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**ORIGINAL ARTICLE**

# Does transfusion improve the outcome for HNSCC patients treated with radiotherapy? – Results from the randomized DAHANCA 5 and 7 trials

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**Abstract**

*Background.* Patients with head and neck squamous cell carcinoma (HNSCC) and a low level of hemoglobin often have a poor response to radiation that may be related to hypoxia-induced radioresistance. We have previously published the importance of hemoglobin level and the effect of transfusion by the results from the randomized DAHANCA 5 trial, including 414 patients in the analysis. Aim of the current analysis was to gain additional power by adding patients from the continued subrandomization in the DAHANCA 7 trial, now including a total of almost 1200 patients. *Material and methods.* Patients were randomized to treatment in the DAHANCA 5 and 7 study (nimorazole vs. placebo and five fx/week vs. six fx/week), and in addition, patients with "low" pre-irradiation hemoglobin values (females $<$13 g/dl; males $<$14.5 g/dl) were subrandomized to plus or minus transfusion. Transfusion was given with packed red blood cells with the aim to achieve a hemoglobin level in the "high" value range. *Results.* A total of 1166 patients were included, 701 patients had high hemoglobin levels and 465 had low hemoglobin levels. Among the low hemoglobin patients, 235 were randomized to receive transfusion. Patient characteristics and treatment arms were well balanced. In the majority of patients, transfusion resulted in increased hemoglobin levels although this decreased slightly throughout treatment as in the non-transfused patients. Overall, the patients with low hemoglobin level had a significant reduced probability of locoregional control, disease-specific and overall survival. In the low hemoglobin group, transfusion did not improve the outcome in locoregional control, disease-specific or overall survival. In multivariate analyses, HPV/p16 status, T and N classification were significant factors for all outcome measures, whereas there was no significant influence of transfusion or hemoglobin level on endpoints. *Conclusion.* Transfusion prior to and during radiation treatment did not improve the outcome in patients with HNSCC and low hemoglobin values, but may have a negative impact on survival.

In a recent publication from the Danish Head and Neck Cancer Group (DAHANCA), the hemoglobin level and transfusion results from the randomized DAHANCA 5 trial has been described [1]. That study showed a prognostic significance of high hemoglobin level in patients with HNSCC (head and neck squamous cell carcinomas) treated with radiotherapy (RT). However, transfusion prior to and during treatment did not improve the outcome in patients with low hemoglobin values. Since the DAHANCA 5 study lacked power to draw final conclusions regarding the effect of transfusion, this subrandomization was continued in the DAHANCA 7 protocol [2]. The purpose of the present analysis is a combined analysis of DAHANCA 5 and DAHANCA 7 results to add knowledge to the conclusions regarding transfusion prior to and during treatment in HNSCC patients with low hemoglobin values. The

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overall hypothesis was that in head and neck cancer patients with low hemoglobin level, an increase in hemoglobin level by transfusion may improve the effect of radiotherapy.

The background for the hypothesis is that HNSCC patients with a low level of hemoglobin often have a poor response when treated with radiation [3–6]. A relationship between hemoglobin level and hypoxia has been shown both experimentally and clinically, but the mechanisms linking these conditions are uncertain [6–10]. Head and neck tumors often present hypoxic radioresistant cells and are heterogeneous as a group [11,12]. Radiosensitivity has been regained in tumor-bearing anemic mice by giving transfusions [13–16] and a correction of anemia in clinical studies has resulted in improvement in tumor oxygenation and a subsequent increase in the therapeutic efficacy of irradiation [4,17–25]. The adverse effects and outcome in this setting when administrating erythropoietin stimulating agents (ESA) [26–32], has reopened the possible advantage of using transfusion to raise hemoglobin levels before and during treatment.

## Methods

### *Patients and treatment*

As part of two randomized trials, DAHANCA 5 & 7 evaluating the role of nimorazole as a hypoxic radiosensitizer and accelerated fractionated RT, respectively, the data to evaluate the importance of hemoglobin level in patients and the modification with transfusion were collected. The protocols have previously been described [2,33–35] and the current evaluation is almost identical to what was done in the previous DAHANCA 5 hemoglobin publication [1].

Patients were prior to randomization stratified according to sex, institution, tumor site, tumor stage and hemoglobin concentration. In DAHANCA 5, the patients were randomized to radiotherapy with nimorazole or placebo, in DAHANCA 7 to nimorazole treatment and radiotherapy with five or six fractions/week. Patients with low pre-irradiation hemoglobin (females $<$13.0 g/dl; males $<$14.5 g/dl) were offered subrandomization to receive or not to receive transfusion. Transfusions were given with packed blood cells (one unit approximately equivalent to 500 ml full blood, leukocyte depletion of the units was not standard) to achieve a hemoglobin concentration in the "high" value range. Transfusion was given before radiotherapy and if during treatment the hemoglobin fell below the values indicated above, the transfusion was repeated. The hemoglobin level was measured at least every two weeks.

Treatment was given according to DAHANCA and protocol guidelines as previously described [2,33–35]. Both studies were performed in accordance with the Helsinki declaration and approved by relevant ethics committees. All patients gave written informed consent [2,33–35].

### *Statistical analyses*

The endpoints used were locoregional control after radiotherapy, disease-specific survival and overall survival. Locoregional control was defined as complete and persistent disappearance of the disease in the primary tumor (T-site) and regional lymph nodes (N-site) after radiotherapy. The evaluation was done by clinical examination and supplemented with relevant tests in case of doubt. Failure was recorded in case of recurrent tumor, or if the primary tumor never completely disappeared. In the latter situation, the tumor was then assumed to have failed at the time of randomization. The endpoint did not include the effect of a successful procedure with salvage surgery. The definition of disease-specific survival was death from or with the actual cancer. The endpoint for overall survival was any death, irrespective of cause. All time estimates were done using the date of randomization as the initial value. Follow-up was completed in connection with the original study [2,33–35].

The treatment effect was evaluated using the intention to treat principle and patients were included in their randomization group irrespective of whether or not they had completed the planned treatment. Time for evaluation of locoregional control, disease-specific survival and overall survival was five years after randomization, since patients were followed regularly only for that period [1].

Patients with high hemoglobin (high) and patients with low hemoglobin, regardless of transfusion status (low all), were compared to determine the effect of hemoglobin level. In addition to this, a comparison between high and low patients without transfusion (low-t) has been done. The effect of transfusion was evaluated by comparing the two low groups with and without transfusion (low+t, low−t), respectively. Patients were defined as having received transfusion during treatment if they received a transfusion in a period of 4–45 days after start of radiotherapy. This subgroup of patients does not include patients receiving transfusion prior to and during the first few days of radiotherapy.

Positive HPV/p16 status was defined retrospectively by positive p16 staining in tumors and compared to tumors with negative staining or unknown status and included due to the positive effect on the outcome of radiotherapy treatment [36,37].

Statistical analyses were done using the STATA 11 software package. Patient characteristics were compared with $\chi^2$-test and a two-sided significance level was chosen at 0.05. The actuarial values of endpoints were evaluated by the Kaplan-Meier plots and compared with the log-rank test for equality of survivor functions. The p-values estimated are those for a two tailed test and the significance level was chosen to be 5%. A multivariate Cox proportional hazards analysis was used to evaluate prognostic factors and treatment with respect to the risk of locoregional failure, disease-specific survival and overall survival. Variables included in the model were selected prior to analysis and included age, gender, site, p16 status, T-classification, N-classification, treatment, protocol, hemoglobin level and transfusion. Data are presented as five-year actuarial hazard ratios (HR) with 95% confidence intervals, unless otherwise mentioned.

The randomized studies were not dimensioned in regard to the present transfusion analysis, but with 230 events in each of the low groups and an expected locoregional control of 70%, we expected to be able to detect a difference of 15% with alpha 0.05 and a power of 0.9.

All diagnostic, therapeutic, and follow-up data were validated and processed by the DAHANCA data centre.

## Results

A total of 1200 patients were eligible; 414/1200 (34.5%) from the DAHANCA 5 and 786/1200 (65.5%) from the DAHANCA 7 study. Of the 1200 patients, 708/1200 (59%) were in the high hemoglobin group and 492/1200 (41%) in the low hemoglobin group. Twenty-six were not randomized to transfusion and therefore excluded from the analysis. Further, eight patients were excluded for having their initial hemoglobin value in the wrong strata. The final analysis included 1166/1200 (97%) patients; 230/1166 (20%) in the low hemoglobin without transfusion (low−t) group, 235/1166 (20%) in the low plus transfusion (low+t) group and 701/1166 (60%) in the high hemoglobin (high) group (Figure 1 and Table I).

Statistically significant differences were found between the high and low hemoglobin groups (high and low all) regarding age, gender, site, T- and

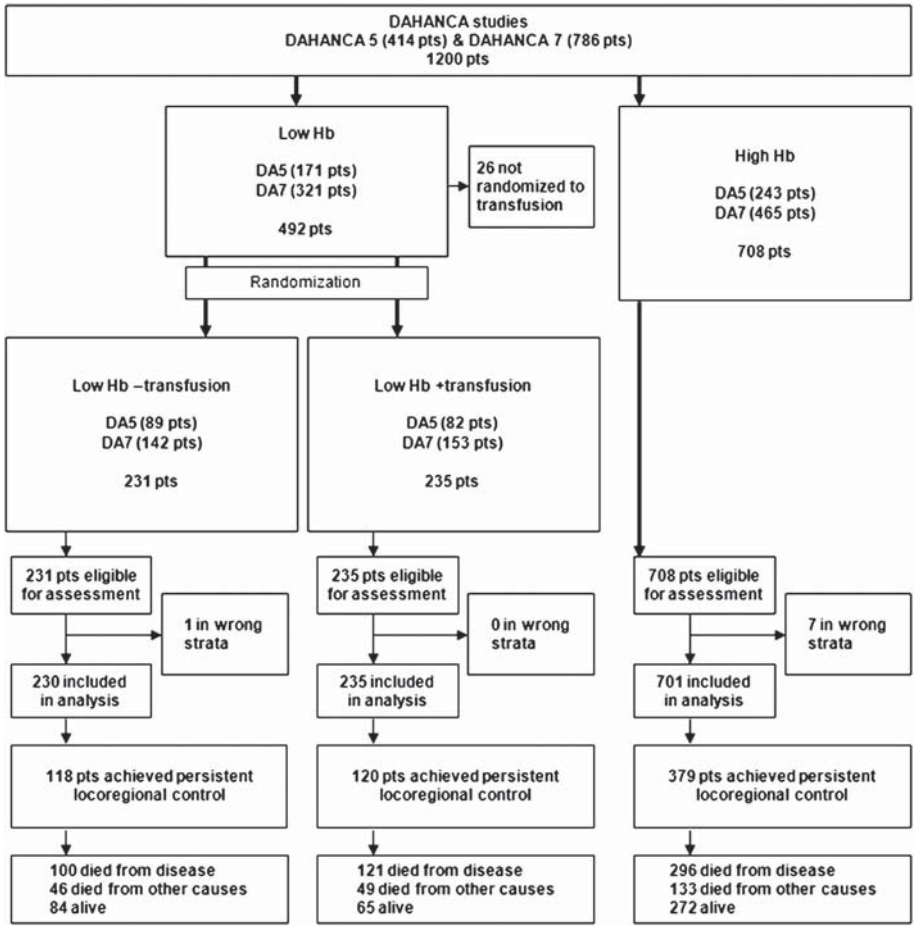


Figure 1. Study flow chart.

Table I. Patient and tumor characteristics.

| Parameter | | Low Hb −transf | | Low Hb +transf | | High Hb | | All patients | p-value (low/high) | p-value (low+/−t) |
|---|---|---|---|---|---|---|---|---|---|---|
| Study/treatment[$] | DAHANCA 5 | 89 | 39% | 82 | 35% | 243 | 35% | 414 | | |
| | DA5Pc5fx | 45 | 20% | 34 | 14% | 116 | 17% | 195 | 0.5[*] | 0.4[*] |
| | DA5Ni5fx | 44 | 19% | 48 | 20% | 127 | 18% | 219 | | |
| | DAHANCA 7 | 141 | 61% | 153 | 65% | 458 | 65% | 752 | | |
| | DA7Ni5fx | 68 | 30% | 79 | 34% | 222 | 32% | 369 | 0.8[#] | 0.5[#] |
| | DA7Ni6fx | 73 | 32% | 74 | 31% | 236 | 34% | 383 | | |
| Age | <60 years | 115 | 50% | 106 | 45% | 375 | 53% | 596 | 0.05 | 0.3 |
| | >60 years | 115 | 50% | 129 | 55% | 326 | 47% | 570 | | |
| Gender | Female | 35 | 15% | 40 | 17% | 226 | 32% | 301 | <0.001 | 0.6 |
| | Male | 195 | 85% | 195 | 83% | 475 | 68% | 865 | | |
| Hb level median (range) | Female | 12.1 (10.6–12.7) | | 12.2 (8.7–12.7) | | 13.8 (12.9–18.4) | | 13.5 | <0.001 | 0.3 |
| | Male | 13.7 (10.0–14.3) | | 13.4 (6.6–14.3) | | 15.1 (14.5–18.2) | | 144 | <0.001 | 0.6 |
| Site | Oral cavity | 21 | 9% | 21 | 9% | 83 | 12% | 125 | | |
| | Supraglottic | 55 | 24% | 57 | 24% | 217 | 31% | 329 | 0.004 | 1.0 |
| | Pharynx | 154 | 67% | 157 | 67% | 401 | 57% | 712 | | |
| HPV status | positive | 51 | 22% | 39 | 17% | 134 | 19% | 224 | 0.9 | 0.1 |
| | neg + UK | 179 | 78% | 196 | 83% | 567 | 81% | 942 | | |
| T-classification | T1 + T2 | 102 | 44% | 104 | 44% | 395 | 56% | 601 | <0.001 | 1.0 |
| | T3 + T4 | 128 | 56% | 131 | 56% | 306 | 44% | 565 | | |
| N-classification | N0 | 98 | 43% | 98 | 42% | 349 | 50% | 545 | 0.01 | 0.8 |
| | $N^+$ | 132 | 57% | 137 | 58% | 352 | 50% | 621 | | |
| Stage | I + II | 43 | 19% | 49 | 21% | 200 | 29% | 292 | 0.001 | 0.6 |
| | III + IV | 187 | 81% | 186 | 79% | 501 | 71% | 874 | | |
| Transfusion during RT | None | 0 | 0% | 154 | 66% | 0 | 0% | 154 | No value | No value |
| | ≥1 | 0 | 0% | 81 | 34% | 0 | 0% | 81 | | |
| Treatment | Nimorazole | 185 | 80% | 201 | 86% | 585 | 83% | 971 | 0.8 | 0.1 |
| | Placebo | 45 | 20% | 34 | 14% | 116 | 17% | 195 | | |
| | 5 fx/week | 157 | 68% | 161 | 69% | 465 | 66% | 783 | 0.5 | 1.0 |
| | 6 fx/week | 73 | 32% | 74 | 31% | 236 | 34% | 383 | | |
| Total | | 230 | | 235 | | 701 | | 1166 | | |

[$]Treatment groups:
1: DAHANCA 5, placebo, 5 fx/week (DA5Pc5fx)
2: DAHANCA 5, nimorazole, 5 fx/week (DA5Ni5fx)
3: DAHANCA 7, nimorazole, 5 fx/week (DA7Ni5fx)
4: DAHANCA 7, nimorazole, 6 fx/week (DA7Ni6fx)
[*]comparing difference between DAHANCA 5 and DAHANCA 7
[#]comparing difference between the four treatment groups (DA5Pc5fx, DA5Ni5fx, DA7Ni5fx and DA7Ni6fx)

N-classification and stage (Table I), i.e. high hemoglobin was associated with more favorable prognostic factors.

Compliance to radiotherapy was high and in both studies, 97% of patients completed the planned radiation treatment [2,33].

Pretreatment hemoglobin was in the low level for 465/1166 (40%) patients; 75/1166 (6%) females and 390/1166 (33%) males. These patients were randomized to transfusion (235/465, 51%) or no transfusion (230/465, 49%). The group given transfusion consisted of 40/235 (17%) females and 195/235 (83%) males.

There was no difference in the distribution of hemoglobin levels in the transfused and non-transfused group prior to treatment, nor were there any significant differences in distribution of patient and tumor characteristics between the two groups (low+t and low−t) before treatment (Table I).

The patients randomized to transfusion received 0–14 units (median two units) of blood. Very few

patients received more than eight units and 95/235 (40%) patients received two units of blood (Appendix 1 to be found online at http://www.informahealthcare.com/doi/abs/10.3109/0284186X.2011.592650).

A total of 210/235 (89%) received transfusion on at least one occasion, 87/235 (37%) at two occasions and 41/235 (17%) at three or more occasions (Figure 2). Transfusion was never received despite randomization in 25/235 (11%) patients.

Most patients 142/235 (60%) received their first transfusion before or on the day of the first radiation fraction; 174/235 (74%) before or within the first week of radiotherapy (Figure 2). Transfusions were with few exceptions given as two units of packed red blood cells.

In patients randomized to transfusion, the first transfusion was on median given one day (range −25–40 days) before start of radiotherapy. In patients receiving their first transfusion prior to radiotherapy, this happened on median four days (range −25–0 days) before start of radiotherapy (Figure 2).

The benefit of transfusion is seen in Figure 3: Measurements of hemoglobin level during radiotherapy showed that the median value increased within the first week in the transfused group. The median hemoglobin level was raised to the same level as the high hemoglobin group. However, after this increase there was a continuous drop throughout the course of radiotherapy for all hemoglobin groups, low+t, high and low−t, respectively (Figure 3 and Appendix 2 to be found online at http://www.informahealthcare.com/doi/abs/10.3109/0284186X.2011.592650). Only 4/40 (10%) females and 76/195 (39%) males had low hemoglobin level after treatment. No cases of venous thromboembolism were found in connection to treatment with transfusion.

Of the 1166 evaluable patients a total of 549 (47%) experienced locoregional failure; 517 (44%)

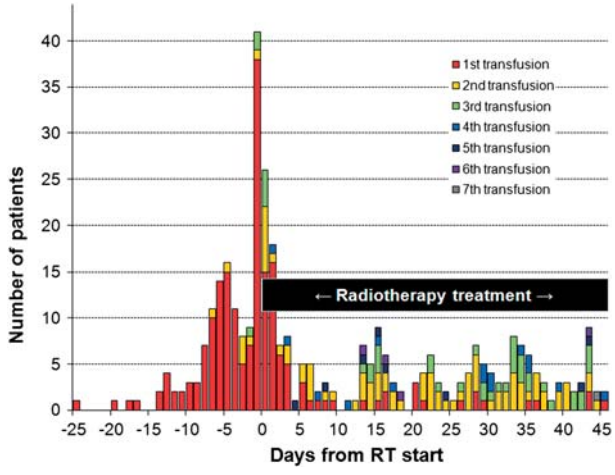


Figure 2. Time of transfusions.

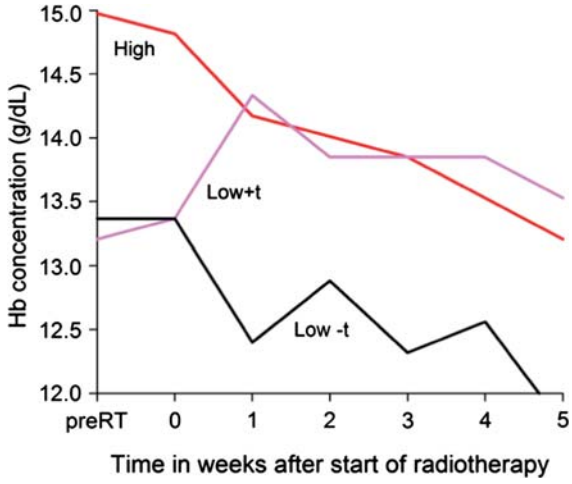


Figure 3. Hemoglobin level during radiotherapy treatment as a function of hemoglobin group.

had died of disease and 745 (64%) had died overall (Figure 1 and Appendix 3 to be found online at http://www.informahealthcare.com/doi/abs/10.3109/0284186X.2011.592650).

Distant metastases was experienced in 117/1166 (10%) patients. Twenty three (10%) failures in the low−t group, 31 (13%) in the low+t group and 63 (9%) in the high group, respectively. However, only 48/1166 (4%) patients experienced an isolated distant failure, with 6/230 (3%) low−t, 15/235 (6%) low+t and 27/701 (4%) high events in the three groups.

Univariate analysis showed statistical significance of low age on overall survival. Female gender, supraglottic location, positive HPV/p16 status, low T-classification, low N-classification and low stage were statistically significant for locoregional control, disease-specific survival and overall survival. When analyzing the treatment groups there was a significant benefit of adding nimorazole to the regimen for both locoregional control and disease-specific survival. There were no differences in outcome measurements when going from the DAHANCA 5 to the DAHANCA 7 protocol. The addition of accelerated radiotherapy did not reach statistical significance, but a tendency towards better outcome measurements was seen.

High hemoglobin level was statistically significant better than low hemoglobin level for locoregional control (HR 0.83; CI 0.70–0.98; p = 0.03), disease-specific (HR 0.77; CI 0.64–0.91; p = 0.003) and overall survival (HR 0.77; CI 0.67–0.89; p = 0.0004). However, analysis of the three hemoglobin groups showed no statistically significant difference between the two non-transfused groups (high vs. low−t) on outcome measurements. No benefit of transfusion (low+t vs. low−t) was observed on locoregional control (HR 1.06; 0.82–1.38; p = 0.7),

disease-specific (HR 1.27; CI 0.97–1.65; p = 0.1) or overall survival (HR 1.24; CI 0.99–1.54; p = 0.08) (Table II and Figure 4a, b and c).

When looking only at the patients randomized to transfusion, transfusion during treatment did not turn out significant for survival measurements when comparing with patients not receiving transfusions during treatment; locoregional control (HR 1.14; CI 0.78–1.67; p = 0.5), disease-specific survival (HR 1.35; CI 0.94–1.95; p = 0.1) and overall survival (HR 1.18; CI 0.86–1.61; p = 0.3). However, this subgroup of patients tends to have a worse prognosis than patients not receiving transfusions during radiotherapy.

The patients received four different treatments defined by their original DAHANCA study, placebo vs. nimorazole and five fx/week vs. six fx/week. In the subgroup analysis it is shown that hemoglobin level is of importance irrespective of hypoxic modification with nimorazole and fractionation schedule (Appendix 3 to be found online at http://www.informahealthcare.com/doi/abs/10.3109/0284186X.2011.592650.).

Multivariate Cox proportional hazards analysis confirmed the statistical significance of low age on overall survival. For locoregional control, disease-specific and overall survival, the statistical significant prognostic effect of positive HPV/p16 status, supraglottic location, low T-classification and low N-classification was also confirmed. The addition of nimorazole and accelerated radiotherapy to treatment protocol was significant for locoregional control when using patients from DAHANCA 5 treated with nimorazole and five fx/week as reference group (Table II).

High hemoglobin level was statistically significant for overall survival, but no positive effect of transfusion was seen on outcome measurements (Table II).

Transfusion during radiotherapy was evaluated in a separate model and patients receiving transfusions during radiotherapy had a statistically significant

Table II. Univariate and multivariate analysis.

| | | Locoregional Control | | | Disease-Specific Survival | | | Overall Survival | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Univariate parameter | | HR | CI | p-value | HR | CI | p-value | HR | CI | p-value |
| Age | >60 vs <60 | 1.09 | (0.92−1.29) | 0.3 | 1.10 | (0.92−1.30) | 0.3 | 1.23 | (1.07−1.42) | 0.004 |
| Gender | female vs male | 0.77 | (0.63−0.94) | 0.009 | 0.78 | (0.63−0.96) | 0.02 | 0.78 | (0.66−0.92) | 0.004 |
| Site | Supraglottic vs pharynx | 0.79 | (0.64−0.96) | 0.02 | 0.58 | (0.47−0.72) | <0.001 | 0.71 | (0.60−0.84) | 0.0001 |
| | Oral cavity vs pharynx | 1.43 | (1.11−1.85) | 0.005 | 1.12 | (0.86−1.46) | 0.4 | 1.06 | (0.84−1.33) | 0.6 |
| | Supraglottic oral cavity | 0.55 | (0.41−0.73) | <0.001 | 0.52 | (0.38−0.71) | <0.001 | 0.67 | (0.52−0.86) | 0.002 |
| HPV status | pos vs neg+UK | 0.51 | (0.40−0.65) | <0.001 | 0.38 | (0.28−0.50) | <0.001 | 0.46 | (0.37−0.57) | <0.001 |
| T-classification | T1 + T2 vs T3 + T4 | 0.60 | (0.51−0.71) | <0.001 | 0.60 | (0.51−0.72) | <0.001 | 0.67 | (0.58−0.78) | <0.001 |
| N-classification | N0 vs $N^+$ | 0.59 | (0.50−0.70) | <0.001 | 0.49 | (0.41−0.59) | <0.001 | 0.65 | (0.56−0.75) | <0.001 |
| Stage | I + II vs III + IV | 0.55 | (0.44−0.68) | <0.001 | 0.49 | (0.38−0.61) | <0.001 | 0.68 | (0.57−0.81) | <0.001 |
| Treatment | DA5Pc5fx vs DA5Ni5fx | 1.43 | (1.10−1.86) | 0.007 | 1.32 | (1.00−1.74) | 0.05 | 1.04 | (0.82−1.32) | 0.7 |
| | DA7Ni5fx vs DA5Ni5fx | 1.01 | (0.79−1.29) | 0.9 | 1.06 | (0.83−1.36) | 0.6 | 1.00 | (0.81−1.22) | 1.0 |
| | DA7Ni6fx vs DA5Ni5fx | 0.81 | (0.63−1.04) | 0.1 | 0.84 | (0.65−1.09) | 0.2 | 0.91 | (0.74−1.12) | 0.4 |
| Hemoglobin level | High vs low all | 0.83 | (0.70−0.98) | 0.03 | 0.77 | (0.64−0.91) | 0.003 | 0.77 | (0.67−0.89) | 0.0004 |
| | High vs low−t | 0.85 | (0.69−1.06) | 0.1 | 0.87 | (0.69−1.09) | 0.2 | 0.86 | (0.71−1.04) | 0.1 |
| | Low+t vs low−t | 1.06 | (0.82−1.38) | 0.7 | 1.27 | (0.97−1.65) | 0.1 | 1.24 | (0.99−1.54) | 0.08 |
| Transfusion during radiotherapy | | 1.14 | (0.78−1.67) | 0.5 | 1.35 | (0.94−1.95) | 0.1 | 1.18 | (0.86−1.61) | 0.3 |
| | | Locoregional Control | | | Disease-Specific Survival | | | Overall Survival | | |
| Multivariate analysis | | HR | CI | p-value | HR | CI | p-value | HR | CI | p-value |
| Age | >60 vs <60 | 1.10 | (0.93−1.30) | 0.3 | 1.11 | (0.93−1.33) | 0.2 | 1.24 | (1.07−1.44) | 0.004 |
| Gender | female vs male | 0.84 | (0.68−1.03) | 0.1 | 0.87 | (0.71−1.08) | 0.2 | 0.84 | (0.70−1.00) | 0.05 |
| Site | Supraglottic vs pharynx | 0.89 | (0.72−1.09) | 0.3 | 0.68 | (0.54−0.86) | 0.001 | 0.75 | (0.63−0.90) | 0.002 |
| | Oral cavity vs pharynx | 1.71 | (1.30−2.25) | <0.001 | 1.27 | (0.96−1.68) | 0.1 | 1.07 | (0.84−1.36) | 0.6 |
| | Supraglottic vs oral cavity | 0.51 | (0.38−0.70) | <0.001 | 0.54 | (0.39−0.74) | <0.001 | 0.7 | (0.54−0.92) | 0.009 |
| HPV status | pos vs neg + UK | 0.53 | (0.41−0.68) | <0.001 | 0.36 | (0.27−0.49) | <0.001 | 0.45 | (0.36−0.56) | <0.001 |
| T-classification | T1 + T2 vs T3 + T4 | 0.63 | (0.54−0.76) | <0.001 | 0.65 | (0.54−0.77) | <0.001 | 0.72 | (0.62−0.83) | <0.001 |
| N-classification | N0 vs $N^+$ | 0.57 | (0.47−0.68) | <0.001 | 0.49 | (0.41−0.60) | <0.001 | 0.64 | (0.55−0.74) | <0.001 |
| Treatment | DA5Pc5fx vs DA5Ni5fx | 1.37 | (1.05−1.78) | 0.02 | 1.22 | (0.92−1.60) | 0.2 | 0.99 | (0.78−1.26) | 0.9 |
| | DA7Ni5fx vs DA5Ni5fx | 0.88 | (0.68−1.13) | 0.3 | 0.91 | (0.71−1.19) | 0.5 | 0.91 | (0.74−1.13) | 0.4 |
| | DA7Ni6fx vs DA5Ni5fx | 0.74 | (0.57−0.96) | 0.03 | 0.83 | (0.63−1.08) | 0.2 | 0.92 | (0.74−1.14) | 0.4 |
| Hemoglobin level | High vs low all* | 0.92 | (0.77−1.11) | 0.4 | 0.86 | (0.72−1.03) | 0.1 | 0.85 | (0.73−0.99) | 0.04 |
| | High vs low−t | 0.93 | (0.74−1.15) | 0.5 | 0.93 | (0.73−1.17) | 0.5 | 0.91 | (0.75−1.10) | 0.3 |
| | Low+t vs low−t | 1.00 | (0.77−1.29) | 1.0 | 1.15 | (0.88−1.50) | 0.3 | 1.14 | (0.91−1.43) | 0.2 |

*When including the high vs. low all parameter in the multivariate model the other parameters HRs are only slightly different from this analysis, which is based on a multivariate model including all three hemoglobin groups. P-values are the same.

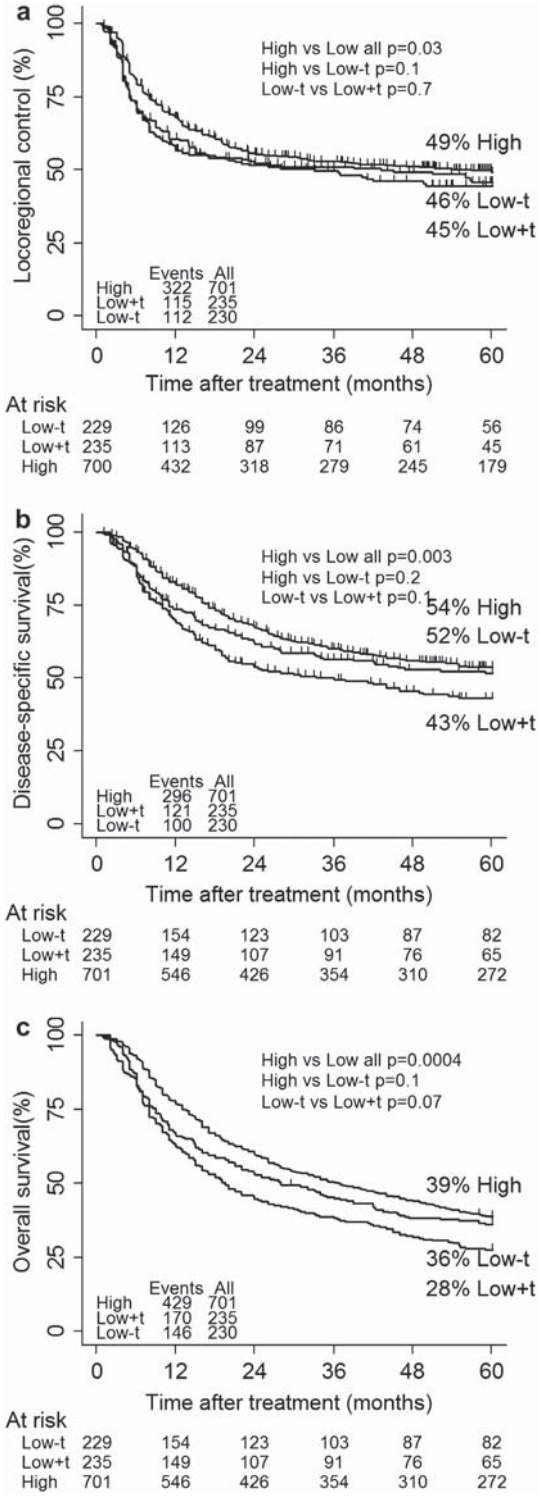


Figure 4. Locoregional control (a), disease-specific (b) and overall survival (c) probability curves (Kaplan-Meier method) according to hemoglobin group.

worse prognosis in disease-specific and overall survival than patients not receiving transfusions during radiotherapy when correcting for other relevant factors (Appendix 4 to be found online at http://www.informahealthcare.com/doi/abs/10.3109/0284186X.2011.592650).

## Discussion

In squamous cell head and neck cancer patients with a low hemoglobin level; transfusion was able to raise the hemoglobin level during radiotherapy, however this increase in hemoglobin level was not able to improve the effect of the radiotherapy treatment. This is in accordance with what was shown in the analysis of the DAHANCA 5 study [1]. The addition of the DAHANCA 7 patients and the total number of almost 1200 patients gives stronger evidence, that transfusion is unable to improve the effect of radiotherapy, but instead, in survival outcome measurements, showing a tendency towards worsening the prognosis.

The tendency to worsening the prognosis when receiving transfusion during radiotherapy could be explained by patients with low hemoglobin levels during treatment, having a worse overall condition and is not necessarily a cancer related transfusion risk.

The known poor prognostic effect of low hemoglobin prior to radiotherapy treatment was confirmed for overall survival in multivariate analysis [6]. This difference in outcome was, however not found when analyzing only the non-transfused patients and comparing the low non-transfused group and the high hemoglobin group. The addition of the low hemoglobin patients receiving transfusion reduced outcome measurements, since these patients have the worst probability for locoregional control, disease-specific and overall survival.

The prognostic effect of low hemoglobin prior to radiotherapy treatment was more pronounced with overall survival as endpoint than with disease-specific survival and locoregional control. This indicates that there may other factors related to hemoglobin level, e.g. comorbidity and overall condition, influencing the prognosis. Work at clarifying the importance of comorbidity in head and neck cancers is currently being done [38].

In addition to what was shown in the DAHANCA 5 analysis, the transfused patients follow the same hemoglobin level pattern as the non-transfused patients throughout the course of radiotherapy. The low patients are by transfusion shifted into the high level, but all groups decrease during treatment. There could be a benefit if this drop was avoided by using erythropoietin stimulation agents, but studies published so far are not encouraging [26–32].

The fact that transfusion have been shown to lower the immune system in patients with kidney transplants may also relate to the poor survival in

transfused cancer patients. The immune system have functions towards the growing cancer, these not fully evaluated, but existing. The beneficial effect of blood transfusions prior to transplantation on graft survival is used. However, in cancer patients this immune regulatory effect may be all but beneficial. It has been shown that allogenic transfusions might affect the overall survival in patients undergoing curative cancer surgery [39]. In a study involving colorectal cancer blood transfusion was shown to be a univariate prognostic negative factor, but when adjusting with well-established prognostic factors in a multivariate prognostic model the negative effect of transfusions disappeared [40].

Even when accomplishing the wanted effect of transfusion by giving the patients the same start values as the high hemoglobin patients, the mechanisms are more complex than just high and low hemoglobin values, and more is needed than "just" raising hemoglobin level by transfusion and probably ESAs. Development of a hypoxia profile, which will be able to single out patients in need of hypoxic modification and offer a more individualized treatment, is ongoing.

There are limitations to the analysis and conclusions drawn from this study, especially in reference to explaining the connection between hypoxia, low hemoglobin levels and transfusion.

We can however conclude that in a large patient material with squamous cell head and neck cancer with a low hemoglobin level; transfusion were able to raise the hemoglobin level during radiotherapy, however this increase in hemoglobin level were not able to improve the effect of the radiotherapy treatment.

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**Supplementary material available online at http://www.informahealthcare.com/doi/abs/10.3109/0284186X.2011.592650.**

Appendix 1. Units of blood.
Appendix 2. Hemoglobin level during RT treatment
Blue = males
Red = females
Showing median Hb level during radiotherapy as a function of hemoglobin group.
Appendix 3. Subgroup analysis by treatment group in the original DAHANCA 5 and 7 trials.
Appendix 4. Multivariate model including transfusion during RT variable.