact of anemia was observed in patients with head and neck cancer participating in a nonrandomized phase II trial when accelerated radiotherapy was combined with carbogen breathing and nicotinamide (ARCON), a hypoxia-modifying regimen. Data from the current phase III trial demonstrate that the poor recurrence-free survival, observed in patients with locally advanced laryngeal cancers presenting with anemia and treated with accelerated radiotherapy, can be corrected by an ARCON regimen. Shorter oxygen diffusion distances in the tumor, higher levels of free oxygen in plasma, and reduced blood viscosity can explain the effect of ARCON in patients with anemia. The potential of ARCON should be further explored in a prospective randomized trial with primary focus on patients with anemia.

**Translational Relevance**

Anemia is a strong prognostic indicator of poor disease control and survival in patients presenting with head and neck cancer. Randomized trials failed to demonstrate outcome improvement with transfusions, and erythropoietin was even counterproductive. In contrast, elimination of the adverse impact of anemia was observed in patients with head and neck cancer participating in a nonrandomized phase II trial when accelerated radiotherapy was combined with carbogen breathing and nicotinamide (ARCON), a hypoxia-modifying regimen. Data from the current phase III trial demonstrate that the poor recurrence-free survival, observed in patients with locally advanced laryngeal cancers presenting with anemia and treated with accelerated radiotherapy, can be corrected by an ARCON regimen. Shorter oxygen diffusion distances in the tumor, higher levels of free oxygen in plasma, and reduced blood viscosity can explain the effect of ARCON in patients with anemia. The potential of ARCON should be further explored in a prospective randomized trial with primary focus on patients with anemia.

Decrease diffusion-limited hypoxia whereas nicotinamide, a vasoactive agent, is added to limit hypoxia caused by intermittent reduction of blood flow (9). The same regimen has subsequently been studied in a randomized phase III trial, comparing ARCON for locally advanced laryngeal cancers. Although local control is not improved, it was demonstrated that ARCON results in a benefit of 8% in regional control and excellent larynx preservation rates without increase of toxicity (10, 11). Translational research using the hypoxia marker pimonidazole demonstrated that proper patient selection based on tumor biology is the key to the success of this approach.

Based on the results of the preceding phase II study the hypothesis was generated that ARCON can abolish the adverse impact of anemia (8). The purpose of this study is to find support for this observation in the data from the randomized phase III trial. This article reports on the impact of ARCON on the prognosis of a cohort of patients with laryngeal cancer presenting with pretreatment anemia.

**Materials and Methods**

**Study design and eligibility**

This was an open-label, randomized phase III trial comparing accelerated radiotherapy with ARCON in patients with cT2-4 laryngeal cancer. The trial (ClinicalTrials.gov NCT00147732) was conducted under the auspices of the Dutch Head and Neck Cancer Group in 7 centers in the Netherlands and in the United Kingdom (Supplementary Table S1). Eligibility criteria are provided in the Supplementary Table S2.

Approval for the study was obtained from the Radboud University Nijmegen Medical Centre Research Ethics Committee with ratification from each center. Written informed consent was obtained before randomization.

**Randomization**

Patients were centrally randomized by phone at the Integraal Kankercentrum Oost trials office. Treatment arm assignments (accelerated radiotherapy vs. ARCON) were stratified for tumor site (glottic vs. supraglottic) and institution. A dynamic allocation method was used to avoid imbalance of treatment assignment within an institution. Randomization took place after all study investigations and no longer than 4 weeks before the anticipated start of treatment.

**Procedure**

A radiation dose of 44 Gy in 22 daily fractions of 2 Gy was prescribed to the primary tumor and neck nodes followed by a boost dose of 24 Gy in twice daily fractions of 2 Gy to the primary tumor and involved lymph nodes. Because a decrease in the radiation tolerance was observed for cartilage and spinal cord in earlier studies with hypoxic sensitization, the total dose to the arytenoid cartilage and the spinal cord in the ARCON arm was limited to 64 and 40 Gy, respectively (12, 13).

Patients allocated to the ARCON arm received carbogen (98% O2 + 2% CO2, 4 minutes before and during radiotherapy) and nicotinamide (60 mg/kg, 1–1.5 hours before fractions) concurrently with radiotherapy. Details of the procedure are described previously (10, 11).

All patients participating in this study were treated in academic hospitals with accreditation in head and neck oncology by the Dutch Cooperative Head and Neck Oncology Group and institution-wide quality assurance programs.

**Hypoxia marker analysis**

After additional informed consent, patients received pimonidazole (Hypoxprobe-1; Natural Pharmacia International) intravenously (500 mg/m²) 2 hours before biopsy taking. Biopsies were snap frozen in liquid nitrogen, immunohistochemically stained, and semi-automatically analyzed (14). Of each biopsy, one complete section was analyzed for the hypoxic fraction, that is, the tumor area positive for pimonidazole relative to the total tumor area.

**Monitoring during treatment and follow-up evaluations**

Before and weekly during treatment hemoglobin, hematocrit, creatinine, and urea levels were obtained. Normal hemoglobin levels were defined as 7.5 to 10 mmol/L for women and 8.5 to 11 mmol/L for men (women, 12–16 g/dL; men, 13.6–17.7 g/dL). Follow-up visits took place every 2, 3, and 4 months during the first, second, and third year, respectively, then every 6 months for another 2 years. The larynx was assessed by fiberoptic or indirect laryngoscopy. Regional control was assessed by palpation of the neck. When tumor recurrence was suspected, imaging (computed tomography scan or MRI) was performed to document the
extent of the disease and biopsies were taken for pathologic confirmation.

Endpoints and statistics
Survival endpoints used were loco-regional control (LRC), metastasis-free survival (MFS), disease-free survival (DFS), and overall survival (OS) at 5 years from randomization. All intervals were calculated from the date of randomization and censored after 60 months or at last follow-up. LRC was defined as freedom of first recurrence at the primary tumor site and complete and persistent disappearance of the pathologic lymph nodes after radiotherapy, not including salvage procedures. MFS was defined as the time from randomization to distant metastasis. DFS was defined as the time to local or regional recurrence, or distant metastasis. OS was defined as time to death.

The primary endpoint of the randomized trial was local control. In the accelerated radiotherapy arm, a local control rate of 60% at 2 years after completion of radiotherapy was expected. An improvement by 15%, resulting in a local control rate of 75% at 2 years, was assumed for the ARCON arm. To detect this difference of 15% with a significance level of 0.05 and a power of 0.80 (2-sided log-rank test), 156 patients were required in each treatment group. To account for a drop out percentage of 10%, an additional 16 patients were added for each group.

Statistical analyses were performed using SPSS 19.0.0. Mann–Whitney U and χ² tests were used at a 2-sided significance level of 0.05. Endpoints were evaluated by the Kaplan–Meier method based on intent-to-treat policy and compared with log-rank testing. A multivariate Cox proportional hazards analysis, with stepwise backward elimination of variables at P > 0.1, was used for both patient groups and included N-classification (N+ vs. N0), performance status (0 vs. 1), and treatment arm (ARCON vs. accelerated radiotherapy). An interaction between treatment arm and pretreatment hemoglobin level was assessed using Cox regression.

Role of the funding source
The study sponsors had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

Results
Patient characteristics and protocol compliance
Between April 2001 and February 2008, 345 patients were randomized to either accelerated radiotherapy (n = 174) or ARCON (n = 171; Fig. 1). The median follow-up time was 44 (range 18–103), 55, and 60 months for the whole group and for patients still alive receiving accelerated radiotherapy and ARCON, respectively. Patient demographics and clinical tumor characteristics were well balanced without significant differences within the whole study population and the group of patients presenting with anemia (Table 1). Patients presenting with anemia were older (P = 0.01), had a poorer performance status (P < 0.01), more N2 stages (P < 0.01), and higher stage grouping (P = 0.02) compared with the whole group of patients. Compliance to radiotherapy, carbogen breathing, and nicotinamide intake was high and comparable between both groups. Detailed information is listed in the Supplementary Table S3.

Figure 1. CONSORT diagram.
Pretreatment hemoglobin levels were available and below normal in 27 of 173 (16%) accelerated radiotherapy and 27 of 167 (16%) ARCON patients (Table 1). Four of them (accelerated radiotherapy, 3; ARCON, 1) received a transfusion, given as 2 units of packed red blood cells. A correlation was observed between low hemoglobin at presentation and lower performance status ($P < 0.01$), and a trend was observed between low hemoglobin and higher N-status ($P = 0.06$).

### LRC and survival

In patients with normal pretreatment hemoglobin levels treatment with ARCON had no significant effect on 5-year LRC (79% vs. 75%, $P = 0.44$) and DFS (75% vs. 70%, $P = 0.46$) compared with accelerated radiotherapy. However, in
patients with low pretreatment hemoglobin levels ARCON treatment significantly improved 5-year LRC (79% vs. 53%, $P = 0.03$) and DFS (68% vs. 45%, $P = 0.04$; Fig. 2A and B). No significant benefit of ARCON was observed for MFS (normal hemoglobin: ARCON vs. accelerated radiotherapy: 91% vs. 89%, $P = 0.56$; low hemoglobin: ARCON vs. accelerated radiotherapy: 87% vs. 69%, $P = 0.10$; Fig. 3A). Patients presenting with low hemoglobin levels had a worse 5-year OS regardless of the treatment regimen (normal hemoglobin: ARCON vs. accelerated radiotherapy: 65% vs. 65%, $P = 0.93$; low hemoglobin: ARCON vs. accelerated radiotherapy: 46% vs. 28%, $P = 0.97$; Fig. 3B).

**Prognostic factors for tumor control**

The impact of various common prognostic factors and hemoglobin levels on LRC, MFS, DFS, and OS is summarized in Table 2. On multivariate analysis after correction for N-stage and hemoglobin level, ARCON treatment remained an independent prognostic factor for LRC ($P = 0.04$) and DFS ($P = 0.09$) in patients with anemia only. A significant interaction between treatment effect and hemoglobin level was found for LRC ($P = 0.02$) and DFS ($P = 0.05$).

**Hypoxia marker study**

Tumor biopsies of 79 patients were available for pimonidazole staining. Characteristics were well balanced between patients receiving accelerated radiotherapy and ARCON (Table 3). However, the group of patients participating in the hypoxia marker study differed in some aspects from the entire study population: patients involved were more frequently female ($P < 0.01$) and presented with higher tumor stage ($P < 0.01$).

The hypoxic fraction, as defined by pimonidazole staining, varied from 0% to 19.4% with a median value of 1.6%. Eleven of the 79 patients had hemoglobin levels below normal at diagnosis. No correlation was observed between pretreatment hemoglobin levels and hypoxic fraction ($P = 0.11$).

**Discussion**

It has been demonstrated by numerous reports that anemia in patients with cancer of the cervix, head and neck, bladder, breast, and lung is associated with poor outcome (2–4). This is also found in the current study for patients with T2-4 laryngeal cancer treated with radiotherapy alone. However, this impaired outcome in patients with anemia is no longer observed when carbogen and nicotinamide are added to radiotherapy, supporting the results of a previous phase II ARCON trial in head and neck cancer (8).

Attempts have been made to improve outcome of cancer patients with anemia using erythropoietin or red blood cell transfusions. A Cochrane review based on 5 randomized
controlled trials found strong indications that for patients with head and neck cancer, the addition of erythropoietin to radiotherapy negatively affects patient outcome in terms of loco-regional progression-free survival and OS (7). Suggested explanations for the lack of benefit of erythropoietin include presence of erythropoietin receptors on the tumor cell membranes stimulating tumor growth, and a decrease of tissue oxygenation because of increased viscosity when hemoglobin concentrations become too high (15–17).

Patients with head and neck cancer with low hemoglobin levels treated in the DAHANCA 5 (radiotherapy and nimorazole vs. radiotherapy and placebo) and DAHANCA 7 (conventional radiotherapy vs. accelerated radiotherapy) studies were subrandomized to receive red blood cell transfusions before and during radiotherapy (3). Also with this approach, the increased hemoglobin level was not able to improve tumor control or survival. Stimulation of inflammatory and immunosuppressive pathways was proposed as a possible factor involved (18). Randomized trials exploring the role of chemotherapy in addition to radiotherapy for patients presenting with anemia are lacking. However, prospective and retrospective studies indicate that the poor prognosis of patients presenting with anemia cannot be improved by a chemoradiotherapy approach (19, 20).

The “reduced cord radius” model described by Hirst and colleagues could explain the success of ARCON in patients with anemia (21). The term “tumor cord” describes the functional unit of a blood vessel and its dependent tumor cell volume*. The model assumes that the ability of cancer cells to survive at a distance from blood vessels is dependent on the local supply and diffusion distance of oxygen and nutrients from each vessel. Histologic examination of tumors in animals exposed to low oxygen tension for several days has shown that the thickness of the tumor cords is less than in animals breathing normal air (22). It has been proposed that anemia causes a reduced cord radius by the same mechanism. Restoration of hemoglobin levels by blood transfusion in animals with anemia produced a markedly increased tumor radiosensitivity supposedly by improved oxygenation of tumor cells in the peripheral zones of the cords. However, this effect was only transient and was lost within 24 hours (23). Tumor cords that are chronically exposed to higher oxygen levels, will adapt and begin to proliferate more actively and will once again outgrow their oxygen supply. In contrast, daily carbogen

*Although the typical corded structure is histologically recognizable in some tumors and tumor types, the cord radius model has a broader application. "Cord radius" is the equivalence of the more generally applicable and easier to recognize "distance from blood vessel to necrosis". However, for readability we use the term "tumor cord" here.
### Table 2. Univariate and multivariate analysis per hemoglobin levels

<table>
<thead>
<tr>
<th></th>
<th>Normal hemoglobin (N = 286)</th>
<th>Low hemoglobin (N = 54)</th>
<th>Normal hemoglobin (N = 286)</th>
<th>Low hemoglobin (N = 54)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>P</td>
<td>HR (95% CI)</td>
<td>P</td>
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<tr>
<td><strong>Univariate parameter</strong></td>
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<td></td>
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<td></td>
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<tr>
<td>Age</td>
<td>&gt;60 vs. ≤60</td>
<td>0.94 (0.57–1.55)</td>
<td>0.81</td>
<td>0.47 (0.18–1.22)</td>
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<tr>
<td>Sex</td>
<td>Female vs. male</td>
<td>0.30 (0.12–0.76)</td>
<td>0.01</td>
<td>1.31 (0.30–5.77)</td>
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<td>Site</td>
<td>Supraglottic vs. glottic</td>
<td>0.81 (0.49–1.33)</td>
<td>0.40</td>
<td>1.45 (0.51–4.13)</td>
</tr>
<tr>
<td>T-classification</td>
<td>T2 vs. T3-4</td>
<td>0.85 (0.52–1.42)</td>
<td>0.54</td>
<td>0.99 (0.37–2.67)</td>
</tr>
<tr>
<td>N-classification</td>
<td>N+ vs. N0</td>
<td>1.85 (1.12–3.06)</td>
<td>0.02</td>
<td>2.91 (1.10–7.68)</td>
</tr>
<tr>
<td>Performance status</td>
<td>0 vs. 1</td>
<td>0.58 (0.25–1.34)</td>
<td>0.20</td>
<td>1.68 (0.65–4.35)</td>
</tr>
<tr>
<td>Treatment</td>
<td>ARCON vs. accelerated radiotherapy</td>
<td>0.82 (0.49–1.33)</td>
<td>0.43</td>
<td>0.31 (0.11–0.89)</td>
</tr>
<tr>
<td><strong>Multivariate analysis</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-classification</td>
<td>N+ vs. N0</td>
<td>1.86 (1.12–3.06)</td>
<td>0.02</td>
<td>3.17 (1.19–8.42)</td>
</tr>
<tr>
<td>Performance status</td>
<td>0 vs. 1</td>
<td>0.60 (0.26–1.39)</td>
<td>0.23</td>
<td>1.91 (0.72–5.06)</td>
</tr>
<tr>
<td>Treatment</td>
<td>ARCON vs. accelerated radiotherapy</td>
<td>0.80 (0.48–1.33)</td>
<td>0.39</td>
<td>0.33 (0.11–0.93)</td>
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<td><strong>Univariate parameter</strong></td>
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<td></td>
</tr>
<tr>
<td>Age</td>
<td>&gt;60 vs. ≤60</td>
<td>0.63 (0.28–1.44)</td>
<td>0.28</td>
<td>0.61 (0.17–2.16)</td>
</tr>
<tr>
<td>Sex</td>
<td>Female vs. male</td>
<td>0.70 (0.24–2.04)</td>
<td>0.51</td>
<td>1.07 (0.14–8.50)</td>
</tr>
<tr>
<td>Site</td>
<td>Supraglottic vs. glottic</td>
<td>1.95 (0.81–4.97)</td>
<td>0.14</td>
<td>1.26 (0.32–4.85)</td>
</tr>
<tr>
<td>T-classification</td>
<td>T2 vs. T3-4</td>
<td>1.47 (0.61–3.54)</td>
<td>0.40</td>
<td>0.86 (0.24–3.07)</td>
</tr>
<tr>
<td>N-classification</td>
<td>N+ vs. N0</td>
<td>4.80 (2.06–11.22)</td>
<td>&lt;0.01</td>
<td>2.82 (0.79–10.06)</td>
</tr>
<tr>
<td>Performance status</td>
<td>0 vs. 1</td>
<td>1.51 (0.56–4.05)</td>
<td>0.41</td>
<td>1.78 (0.51–5.14)</td>
</tr>
<tr>
<td>Treatment</td>
<td>ARCON vs. accelerated radiotherapy</td>
<td>0.78 (0.34–1.76)</td>
<td>0.55</td>
<td>0.35 (0.09–1.33)</td>
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<tr>
<td><strong>Multivariate analysis</strong></td>
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<td></td>
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<tr>
<td>N-classification</td>
<td>N+ vs. N0</td>
<td>4.80 (2.06–11.22)</td>
<td>&lt;0.01</td>
<td>3.31 (0.95–11.5)</td>
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<tr>
<td>Performance status</td>
<td>0 vs. 1</td>
<td>1.78 (0.66–4.78)</td>
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<td>Treatment</td>
<td>ARCON vs. accelerated radiotherapy</td>
<td>0.74 (0.33–1.66)</td>
<td>0.46</td>
<td>0.41 (0.11–1.55)</td>
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</table>
breathing immediately followed by radiotherapy will not cause this adaptive response because the oxygenation increase is too short and only for the duration of the radiation treatment (10–15 minutes). The status quo of reduced cord radius and, consequently, shorter oxygen diffusion distances in combination with higher levels of free oxygen in plasma explains how ARCON can exploit adaptive mechanisms in patients with anemia. In addition, because the effect of carbogen relies on oxygen transport by the plasma, the reduced blood viscosity and consequent increased flow through the tumor microvasculature will further benefit the patients with anemia. Finally, other compensatory mechanisms such as a shift in the oxygen–hemoglobin dissociation relationship may contribute as well.

Although the effect of hyperbaric oxygen in patients with head and neck cancer has recently been demonstrated in a systematic review and meta-analysis, prospective data testing hyperbaric oxygen in relation to anemia are lacking (24). However, a retrospective analysis of patients with carcinoma of the uterine cervix treated with radiotherapy in hyperbaric oxygen also revealed a marked improvement in local tumor control in patients with severe anemia before radiotherapy (25). Additional support comes from a preclinical study demonstrating that hyperbaric oxygen was successful in overcoming the increased radioresistance associated with anemia in mouse mammary adenocarcinomas (26). These observations are of interest because several mechanisms of action of ARCON discussed above also apply to hyperbaric oxygen.

Another issue is why patients with anemia have an inferior outcome compared with patients without anemia in the first place. It is generally assumed that this is primarily a consequence of the impaired tumor oxygenation resulting in a more aggressive and treatment resistant tumor phenotype (15, 27). Indeed, clinical and preclinical studies do provide evidence for a correlation between hemoglobin level and tumor hypoxia measured by polarographic \(pO_2\) electrodes (15, 16, 28, 29). In particular, this association is present in patients with severe anemia \([\text{hemoglobin} < 11.0 \text{ g/dL} \leq [6.9 \text{ mmol/L}])\], whereas it is much weaker in patients with mild anemia or low normal hemoglobin levels. One study did not demonstrate such correlation, but it should be noted that in that study there were hardly any patients with severe anemia (30). This was also the case in the current study: in the subgroup of patients, participating in the hypoxia marker side study, no correlation was found between tumor hypoxia as measured by pimonidazole binding and hemoglobin level but there was only one patient with a hemoglobin level below 11.0 g/dL. Only 79 of 345 patients took part in this side study. This is mainly because of a late amendment to the initial protocol and the limited number of participating centers to the side study. However, we argue that it is not the absolute amount of hypoxia but the distribution as function of distance to the vessels that is relevant for the response to ARCON.

Tumor hypoxia may be one reason for the poor prognosis of patients with anemia but most likely not the only one. A typical molecular feature of malignancies is the switch in tumor cell metabolism from oxidative to glycolytic pathways (31, 32). Anaerobic glycolysis provides cells with a growth advantage in the tumor microenvironment and promotes metastasis formation. With decreased blood viscosity and increased plasma flow in patients with anemia there will be greater availability of glucose, fueling the process of malignant progression. This can explain the

### Table 3. Demographics and clinical characteristics of patients \((N = 79)\) participating in the hypoxia marker study

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Accelerated radiotherapy ((N = 38))</th>
<th>ARCON ((N = 41))</th>
<th>(P)</th>
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<td>61</td>
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<tr>
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<tr>
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<td>Female</td>
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<td>Performance status, number (%)</td>
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<td>34 (83)</td>
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<td>1</td>
<td>9 (24)</td>
<td>7 (17)</td>
<td></td>
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<td>Site of the primary tumor, number (%)</td>
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<tr>
<td>Supraglottic</td>
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<td>27 (66)</td>
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<tr>
<td>Glottic</td>
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<td>14 (34)</td>
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<td>T-stage, number (%)</td>
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<td>T3</td>
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<td>N-stage, number (%)</td>
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<td>Pretreatment hemoglobin, number (%)</td>
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<tr>
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<td>37 (90)</td>
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<td></td>
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<tr>
<td>Male, mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>9.0</td>
<td>9.1</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>7.7–10.3</td>
<td>6.8–10.4</td>
<td></td>
</tr>
<tr>
<td>Female, mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>7.9</td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>7.4–9.3</td>
<td>7.5–9.9</td>
<td></td>
</tr>
</tbody>
</table>

\(a\) Mann–Whitney \(U\) test.  
\(b\) \(\chi^2\) test.  

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observation of an association between anemia and the trend toward a higher N-stage ($P = 0.06$) in this study. Apart from a causative factor, anemia can also be an epiphenomenon of aggressive tumor behavior. Activation of the immune and inflammatory system by the malignant disease produces cytokines, including interferons, TNF, and interleukin-1 (33). These cytokines inhibit erythropoiesis, affect the life span of erythrocytes and impair iron metabolism.

Despite reduction of tumor recurrence, no benefit of ARCON was observed on OS in patients with anemia. The correlation between low hemoglobin levels and a poorer performance status ($P < 0.01$), observed in this study, suggests that associated comorbidity in patients with low hemoglobin levels at diagnosis may affect survival independent of tumor control. Supportive evidence for this comes from the GORTEC 94-01 trial comparing radiotherapy alone with concomitant radiochemotherapy in advanced-stage oropharynx carcinoma. This study showed that anemia was associated with a higher probability of death caused by intercurrent disease (20). In our cohort of patients presenting with anemia, approximately 40% exhibit significant comorbidity. It is obvious that the survival impact of comorbidity cannot be influenced by ARCON or any other cancer treatment (34).

In conclusion, the poor prognosis of patients with laryngeal cancer with pretreatment anemia is no longer observed when radiotherapy is combined with carbogen breathing and nicotinamide. This observation of improved outcome in patients with anemia supports an earlier proposed hypothesis (8). Reduced oxygen diffusion distances in the tumor and improved oxygen transportation by the plasma because of reduced blood viscosity can explain the effectiveness of ARCON in patients with anemia. The potential of ARCON should be further explored in a large prospective randomized trial with focus on patients presenting with anemia.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Authors’ Contributions**

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): G.O. Janssens, S.E. Rademakers, J.H. Hoogsten, P.N. Span, J.H. Kaanders
Writing, review, and/or revision of the manuscript: G.O. Janssens, S.E. Rademakers, C.H. Terhaard, P.A. Doornaert, H.P. Bijl, A. Chin, R.P. Takes, R. de Bree, J. Bussink, P.N. Span, J.H. Kaanders
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**References**


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