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Meta-analysis of hypoxia in HNSCC

Hypoxic modification of radiotherapy in squamous cell carcinoma of the head and neck – A systematic review and meta-analysis

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ABSTRACT

Background: The importance of tumour hypoxia for the outcome of radiotherapy has been under investigation for decades. Numerous clinical trials modifying the hypoxic radioresistance in squamous cell carcinoma of the head and neck (HNSCC) have been conducted, but most have been inconclusive, partly due to a small number of patients in the individual trial. The present meta-analysis was, therefore, performed utilising the results from all clinical trials addressing the specific question of hypoxic modification in HNSCC undergoing curative intended primary radiotherapy alone. *Methods:* A systematic review of published and unpublished data identified 4805 patients with HNSCC treated in 32 randomized clinical trials, applying, normobaric oxygen or carbogen breathing (5 trials); hyperbaric oxygen (HBO) (9 trials); hypoxic radiosensitizers (17 trials) and HBO and radiosensitizer (1 trial). The trials were analysed with regard to the following endpoints: loco-regional control (32 trials), disease specific survival (30 trials), overall survival (29 trials), distant metastases (12 trials) and complications to radiotherapy (23 trials). *Results:* Overall hypoxic modification of radiotherapy in head and neck cancer did result in a significant improved therapeutic benefit. This was most dominantly observed when using the direct endpoint of loco-regional control with an odds ratio (OR) of 0.71, 95% cf.l. 0.63–0.80; $p < 0.001$), but this was almost mirrored in the disease specific survival (OR: 0.73, 95% cf.l. 0.64–0.82; $p < 0.001$), and to a lesser extent in the overall survival (OR: 0.87, 95% cf.l. 0.77–0.98; $p = 0.03$). The risk of distant metastases was not significantly influenced although it appears to be less in the tumours treated with hypoxic modification (OR: 0.87, 95% cf.l. 0.69–1.09; $p = 0.22$), whereas the radiation related late complications were not influenced by the overall use of hypoxic modifications (OR: 1.00, 95% cf.l. 0.82–1.23; $p = 0.96$). The improvement in loco-regional control was found to be independent of the type of hypoxic modification. The trials have used different fractionation schedules, including large doses per fraction, which may result in relatively more hypoxia and greater benefit. However, analysis of HNSCC trials using conventional fractionation only, showed that the significant effect of hypoxic modification was maintained. *Conclusion:* The meta-analysis thus demonstrates that there is level 1a evidence in favour of adding hypoxic modification to radiotherapy of squamous cell carcinomas of the head and neck.

Squamous cell carcinoma in the head and neck region (HNSCC) is becoming a still more frequent disease which constitutes approximately 7% of all cancers worldwide [1]. The treatment is directed towards achieving loco-regional tumour control since primary distant metastases are infrequent. Thus, treatment is typically surgery and/or radiotherapy directed towards controlling the disease in the T- and N-position. The use of primary radiotherapy has often been the preferred treatment due to its known efficacy in eradicating the malignant cells and at the same time securing the optimal organ-conserving outcome [2,3].

The radiotherapeutic treatment has over the years developed at both the technical and biological levels. Technically modern radiotherapy aims at securing inclusion of the relevant target with minimal irradiation to unnecessary normal tissue structures such as, e.g. salivary glands. This can among other things be secured by using image guided intensity modulated radiotherapy (IMRT) [3–5]. Biologically the optimisation has taken place through improving of the radiotherapy fractionation schedules by the use of so-called altered fractionation which includes more fractions per day in order to reduce the overall treatment time (accelerated fractionation) and/or the use of multiple small fraction doses (hyperfractionation) which allows a higher total dose to be given without enhancing the risk of radiation-induced morbidity [6]. The third radiobiological problem which is frequently seen in head and neck cancer is related to radioresistance due to the presence of

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tumour hypoxia. The biological background and clinical implications of hypoxia have been described in detail previously [7–10].

It is characteristic that the different biological principles, which may influence the outcome of radiotherapy in head and neck cancer to a large extent, have been subjected to numerous and frequently large randomized trials and, therefore, there exists abundant information to evaluate the evidence for such therapeutic interventions. This has especially been the case for issues related to accelerated fractionation and chemoradiotherapy and both principles have, therefore, previously been evaluated in systematic reviews and meta-analyses which have defined the evidence and indication for the use of such strategies [11,12]. Despite the fact that hypoxic modification in HNSCC has been investigated over the longest time period [9,10], the benefit of this therapeutic intervention has not yet been subjected to a full evaluation in a systematic review taking all available data from randomized trials into consideration.

The purpose of the present analysis is to overview the past experience with hypoxic modification of the primary radiotherapy treatment of HNSCC, in order to supplement the more recent meta-analysis on altered fractionation [11] and chemoradiotherapy [12] with information of the importance of hypoxic modification of radiotherapy in head and neck cancer.

In contrast to the other systematic reviews of radiotherapy in HNSCC, most trials with hypoxic modification have taken place more than 30 years ago and, therefore, suffer from being relatively small in number and below current power dimensions. Furthermore detailed patient data from the individual trials have not been available, and the present knowledge has, therefore, been derived from data collected from publications rather than from the individual data set.

Material and methods

The criterion for inclusion in the present overview analysis has been that the treatment should be curative intended primary radiotherapy alone with randomization to a hypoxic modifier which should be known only to influence hypoxic radioresistance and have no other cytotoxic effect. Thus, studies involving chemoradiotherapy either as baseline treatment or as an intended hypoxic modifier, (e.g. mitomycin C), or hyperthermia are not included [13–17]. Neither are studies of patients with metastatic disease included since the analysis focuses on the effect of curatively intended radiotherapy.

The hypoxic modification in the trials has been either oxygen breathing under normobaric or hyperbaric pressure or the use of nitroimidazoles. The few studies with haemoglobin modification by either transfusion or the use of EPO [18–20] are not included because there have been some uncertainty about their interpretation, and especially the EPO-related studies are not available in sufficient detail, but are currently under intense scrutinisation.

Search strategy

The present knowledge about the randomized trials with hypoxic modification has been gained by a systematic search through Medline and SCOPUS using the search terms: hypoxia OR hypoxic OR oxygen OR hyperbaric OR nitroimidazoles AND (radiotherapy OR irradiation) AND (cancer OR neoplasms) AND human AND (laryngeal OR pharyngeal OR oral cavity OR head Or neck) AND randomized clinical trials. This search yielded initially 42 references to 27 trials; and included all but one previously (to the author) known published paper in the peer reviewed literature (only one paper in French language was not detected). The search further included all relevant referred literature found in the identified publications plus a scan of abstracts from relevant scientific meetings (e.g. ASTRO, ESTRO or specific meetings on hypoxic modification, etc.) which added 4 additional trials. Also investigators from large multicentre cooperative groups were contacted as well as the relevant pharmaceutical companies. The author has been active in this field for more than 30 years and has in addition a wide personal network within this field of research which further was used to explore unknown trials. The use of the described search strategy, however, identified all the included studies, although a few were in the format of abstracts of which some had subsequently been published, and some other studies have later been updated and published in more detail.

The following overview of the literature must, therefore, be considered to be covering the international experience so far. An overview of all the trials [21–56] can be found in Table 1, and in addition more detailed references to especially the older trials are to be found in previously published overview analyses [8–10,57–63].

Unfortunately, it has been impossible to collect individual patient data from many, especially elderly trials, because much of the material no longer exists and the overview is, therefore, based on an extract of information from the published papers. Since there is some variation in the observation time the studies are evaluated by the use of an odds ratio analysis which is considered to be one of the more robust although crude methods [64–66]. The numbers of events are either taken directly from the published information or, if not possible, from measurement from published survival curves as previously described [65]. The analysis has been performed using the "Comprehensive Meta-analysis" program v.2.0 [67], and presented as Forrest plots. The following endpoints have been addressed: loco-regional control, disease specific and overall survival, occurrence of distant metastases, and radiation-related complications.

Results

A total of 32 randomized trials with 4805 patients have been identified (Table 1). Thus many trials have included only a small number of patients (median number of patients per trial: 73, range 17–622) and are in general not conclusive by themselves. This reflect the [lack of] clinical trial methodology performed in the 1970s and 1980s where many trials were performed without proper power calculations, and further underlines thereby the need for a meta-analysis.

Overall hypoxic modifications of radiotherapy in head and neck cancer did result in a significant benefit and in both loco-regional control and survival (Fig. 1). The benefit was most dominant in the direct endpoint of loco-regional control with an odds ratio (OR) of 0.71, 95% cf.l. 0.63–0.80; $p < 0.001$), but this was almost mirrored in the disease specific survival (OR: 0.73, 95% cf.l. 0.64–0.82; $p < 0.001$), and to a lesser extent also in the overall survival (OR: 0.87, 95% cf.l. 0.77–0.98; $p = 0.03$). The risk of distant metastases was not significantly influenced although it appears to be less in the tumours treated with hypoxic modification (OR: 0.87, 95% cf.l. 0.69–1.09; $p = 0.22$), whereas the radiation related late complications were not influenced by the overall use of hypoxic modifications (OR: 1.00, 95% cf.l. 0.82–1.23; $p = 0.96$). On this basis, the current meta-analysis demonstrates an improved therapeutic benefit with *level 1a evidence in favour of adding hypoxic modification to radiotherapy of squamous cell carcinomas of the head and neck* [68].

Loco-regional tumour control

The effect on loco-regional tumour control could be evaluated in all 32 investigated trials. Fig. 2 shows the overall loco-regional

Table 1
Randomized clinical trials with hypoxic modification of radiotherapy in HNSCC.

References	Trial acronym	Year	No. pts	fx[a]	RT schedule	Hypoxic modification	Endpoint[b]					Obs. time
[21]	van den Brenk	1968	30	HH	7.75 Gy x4vs7.25 Gy x4 with HBO	HBO 4 atm	L	D	S			2 + years
[22]	Evans 1	1970	40	LL	60 Gy/30 fx	Normobaric 02	L	D	S			2 + years
[23]	Tobin	1971	17	LL	60 Gy/30 fx	HBO 3 atm	L	D	S			2–3 years
[24]	Chang	1973	51	HHL	6 Gy x6+ HBO vs 6 Gy x7 or 60 Gy/30 fx	HBO 3 atm	L	D	S	M	C	5 years
[25]	Shigamats u	1973	31	HH	60–79 Gy/1O fx vs. 40–50 Gy/8–10 fx + HBO	HBO	L	D	S			2 + years
[26]	Evans 2	1975	44	LL	60 Gy/30 fx	Normobaric 02	L	D	S	M	C	2 + years
[27]	MRC 1 trial	1977	276	HH	35–45 Gy x10	HBO 3 atm	L	D	S	M	c	4 + years
[26]	MRC 3, trial	1979	24	HL	45–50/15 el 48.5–55/20 air vs. 40–45/10 HBO	HBO	L	D	S		c	5 years
[29]	RTOG 70-02	1979	254	LL	60–70 Gy/30 fx	Carbogen	L	D	S	M	c	2 + years
[30]	Sause	1979	44	HL	48 Gy/12 fx + HBO vs. 62 Gy/25 fx	HBO 3 aim	L	D	S		c	2 + years
[31]	Giaux	1962	56	II	50 Gy/16 fx	MISO	L	D	S			34 months
[32]	Sealy 1	1962	97	HH	36 Gy/6 fx/17 days	MISO	L					>1 year
[33]	B run in	1963	101	LL	72 Gy/36 fx	MISO	L	D	S			2 years
[34]	MRC 10 fx	1964	162	HH	40–45 Gy/10 fx	MISO	L	D	S		c	3 + years
[34]	MRC 20 fx	1964	89	LL	50–57 Gy/20 fx	MISO	L	D	S			3 + years
[35]	Panis	1964	52	MM	Split-course 1.1 Gy x6 daily/ 5 days – 4 weeks split-repeat	MISO	L	D	S		c	2 + years
[36,37]	EORTC 22S111	1966	330	MM	1.6 Gy x3/10 days – 3 weeks split + same to total of 67–72 Gy	MISO	L	D	S		c	5 + years
[38,39]	MRC 2, trial	1966	103	HL	64 Gy/30 fx vs. 41–44 Gy/10 fx + HBO	HBO 3 aim	L	D	S	M	c	4 + years
[40]	Sealy 2	1966	124	HL	63 Gy/30 fx (air); 36 Gy/6 fx (HBO)	HBO/MISO	L	D	S	M	c	1–2-year
[41,42]	IAEA study	1967	36	LL	70 Gy/35 fx	On ids zo e	L	D	S		c	2 + years
[43,44]	RTOG 79-15	1967	297	LL	66–74/33–37 fx	MISO	L	D	S	M	c	2 + years
[45]	Galecki	1969	35	LL	70 Gy/35 fx vs. 66 Gy/30 fx vs. 80.5 Gyx 70 fx	Metronidazole	L	D	S		c	3 + years
[46]	Dahanca 2	1969	622	LL	68-72/34–36 fx eller 61/22/9.5 weeks	MISO	L	D	S	M	c	5 + years
[47]	RTOG 79-04	1969	40	HH	4 Gy 11–13 fx	MISO	L	D	S		c	2 + years
[48]	RTOG 8S-27	1995	504	LL	66–74 Gy/33–37 fx	Etanidazole	L	D	S	M	c	5 + years
[49]	Huilgol	1996	18	LL	54 Gy/45 fx/22 days	AK-2123	L	D	S			2 + years
[50]	European trial	1997	374	LL	66–74 Gy/33–37 fx	Etanidazole	L	D	S		c	5 + years
[51,52]	Dahanca 5	1998	414	LL	66–68/33–34	Nirnorazole	L	D	S	M		5 years
[53]	Haffty	1999	48	HH	12.65 Gy x2 vs. 11.50 Gy x2 + HBO	HB04 atm	L	D		M	c	5 + years
[54]	Mendenhall	2005	101	MM	76 Gy/1.2 Gy fx BID	02 Carbogen	L	D	s	M		5 + years
[55]	Ullal	2006	46	LL	60 Gy/30 fx	AK-2123	L					3 + months
[56]	ARCON	2010	345	LL	64–68 Gy/32–34 fx accelerated fx	Nicotinamide	L	D	s			2 years

[a] H: Hypofract; L: conventional tract; M: hyperfract (multiple fx/day).
[b] L: Loco-regional failure; D: disease specific death; S: overall death; M: distant metastasis; C: complications.

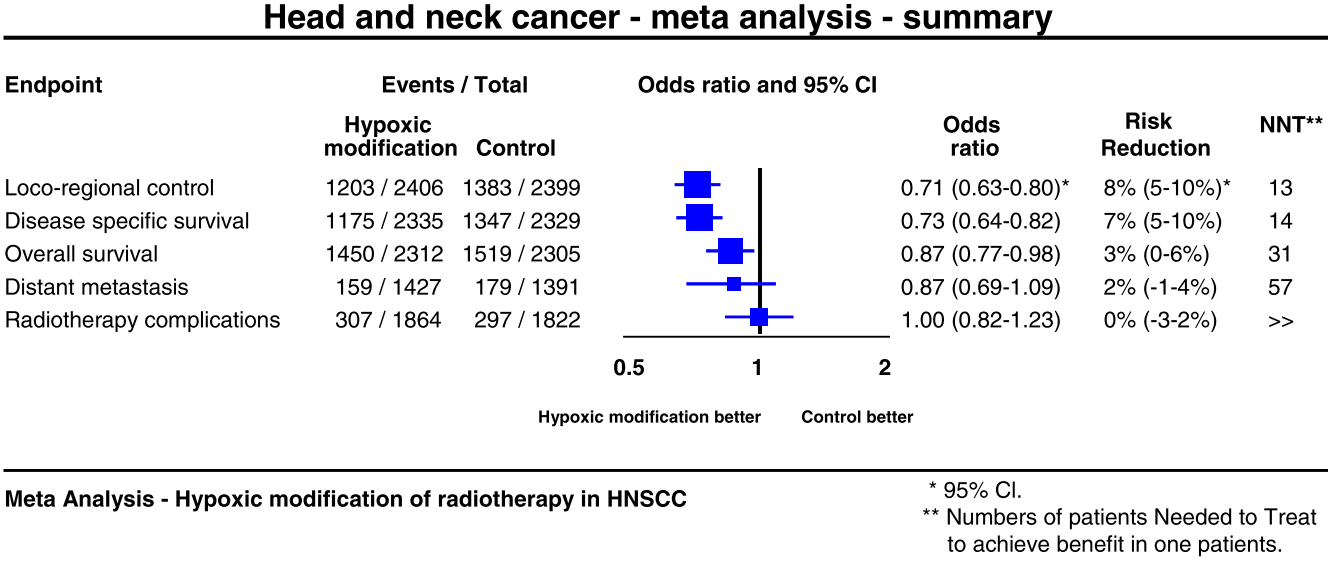

Fig. 1. Overview of hypoxic modification of radiotherapy in head and neck squamous cell carcinoma. Summary of data from 32 randomized trials including 4805 patients.

outcome which indicates that the benefit appears to be present irrespective of the mode of hypoxic modification.

The number of patients in the trials using normobaric oxygen were relatively sparse and dominated by the recently reported ARCON study in T2-T4 laryngeal tumours [56,69,70,71] which in addition to carbogen added nicotinamide [71], and also used a slightly lower dose to the T-site in the patients who were randomized to hypoxic modification.

The studies using hyperbaric oxygen (HBO) was by and large performed in the seventies. The overview is dominated by a series of trials performed in UK by the Medical Research Council (MRC). Overall the hyperbaric oxygen trials demonstrate the most pronounced effect of hypoxic modification, but several of the trials are difficult to interpret because the patients given HBO often were treated with large doses per fraction and in some studies compared to control groups given conventional fractionation [24,28,30,38,39]

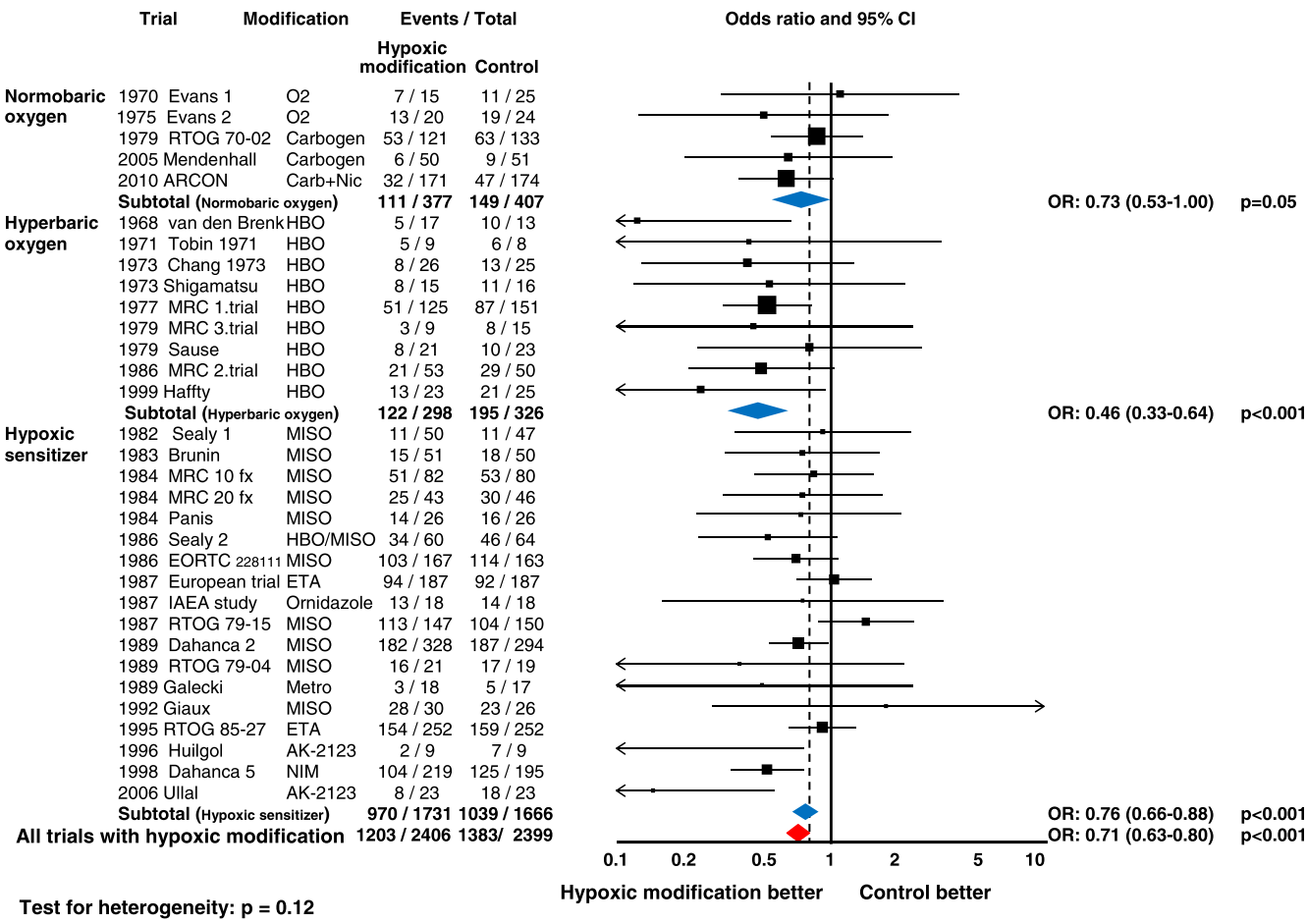

Fig. 2. Hypoxic modification of radiotherapy in HNSCC. Influence on loco-regional control as a function of type of hypoxic modification.

(Table 1). The substantial benefit found in such trials may partly be caused by a more pronounced hypoxic sensitisation which has been associated with the use of hypofractionation [8,72].

In the late-1970s, the use of hypoxic radiosensitizers became dominant, while the use of HBO faded out, partly due to the complex nature of the treatment, partly due to an explosive accident [73] with hyperbaric oxygen. Most hypoxic sensitizer trials have been performed with nitroimidazoles, which after an initial short period with the use of Flagyl (metronidazole) became dominated by trials using the 2-nitroimidazole: Misonidazole, which became the most common hypoxic sensitizer to be investigated in clinical trials. Due to frequent and significant side effects, especially in form of delayed peripheral neuropathy the use of Misonidazole was subsequently ceased. This was replaced by a third generation of nitroimidazoles which was expected to yield a more powerful hypoxic sensitisation (e.g. Etanidazole) [48,50] or having less severe morbidity (e.g. Nimorazole) [52].

A total of 18 studies have been reported using various hypoxic sensitizers [31–37,40–52,55]. In general the outcome seems to be irrespective of the use of the specific sensitizer although the use of Etanidazole [48,50] tend to show a limited, if any, benefit (Fig. 2). Although only two hypoxic sensitizer trials by themselves showed a significant improvement in loco-regional control, namely the DAHANCA 2 and DAHANCA 5 [46,52], this was probably more a consequence of these trials being among the few with a sufficient large number of patients and thereby statistical power to yield such outcome.

Overall, however, it can be concluded from the meta-analysis that hypoxic modification is able to reduce the risk of having loco-regional failure after radiotherapeutic treatment for head and neck cancer. The benefit of such treatment appears to be irrespective of the methods used for hypoxic modification.

Disease specific death

Thirty of the randomized studies could be evaluated for the endpoint of disease specific death (Fig. 3). The outcome was of almost the same magnitude as the loco-regional failure rate. When comparing the rates of loco-regional failure and disease specific death in the individual studies, a prominent relationship was found. Supplementary Fig. 1 shows the difference in loco-regional control and its related difference in disease specific survival. On an average, the loco-regional improvement translated into a survival benefit in approximately 60% of the patients. The outcome as a function of the various forms of hypoxic modification was consequently similar to that observed for loco-regional failure with a tendency that patients treated with normobaric oxygen had less benefit and those treated with hyperbaric oxygen seem to have

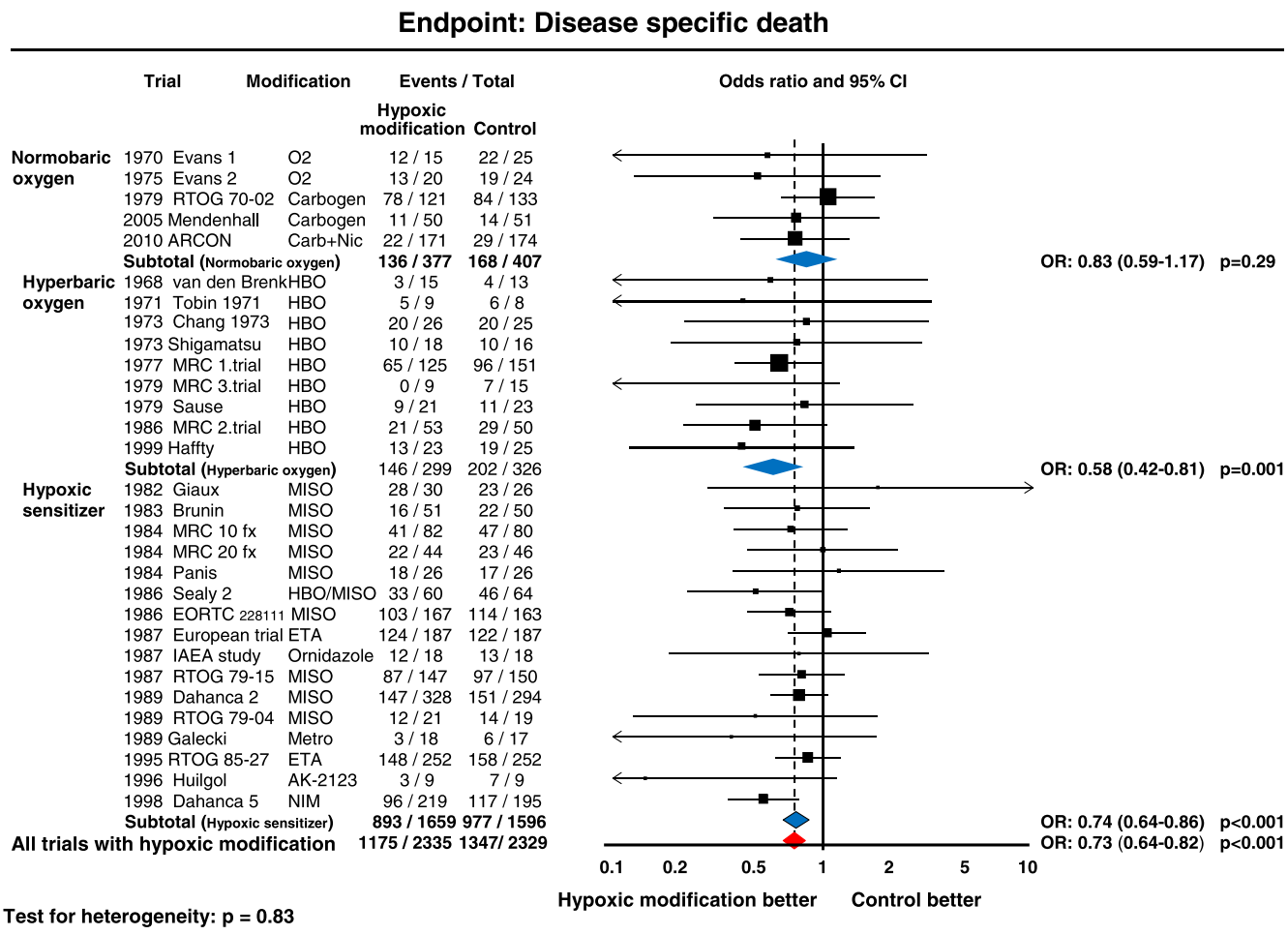

Fig. 3. Hypoxic modification of radiotherapy in HNSCC. Influence on disease specific survival as a function of type of hypoxic modification.

the best outcome (Fig. 3). This support that there is a strong relationship between loco-regional control and probability of surviving the disease because head and neck cancer in general is characterised by a pattern of failure which is limited to the T- and N-position [74,75].

Overall survival

By using overall death as the endpoint a similar although less prominent pattern was found in the analysis of all 29 trials where this endpoint could be evaluated; namely that the use of hypoxic modification results in a significant improved overall survival (Fig. 4). This was, however, less prominent than the improvement in disease specific survival and may reflect that the cohort of patients with HNSCC have a relatively high risk of dying from other diseases, especially those related to excess use of tobacco and alcohol. However, also the overall survival improvement showed a significant relationship with loco-regional tumour control (Supplementary Fig. 2). Again the benefit was mainly related to patients treated with hyperbaric oxygen and hypoxic sensitizers whereas it was not observed in the patients treated with normobaric oxygen. The number of patients in the latter trials was relatively sparse, but the observation may also be due to the fact that the patients in these studies predominantly have laryngeal carcinomas which tend to have higher co-morbidity [76].

Distant metastases

Also the potential effect of hypoxic modification in the treatment of the primary tumour was investigated on the risk of developing distant metastases (Supplementary Fig. 3). Distant metastases are generally a consequence of loco-regional failure in head and neck cancer, but they occur, on the other hand, rather infrequently. When analysing 12 trials where such data were available, a slight but not significant relationship in favour of hypoxic modification could be observed (Fig. 1). This highlights the fact that that benefit of hypoxic modification obviously is a consequence of an improved loco-regional control and that the potential reduction in distant metastases in turn most likely was due to improved tumour control in the loco-regional site.

Radiation related late complications

The various forms of hypoxic modifications may possess side effects related to the use of especially nitroimidazoles in the form of acute gastrointestinal toxicity expressed by nausea and vomiting and for the 2-nitromidazoles also peripheral neurological complications. Similarly, there may be problems related to the use of carbogen, nicotinamide or HBO, but all such complications were temporary and mainly relate to compliance with treatment. Of greater importance may be the potential influence on the radiotherapy related late morbidity. Information of such parameters

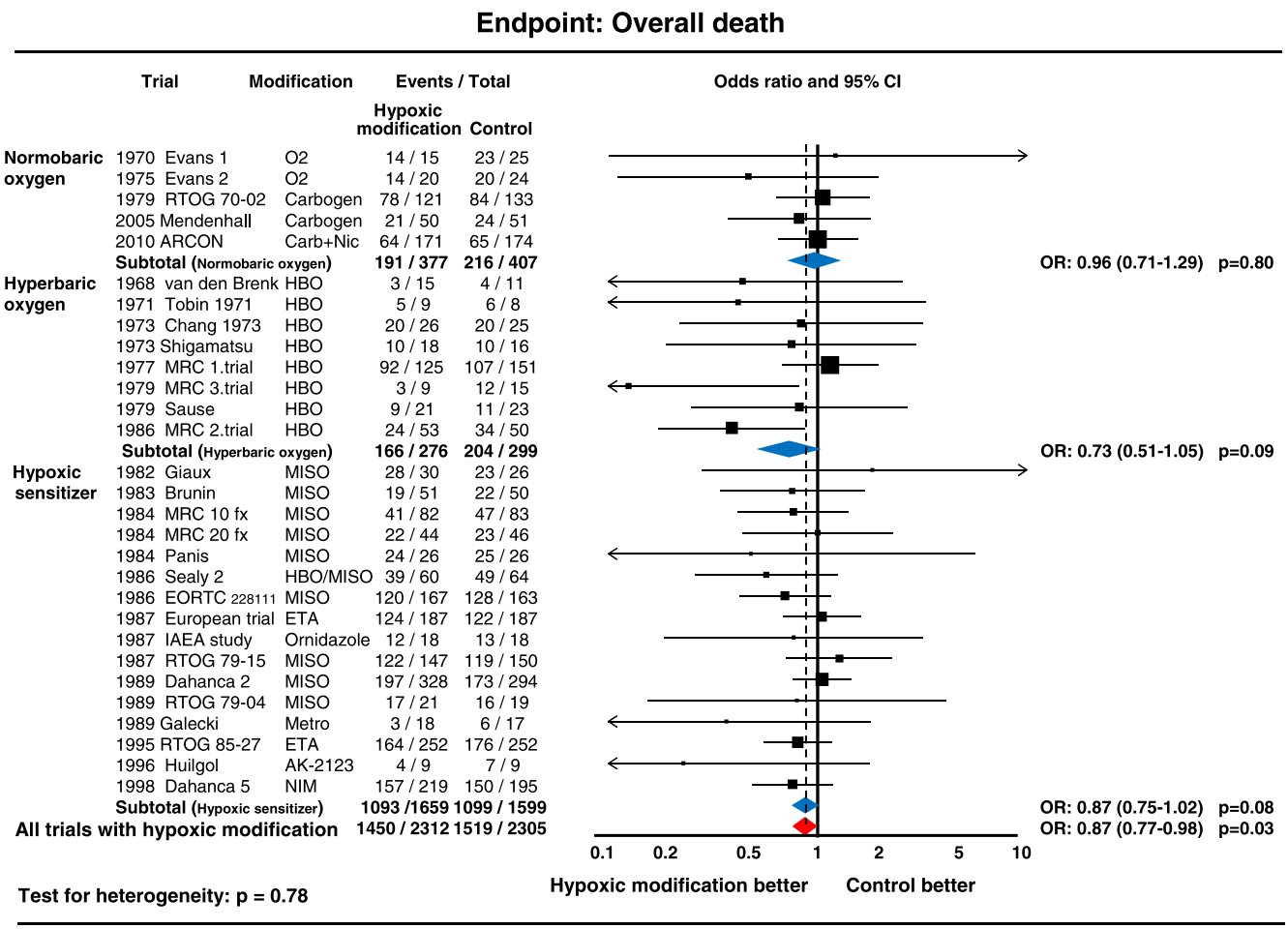

Fig. 4. Hypoxic modification of radiotherapy in HNSCC. Influence on overall survival as a function of type of hypoxic modification.

could be obtained from 23 trials. Overall there was no difference between the use and no use of hypoxic modifications (odds ratio: 1.00, 95% cf.l. 0.82–1.23; $p = 0.96$). A similar conclusion was also observed for the use of hypoxic sensitizers, whereas the studies using HBO showed a significant increase in the risk of radiation related complications ((OR: 2.43, 95% cf.l. 1.43–4.12; $p < 0.01$) (Supplementary Fig. 4). However, this excess morbidity was mainly observed in trials using a large dosage per fraction where the risk of late morbidity is known to be relatively high), and where the additional influence of hyperbaric oxygen may add to such an outcome [27]. In some trials, this was further enhanced by the before mentioned difference in trial design where patients given HBO also were treated with large doses per fraction and thereby in turn developed a higher probability of late radiation related morbidity when compared with control patients given conventional fractionation. Consequently, there is no definitive evidence of any excess morbidity associated with the hypoxic modification itself, although it has been suggested hat HBO may result in some increase in complications related to structures assumed to be potentially hypoxic, such as, e.g. laryngeal cartilage [27].

Influence of dose per fraction

As previously indicated, the dose per fraction may influence the magnitude of the benefit from hypoxic modification. Table 1 shows the hypoxic modification as a function of whether it was included in trials given with hypofractionation or applied in a conventional fractionation scheme.

The use of hypofractionation results in a more pronounced benefit of hypoxic modification, but also the use of hypoxic modification in connection with the use of conventional 2 Gy per fraction, yielded a significant difference, both when using loco-regional failure or disease specific death as the endpoint (Table 2). On the contrary, radiation related complications were significantly enhanced after hypoxic modification given with hypofractionation, whereas no influence on late radiation related morbidity was found in trials using conventional schedules (Supplementary Fig. 4). This indicates that hypoxia may be a greater problem in hypofractionated radiotherapy and should, for instance, be considered when treating potential hypoxic tumours with large doses per fraction, such as in treatments using stereotactic body radiotherapy, high dose rate brachytherapy or IMRT with integrated boost.

The excess radiation related morbidity seen after hypofractionated treatment, is mainly observed in HBO trials where hypoxic modification is given together with hypofractionation but compared with normal fractionated controls, thus it may more be a consequence of the fractionation regime, rather than an effect induced by the hypoxic modification (Supplementary Fig. 4). Although the curative benefit of hypoxic modification may be more pronounced with increasing dose per fraction, there is also strong evidence from the current overview that such hypoxic modified radiotherapy is significantly beneficial when applied together with

Table 2
Effect of hypoxic modification of radiotherapy of HNSCC given with different dose per fraction schedules.

Fractionation pattern	Endpoint and Odds Ratio (95% CI)		
	Loco-regional failure	Disease specific death	Late radiation related morbidity
Hypo-fractionation[a]	0.56 (0.40–0.77) $p > 0.001$	0.62 (0.44–0.86) $p > 0.001$	1.83 (1.05–3.18) $p > 0.03$
Conventional fractionation[a]	0.77 (0.67–0.89) $p > 0.001$	0.78 (0.67–0.90 $p > 0.001$	0.90 (0.71–1.14) $p > 0.39$

[a] The same fractionation pattern has been applied in hypoxic modification and control arms.

radiotherapy given with conventional fractionated schedules in the treatment of head and neck cancer (Table 1).

Magnitude and cost of hypoxic modification

The magnitude of hypoxic modification resulted in a risk reduction of approximately 8% for loco-regional failure and disease specific death (Fig. 1), which was of the same magnitude as that achieved by accelerated fractionation [11], but slightly less than that obtained by simultaneous chemoradiotherapy [12] or hyperfractionated radiotherapy [11]. This benefit is, however, achieved without any detectable enhancement of radiation related morbidity and as such, it represents a pure long-term gain although the acute morbidity linked with some of the sensitizers may result in some discomfort, but without life threatening or persistent morbidity.

To understand the clinical magnitude of this risk reduction using hypoxic modification was the "number of patients needed to treat" to achieve benefit (NNT) calculated for the various endpoints (Fig. 1). For the primary cancer related endpoints of loco-regional control and disease related survival it was estimated that every time approximately 13 patients were treated did on average one patient benefit from the use of hypoxic modification. Since it does not cause any persistent or serious side effects, does it in full justify the use of hypoxic modification, also because the other (economical and labour) related costs are small, especially when compared to the treatment with, e.g. biological modifiers or chemotherapy.

Discussion

Squamous cell carcinoma of the head and neck has been a paragon tumour for studying the effect of clinical radiobiological modification, and many of our biologically based treatment strategies have been derived from studies in head and neck cancer. This includes trials with targeted radiotherapy, chemoradiotherapy, altered fractionation and hypoxic modification. Thus recent meta-analyses have overviewed the experience with chemoradiotherapy and altered fractionation [11,12]. The current analysis adds to this experience by giving the first complete overview of randomized controlled clinical trials investigating the use of hypoxic modification.

Hypoxia was the first radiobiological topic which was studied in large scale in randomized clinical trials and most of the studies have been performed for 25 or more years ago (Table 1). A meta-analysis of such trials, therefore, suffer from limited access to individual patient data and often also from more sporadic reporting of the studies. It has consequently not been possible to perform an individual patient analysis as it had been the case with the other meta-analyses in radiotherapy of HNSCC [11,12]. Therefore, the current report is primarily based on data obtained from published material. Nevertheless, the network of investigators related to the trials with hypoxic modification was rather close, and it is the impression that the current report contains all of the completed controlled clinical trials performed and reported within this topic. This is partly one of the reasons why some of the studies have a small number of patients, as they have simply not been performed or completed in a sufficiently large scale. The report is consequently also limited by difficulties in obtaining homogenous endpoints, and especially as the observation time described in the various studies is not uniform, although by far most of the data, are based on reports giving at least 2–5 years of follow up time. (Table 1). To the extent it has been possible and needed, the collection of data has been followed by personal contacts to the trial investigators, in order to achieve additional information or clarify potential issues of doubt.

The current meta-analysis has been limited to evaluation of hypoxic modification of radiotherapy only. Thus more recent studies involving chemoradiotherapy have not been included [16,17,77]. This is in order to investigate the proper radiobiological effect of hypoxic modification, but also because the number of studies dealing with chemoradiotherapy as hypoxic modifier is rather limited, and further have shown the importance and need of securing proper quality assurance in the evaluation [78,79].

Overall the meta-analysis shows that radiotherapy of HNSCC significantly benefits from hypoxic modification in the form of a significantly improved loco-regional control, disease specific control and overall survival. This happens irrespective of the type of hypoxic modification although most data have been obtained by hypoxic sensitizers and consequently the information from such a modification seems most stable. The number of trials and patients treated with normobaric oxygen was rather limited. The studies with hyperbaric oxygen were in general of older data as they constitute the first hypoxic modification investigated in clinical trials. Many of these trials have been performed with high doses per fraction and the interpretation of the outcome should, therefore, take such fractionation into consideration. However, the meta-analysis has also shown that although hypofractionated tumours may have greater need for hypoxic modification, the benefit is also present in tumours treated with conventional fractionation and, therefore, hypoxic modification is an issue to be considered in modern radiotherapy.

The meta-analysis presents also for the first time a significant benefit in overall survival and consequently the current meta-analysis yield level 1a evidence for using hypoxic modification as a part of radiotherapy treatment for head and neck cancer.

The findings also add to the arguments for optimal radiobiologically based treatment of HNSCC. The interaction between the different variations of radiobiological based therapeutic strategy has not yet been fully clarified, but as such, there seems to be no negative interaction between the strategies, and hypoxic modification may be expected to yield additional therapeutic benefits when added to treatment regimens using both modified fractionation, chemoradiotherapy and biological modifiers although no specific controlled trials so far have addressed such options.

The potential benefit of using hypoxic modification has also been evaluated in other tumour sites and types, and a benefit have

especially been associated with squamous cell carcinomas [8–10], whereas the data from other tumour sites with different histopathology are too sparse and need further studies to be conclusive [8–10]. The reason why squamous cell carcinomas are likely to include clonogenic hypoxic stem cells may be found in the natural history of the epithelium from which the tumours have been derived. This may contain relatively hypoxic cells due to the original avascular nature of the epithelium. Therefore, it is likely that tumour cells derived from such a tissue posses the ability to withstand low oxygen tensions in the range of radiobiological hypoxia. These tumour cells may consequently not necessarily turn into necrosis under short duration of hypoxia, but may be able to survive for a long time and thereby become a nidus for recurrence. The killing of such hypoxic tumour stem cells by radiotherapy is essential to secure tumour control [80–83], and the use of hypoxic modification may add to this effect.

Does all patients need hypoxic modification?

Although the meta-analysis shows a global benefit of hypoxic modification, it has not been clarified whether this benefits all patients with HNSCC, or if it is associated with specific tumour subtypes or biological features. The scenario of head and neck cancer is currently undergoing an epidemiological change and HPV/p16 positive tumours of especially the oropharynx are fast increasing to become the most frequent type of head and neck cancer in the western world, also because the traditional smoking related tumours are on a decline [84–86]. A recent re-analysis of a large randomized trial with the hypoxic modifier Nimorazole suggests that HPV/p16 positive tumours may not be in need of such hypoxic modification [87]. Unfortunately the power of this analysis was limited due to the relatively small fraction of HPV/p16 positive tumours in the study, and the issue may demand further confirmatory exploration. Several other analyses have also pointed towards prognostic and predictive biological identification of HNSCC which may request hypoxic modification [88–90]. This include studies of hypoxic markers, such as direct oxygen measurement [91–94], detection of pimonidazole by immunohistochemistry [95–97], or biological PET-imaging with MISO or FAZA [98–106], plasma measurements of osteopontin [107,108] and most recently, identification of hypoxic related proteins and genes [109–116], of which one gene expression profile has shown a strong predictive value for the use of the hypoxic modifier Nimorazole [116]. However, it should be noted that the prognostic and predictive value of such markers may not may be valid for the evaluation of HPV positive tumours [86,116,117].

Concluding remarks

With the information from the present meta-analysis, and the potential future ability to identify subsets of tumours which may especially benefit from hypoxic modification of radiotherapy, the focus is once again on the issue of hypoxia in head and neck tumours, and on how it can be modified. The current information strongly supports that there is a biological rationale and a valid treatment strategy, and when used it may result in improved loco-regional tumour control and consequently an improved survival probability.

Hypoxia is by far the most explored, and most widely cited [10], biological phenomena in radiotherapy, but yet with limited impact on daily routine practice, or as it has been expressed: hypoxia is "adored and ignored" [10]. That hypoxia can cause clinical radioresistance has been known for more than a century [8–10], and since the pivotal work by Gray and colleagues [7] have attempts to overcome it been explored in controlled clinical trials. More than 10,000 patients have been included in these studies, most of those being trials including patients with head and neck carcinoma. Although the reports of most trials suffer from being performed long time ago and, therefore, only includes limited information, does the present meta-analysis obviously contain sufficient data to yield convincing evidence in favour of an improved overall loco-regional tumour control and survival. Thus, hypoxic modification should be a part of the optimal radiotherapeutic treatment strategy of head and neck cancer, and omission of this will bear the risk of a poorer outcome. Furthermore does the apparent relationship with dose per fraction point towards an even stronger need for hypoxic modification when a fraction size above 2 Gy is used. Since this often happens in current IMRT treatments when using an integrated boost, does it further underline the need for using hypoxic modification.

That some patient groups may be identified to have more or less need for hypoxic modification does not change the overall concept: that hypoxic modification of radiotherapy for HNSCC is indicated unless *lack* of hypoxic resistance can be demonstrated by biological profiling, by specific tumour characteristics (e.g. HPV/p16 status), etc. Although the current meta-analysis only investigates the use of hypoxic modification given in conjunction with primary curative intended radiotherapy alone, are there no indications that hypoxic modification should not be equally needed when radiotherapy is given together with biological modifiers or chemotherapy.

In conclusion, the meta-analysis gives level 1a evidence for an improved tumour control and survival when hypoxic modification is given in conjunction with curative intended radiotherapy of squamous cell carcinoma of the head and neck. This is obtained with out excess radiation related late morbidity, and the use of hypoxic modification does, therefore, yield a true therapeutic gain. Unless lack of hypoxic radioresistance can be demonstrated in the tumours, hypoxic modification must be needed as a part of the optimal radiotherapeutic treatment strategy in patients with squamous cell carcinoma of the head and neck.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.radonc.2011.03.004.

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