



HAL
open science

TORquegueusie avant, pendant et après traitement d'un Cancer des voies AéroDigestives supérieures : étiologies, impact sur la qualité de vie (TORCAD)

Guillaume Buiret

► To cite this version:

Guillaume Buiret. TORquegueusie avant, pendant et après traitement d'un Cancer des voies AéroDigestives supérieures : étiologies, impact sur la qualité de vie (TORCAD). Biodiversité et Ecologie. Université Bourgogne Franche-Comté, 2024. Français. NNT : 2024UBFCI009 . tel-04774173

HAL Id: tel-04774173

<https://theses.hal.science/tel-04774173v1>

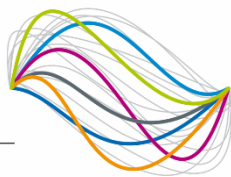
Submitted on 8 Nov 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

UBFC

UNIVERSITÉ
BOURGOGNE FRANCHE-COMTÉ



**THESE DE DOCTORAT DE L'ETABLISSEMENT UNIVERSITE BOURGOGNE FRANCHE-COMTE
PREPAREE AU CENTRE DES SCIENCES DU GOUT ET DE L'ALIMENTATION**

Ecole doctorale n°554

Ecole doctorale Environnements Santé

Doctorat de Sciences agronomiques

Par

M. BUIRET Guillaume

TORquegueusie avant, pendant et après traitement d'un Cancer des voies AéroDigestives
supérieures : étiologies, impact sur la qualité de vie (TORCAD)

Thèse présentée et soutenue à Dijon, le 25 octobre 2024.

Composition du Jury :

Pr Claire Falandry
Pr Thomas Hummel
Dr Agnès Giboreau
Dr Jérôme Fayette
Pr Florian Scotté
Dr Gilles Feron
Dr Thierry Thomas-Danguin

Oncogériatre, HCL / UCBL1
Oberarzt , Université Dresde
Directrice, Institut Lyfe
Oncologue, Centre Léon Bérard
Oncologue, Institut Gustave Roussy
Directeur de Recherche, CSGA
Directeur de Recherche, CSGA

Présidente
Rapporteur
Rapporteuse
Examineur
Examineur
Directeur de thèse
Codirecteur de thèse



ÉCOLE DOCTORALE Environnements - Santé

Bourgogne | Franche-Comté

Titre : TORqueueusie avant, pendant et après traitement d'un Cancer des voies AéroDigestives supérieures : étiologies, impact sur la qualité de vie (TORCAD)

Mots clés : torqueueusie, goût métallique, cancer des voies aérodigestives supérieures, malondialdéhyde

Résumé :

Contexte : Le goût métallique est fréquemment rapporté par les patients pendant le traitement d'un cancer des voies aérodigestives supérieures. On sait très peu de choses sur ce symptôme. Une étude prospective d'observation a été menée pour en déterminer l'incidence et la ou les causes, ainsi qu'un traitement potentiel. Deux hypothèses ont été avancées : la lipoperoxydation de la muqueuse buccale et la libération de l'inhibition du nerf facial sur le nerf glossopharyngien.

Méthodes : 44 patients ont été suivis avant, pendant et jusqu'à un an après la fin du traitement pour un cancer de la tête et du cou. Des évaluations par questionnaires, des prélèvements salivaires et des tests supraliminaires d'intensité et de reconnaissance du goût ont été effectués régulièrement, jusqu'à huit fois, pendant un an. Un antidote potentiel, le bain de bouche à la lactoferrine, a également été proposé en cas de goût métallique.

Résultats : Le « goût » métallique a affecté 27,2% des patients, avec une intensité

Résultats : Le « goût » métallique a affecté 27,2% des patients, avec une intensité modérée

à sévère, pendant la radiothérapie ou la radiochimiothérapie, avec une association significative avec la présence d'une mucite orale et un stress oxydatif sur les marqueurs salivaires. Le goût métallique a eu un impact significatif sur la qualité de vie liée au goût, mais pas sur l'échelle visuelle analogique de l'ingestion ou du poids. L'hypothèse étiologique de la libération de l'inhibition du nerf facial sur le nerf glossopharyngien n'a pas été vérifiée. Il n'y avait pas plus de stress oxydatif dans le groupe goût métallique que dans le groupe sans goût métallique. Enfin, le goût métallique a pu être atténué par un bain de bouche à la lactoferrine dans 63 % des cas (complètement dans 16 % et partiellement dans 47 %).

Conclusion : le goût métallique est une plainte fréquente et intense chez les patients atteints de cancer des voies aérodigestives supérieures, pendant la radiothérapie ou la radiochimiothérapie, en particulier en cas de mucite. Les causes précises n'ont pas encore été entièrement identifiées. Le bain de bouche à la lactoferrine semble atténuer ce symptôme.

Title : Metallic taste before, during and after treatment of head and neck cancer : causes, impact on quality of life (TORCAD)

Keywords : metallic taste, head and neck cancer, malodialdehyd

Abstract :

Background: Metallic taste is frequently reported by patients during treatment for head and neck. Very little is known about this symptom. A prospective observational study was carried out to determine the incidence and cause(s) and a potential treatment. Two hypotheses were put forward: lipoperoxidation of the oral mucosa, and release of facial nerve inhibition on the glossopharyngeal nerve.

Methods: 44 patients were followed before, during and up to one year after completion of treatment for head and neck cancer. Evaluations by questionnaires, salivary sampling and supraliminal taste intensity and recognition tests were carried out regularly, up to eight times, for one year. A potential antidote, lactoferrin mouthwash, was also proposed in case of metallic taste.

Results: The metallic “taste” affected 27.2% of patients, with moderate to severe intensity,

during the radiotherapy or radiochemotherapy, with a significant association with the presence of oral mucositis and an oxidative stress on salivary markers. The metallic taste had a significant impact on taste-related quality of life, but not on the visual analog scale of ingestion or weight. The etiological hypothesis of release of facial nerve inhibition on the glossopharyngeal nerve was not verified. There was no more oxidative stress in the metallic taste group than in the no-metallic taste group. Finally, the metallic taste could be alleviated by lactoferrin mouthwash in 63% of cases (completely in 16% and partially in 47%).

Conclusion: metallic taste is a frequent and intense complaint in patients with head and neck cancer, during the radiotherapy or radiochemotherapy, especially in cases of mucositis. The precise causes have not yet been fully identified. Lactoferrin mouthwash seems to alleviate this symptom

REMERCIEMENTS

Aux membres du jury :

To Pr Thomas HUMMEL

I am deeply honored to have had you as a raporter of my PhD on the metallic taste in head and neck oncology. Your pioneering work at the Smell & Taste Clinic at TU Dresden and your extensive research on the physiology and pathology of the human chemical senses have been incredibly influential. Your development of the smell training approach, now a global standard, and your insights into the chemosensory systems have profoundly shaped my understanding and approach to this research. Your valuable feedback and expert guidance have been indispensable in refining my work. Furthermore you are an very approachable person, when I asked you pieces of advice by mail or when we personally met in Reykjavik. Thank you for your time and invaluable contributions.

Au Dr Agnès GIBOREAU

Je tiens à t'exprimer ma profonde reconnaissance d'avoir accepté d'être rapporteuse pour ma thèse sur le goût métallique en cancérologie ORL. Même si nous n'avons jamais pu travailler ensemble, nos contacts réguliers durant ces années m'ont toujours été utiles. Tes recherches innovantes sur la perception sensorielle et l'alimentation à l'Institut Paul Bocuse / Lyfe ont été une source d'inspiration majeure pour mon travail. Merci infiniment pour tes remarques constructives et ton soutien continu.

Au Pr Claire FALANDRY

Nous nous sommes rencontrés au congrès de l'AFSOS avant l'épisode COVID. Vos contributions à l'oncogériatrie et votre engagement à améliorer les traitements pour les patients âgés en oncologie sont admirables. Votre expertise unique et vos recherches approfondies sur l'intersection entre vieillissement et cancer ont offert des perspectives précieuses et inspirantes à mon travail en soins oncologiques de support en général. Je vous adresse mes sincères remerciements d'avoir accepté de siéger dans mon jury de ma thèse.

Au Dr Florian SCOTTE

Monsieur le Président, je souhaite te remercier chaleureusement pour ta participation en tant que membre du jury de ma thèse sur le goût métallique en cancérologie ORL. Ton engagement envers les soins de support en oncologie et ton approche centrée sur le patient à l'HEGP, l'Hôpital Foch et l'IGR ont profondément influencé mon approche de la recherche en soins oncologiques de support. Ton expertise clinique et ta passion communicative pour l'amélioration de la qualité de vie des patients sont un exemple pour moi. Merci pour tes précieux conseils et ton soutien constant.

Aux membres du jury et de mon comité de suivi de thèse

Au Dr Jérôme FAYETTE

Je te suis profondément reconnaissant pour ton rôle en tant que membre du jury et de comité de suivi de ma thèse. Ton expertise internationale en cancérologie médicale au Centre Léon Bérard, particulièrement dans le traitement des cancers ORL, a été une source d'inspiration majeure pour mes travaux de recherche et une source inépuisable de conseils de traitement pour mes patients. Merci pour tes commentaires éclairés et ton soutien indéfectible.

A mes directeurs de thèse

Au Dr Gilles FERON

Je tiens à t'exprimer ma plus profonde gratitude pour ta direction de thèse et ton soutien indéfectible tout au long de ma thèse sur le goût métallique en cancérologie ORL. Ton expertise au sein du CSGA, à l'INRAE et maintenant à l'Agence Nationale de la Recherche et ton travail sur la salive, les interactions entre la perception sensorielle et les aliments ont été d'une grande

inspiration. Ton encadrement et tes conseils ont été essentiels pour mener à bien cette recherche. Merci infiniment pour ta patience et ton engagement. Je suis particulièrement touché car a priori, je serai ton dernier thésard.

Au Dr Thierry THOMAS-DANGUIN

Je t'adresse mes sincères remerciements pour ta co-direction et ton soutien continu pendant ma thèse. Ton expertise en psychophysique sensorielle et en analyse des interactions multisensorielles au CSGA a été un support essentiel à mon travail. Tes conseils avisés et ta rigueur scientifique ont été des atouts précieux dans la réalisation de cette recherche, surtout sur la dernière ligne droite avant soumission. Merci pour ta disponibilité, pour m'avoir poussé dans mes retranchements biostatistiques ainsi que pour m'avoir toujours encouragé mon développement académique et personnel.

Aux personnes ayant participé, de près ou de loin à cette thèse :

Aux patients, pour leur grande patience

A Blandine Molandre et Clémence Aubenas :

Merci d'avoir fait l'acquisition de toutes les données. Votre rigueur dans cette étude de recherche clinique, nouveauté pour vous, ont été la pierre angulaire de ce travail.

A Hélène BRIGNOT :

Je souhaite exprimer ma plus profonde gratitude pour votre aide précieuse dans la réalisation des analyses salivaires. Votre expertise en métabolomique salivaire et en enzymes salivaires a été essentielle pour obtenir des résultats de haute qualité, qui ont constitué le critère principal de mesure de mon étude. Votre soutien technique, les travaux préparatoires et vos conseils avisés ont rendu possible de cette recherche. Merci infiniment pour votre temps et votre dévouement.

A Chantal SEPTIER :

Je tiens à vous exprimer mes plus sincères remerciements pour votre aide inestimable tout au long de ma thèse. Votre disponibilité et votre soutien pour l'envoi des testants ont été cruciaux pour la progression de mes recherches. Je vous suis profondément reconnaissant pour votre engagement et votre bienveillance.

A Justine PARISE, étudiante en M1, qui a réalisé la partie préparatoire pour les tests sensoriels gustatifs

A Clémence RAYOT, étudiante en M1, qui a réalisé la partie préparatoire pour les dosages salivaires de malondialdéhyde

A Sandrine BEAUCHARD, pour ton soutien logistique sans faille

A Charles MOLTA pour ton aide à la traduction anglaise et tes conseils précieux

A Julie AUGER et Audrey BOULIER, de la société Ingredia, pour avoir soutenu le projet en fournissant la lactoferrine.

Aux comités de Lutte contre le Cancer de la Drôme et de l'Ardèche pour leur soutien financier et leur curiosité quant aux résultats de cette étude. Merci également pour tout le bien que vous faites à tous les patients que vous aidez.

A Freddy SERVEAUX, ancien Directeur du CH de Valence :

Je tiens à vous exprimer ma profonde gratitude pour le soutien que vous m'avez apporté pendant votre direction à Valence. Votre analyse perspicace et vos compétences en gestion ont été d'une aide précieuse, non pour ces travaux mais pour la gestion de mon service. Votre expérience à la tête du centre hospitalier de Valence et maintenant du CHU de Dijon Bourgogne témoigne de votre engagement envers l'excellence dans le domaine de la santé, et j'ai eu la chance de bénéficier de vos conseils. Merci infiniment du temps que vous m'avez consacré et pour vos encouragements.

A mes collègues de travail, passés, présents et futurs

A la formidable équipe médicale et paramédicale d'ORL du CH Valence, qui font que tous les matins sont une joie d'aller travailler.

A l'ensemble des internes et assistants : participer à votre formation, vous voir grandir et vous autonomiser est un pur bonheur.

A ma famille

A Claire

Je veux te dire merci du fond du cœur pour tout ton soutien et ta patience tout au long de cette aventure scientifique, et pour mon travail hospitalier plus généralement. Sans ton aide, tes conseils, tes encouragements et ta compréhension, je n'aurais pas pu mener à bien tous ces travaux. Tu es mon roc, mon soutien inébranlable, et je suis infiniment reconnaissant de t'avoir à mes côtés.

Avec tout mon amour

A mes enfants Thomas et Romane,

Vous avez apporté tant de joie, de rires et de lumière dans ma vie. Merci pour votre patience et pour votre soutien silencieux. Votre présence a été une source de motivation constante et je suis tellement fier de vous. Vous êtes ma force et mon inspiration.

A ma mère

Je tiens à te remercier pour votre soutien et ton amour, même si ce travail peut sembler difficile à comprendre. Tes encouragements m'ont aidé à traverser cette période exigeante.

A ma sœur

Merci pour ton soutien. Tes encouragements ont été d'une grande aide pour moi. Ton soutien constant a été très précieux.

A mon père, qui me manque tous les jours.

Table des matières

REMERCIEMENTS	5
Table des matières	9
Table des figures.....	11
Table des tableaux.....	13
INTRODUCTION	15
ETAT DE L'ART	19
1. Causes et traitements de la dénutrition et des altérations sensorielles chez les patients atteints d'un cancer des voies aérodigestives supérieures.....	20
2. Perception du goût métallique.....	31
3. Problematics on metallic taste	37
MATERIEL ET METHODES	39
1. Partie réglementaire	40
2. Tests sensoriels.....	41
3. Dosages salivaires du malondialdéhyde.....	43
4. Questionnaires	59
RESULTATS.....	61
1. Metallic taste prevalence in patients treated for cancer: A systematic literature review and meta-analysis.....	62
2. The Nutritional Impact of Metallic Taste in Head and Neck Cancer Patients: Explorations and Clinical Implications.....	74
3. Is metallic perception a taste, an aroma or a flavor?.....	83
4. Evaluating the Etiology of Metallic Taste During Head and Neck Cancer Treatments: A Study of Facial and Glossopharyngeal Nerve Interactions	89
5. Exploring the Role of Oxidative Stress in Metallic Taste During Head and Neck Cancer Treatment: A Study of Salivary Malondialdehyde and Therapeutic Interventions.....	99
6. Evolution des capacités de reconnaissance et d'intensité gustatives sur différentes régions de la langue chez des patients atteints de cancer des voies aérodigestives supérieures	108
DISCUSSION - SYNTHESE	117
CONCLUSION	121
BIBLIOGRAPHIE.....	125
ANNEXES.....	141
ANNEX 1: Traduction anglaise de l'article : causes and treatments for undernutrition and sensory alterations in patients with head and neck cancer	142
ANNEX 2. Traduction en anglais du chapitre sur la perception du goût métallique de l'Encyclopédie des Sciences.....	152
ANNEX 3 : Mode opératoire des tests gustatifs.....	158
ANNEX 4: final protocol of MDA dosage	161

ANNEX 5: questionnaires	168
ANNEX 6: Supplementary appendix to Support Care Cancer 2022;30:5691-702 (11)	179
ANNEX 7: Supplementary files to the manuscript “Evaluating the Etiology of Metallic Taste During Head and Neck Cancer Treatments: A Study of Facial and Glossopharyngeal Nerve Interactions”	196
ANNEX 8: Supplementary files to the manuscript “Exploring the Role of Oxidative Stress in Metallic Taste During Head and Neck Cancer Treatment: A Study of Salivary Malondialdehyde and Therapeutic Interventions”	200
ANNEX 9 : Traduction en anglais de l’article sur l’évolution des capacités de reconnaissance et d’intensité gustatives sur différentes régions de la langue chez des patients atteints de cancer des voies aérodigestives supérieures	206
ANNEX 10: supplementary files to the manuscript “evolution of taste recognition and intensity in different regions of the tongue in patients with head and neck cancer”	214

Table des figures

Figure 1 : schématisation des causes de dénutrition chez un patient atteint d'un cancer des voies aérodigestives supérieures.....	22
Figure 2: schéma de l'hypothèse de la levée d'inhibition du nerf facial sur le nerf glossopharyngien.	28
Figure 3: Rapports de la corde du tympan et du tympan.....	33
Figure 4: Schéma de la levée d'inhibition.....	34
Figure 5: composés volatils.....	35
Figure 6: Structure de la lactoferrine.....	36
Figure 7: Temporalité des tests.....	40
Figure 8: order and location of application of sapid compounds on the tongue	42
Figure 9: Illustration d'un test sensoriel.....	43
Figure 10: graphical abstract	44
Figure 11: protocol validation (1/3)	51
Figure 12: protocol validation (2/3)	54
Figure 13 : step-by-step approach of the analytical method.....	55
Figure 14: optimized sample MDA yield considering the study conditions and context.....	57
Figure 15: protocol validation (3/3) :	58
Figure 16: PRISMA flowchart.	65
Figure 17: Review authors' judgments of the risk of bias for each item, presented as percentages across all included studies.	66
Figure 18: Random effect meta-analysis of MT prevalence in cancer patients.	67
Figure 19: Funnel plot for random effects of meta-analysis of MT prevalence rate versus standard error	67
Figure 20: Study flowchart.....	76
Figure 21: evolution of MT intensity according to the patients who experienced it.	78
Figure 22: weight (kg) evolution.....	79
Figure 23: Changes in Visual Analog Scale scores for food intake according to Time Point.....	80
Figure 24: Changes in EORTC HN35 question #44 ("During the last week, did you have trouble with taste?") according to Metallic Taste status and time point.....	81
Figure 25: test location.....	84
Figure 26: Age according to the nasal occlusion.....	85
Figure 27: metallic intensity perception according to the test location.....	86
Figure 28: metallic intensity perception according to the test location.....	87
Figure 29 : study flowchart	91
Figure 30: evolution of results for bitter taste according to the precise location on the tongue.....	93
Figure 31: evolution of taste results for salty taste according to the precise location on the tongue	94
Figure 32: evolution of taste results for sour according to the precise location on the tongue	95
Figure 33: evolution of taste results for sweet according to the precise location on the tongue	96
Figure 34: study flowchart	102
Figure 35: evolution of salivary MDA concentration ($\mu\text{mol/l}$) during the follow-up.	104
Figure 36: qualitative efficacy of lactoferrin mouthwash in patients with metallic taste according to the evaluation moment, based on patient-reported perceptions of attenuation across three modalities....	105
Figure 37: diagramme de flux de l'étude	110
Figure 38 : évolution de la réponse à la question « Durant la semaine passée, avez-vous eu des problèmes de goût ? » (EORTC HN35 n°14).....	111
Figure 39: évolution de la reconnaissance des saveurs en fonction du moment de l'évaluation et selon la localisation sur la langue	112

Figure 40: évolution de l'intensité des saveurs en fonction du moment de l'évaluation et selon la localisation sur la langue 113

Table des tableaux

Tableau 1: Principaux mécanismes et causes des troubles de perception des saveurs en cancérologie des voies aérodigestives supérieures	25
Tableau 2: Seuils de détection par voie rétronasale (nez ouvert) ou gustative (nez fermé) de solution de fer et de cuivre.....	35
Tableau 3 : substances retenues pour les tests gustatifs.....	41
Tableau 4 : premier essai de concentration des composés sapides	41
Tableau 5 : deuxième essai de concentration des composés sapides, avec des concentrations supérieures.....	41
Tableau 6: Specifications table.....	45
Tableau 7: Fluorescence values for reaction mixtures with and without TBA.	52
Tableau 8: Presentation of blank values and pH before and after incubation	53
Tableau 9: list of the 22 selected articles.....	73
Tableau 10: studied population characteristics at inclusion according to metallic taste status.	77
Tableau 11: Impact of Metallic Test as assessed by the specific metallic taste questionnaire.....	78
Tableau 12: population characteristics	92
Tableau 13: intensity differences (posterior minus anterior) of tastes according to metallic taste (MT) status.....	93
Tableau 14: studied population characteristics at inclusion according to metallic taste status.	103
Tableau 15: caractéristiques démographiques de la population et traitements réalisés.....	111

INTRODUCTION

Les troubles de la perception de flaveur (goût, odorat et chemesthésie orale (1)) sont des plaintes fréquentes en oncologie, quel que soit le type de cancer, solide ou hématologique (2-6). Ils peuvent être spontanés ou causés par les traitements, quels qu'ils soient. Toutes les sortes de modifications sensorielles peuvent être rencontrées, de façon unique, séquentielle ou concomitante : modification des seuils de perception (qui peuvent augmenter ou diminuer) et/ou confusion des perceptions (parageusie et/ou parosmie) et/ou hallucinations avec des perceptions spontanées qui n'existent pas (phantogeusie ou phantasmie). Ces troubles des perceptions chimiosensorielles ont un impact sur le statut nutritionnel par la limitation des ingesta (7, 8) et diminuent la qualité de vie (3, 9, 10).

Parmi les parageusies, la torquegueusie, ou goût métallique est assez peu décrite et étudiée. Tout d'abord, il existe très peu de données établissant l'incidence en cancérologie (11). Parfois de diagnostic et de traitement aisés lorsqu'elle est générée par une candidose orale, ses causes sont rarement aussi évidentes. Plusieurs hypothèses ont été évoquées : lipopéroxydation orale (12), levée d'inhibition du nerf facial sur le nerf glossopharyngien (13-15), galvanisme spontané (16), surexpression de récepteurs de l'amertume sur la langue (17, 18). Mais aucune n'a été définitivement prouvée. Enfin son retentissement sur les apports oraux, le statut nutritionnel et la qualité de vie n'a (quasiment) pas été étudié.

Durant ma carrière professionnelle en chirurgie oncologique ORL, j'ai pu constater qu'une prise en charge globale, précoce et multidisciplinaire (médicale, infirmière, diététique, activité physique adaptée) permettait au patient de mieux aborder les épreuves qui l'attendaient. Cependant, lors des traitements, en particulier à la fin de l'irradiation, la plainte d'un goût métallique générant une anorexie était fréquemment émise par les patients, sans particulièrement de mucite ou de candidose orale. Plusieurs de mes patients, alors que je les sermonnais pour qu'ils mangent plus et que l'on puisse se passer de la nutrition entérale, me disaient qu'ils n'avaient pas envie de manger avec ce goût métallique.

Afin de mieux aborder cette plainte, nous avons mené un travail de recherche depuis 2017, découpé en plusieurs phases, en fonction de l'attente de financements et afin de permettre de saisir l'ampleur du sujet, en :

- (i) une phase initiale d'analyse de la littérature sur les causes et l'incidence du goût métallique,
- (ii) une phase préparatoire avec essai des questionnaires chez 17 patients en 2018, rédaction des protocoles de tests gustatifs en 2021, tests de goût métallique chez 120 volontaires sains en 2021
- (iii) l'étude proprement dite à la recherche de l'incidence, des causes, du retentissement sur le statut nutritionnel et la qualité de vie et de l'efficacité éventuelle de la lactoferrine bovine en bain de bouche, pour atténuer ce symptôme.

Les travaux sont présentés par ordre chronologique, qui se trouve être aussi l'ordre de complexité croissante. Les numéros des références bibliographiques, des tableaux et des figures sont mis en commun pour l'ensemble des articles écrits, même lorsqu'ils ont été publiés, afin de pouvoir facilement se reporter à la table des figures ou tableaux en début de thèse, ou aux références bibliographies en fin de thèse.

Notre recherche visait à répondre à quatre questions clés :

- Quelle est la fréquence du goût métallique chez les patients traités pour un cancer des voies aérodigestives supérieures ?
- Quel est l'impact de ce symptôme sur leur qualité de vie et leur état nutritionnel ?
- Quelles sont les causes potentielles du goût métallique ?
- Existe-t-il un traitement susceptible de réduire ce symptôme ?

Ainsi, après avoir détaillé les Méthodes, nous commencerons par une estimation du goût métallique en oncologie générale avec la revue systématique de la littérature et la méta-analyse que nous avons réalisées. Ensuite, les résultats de la fréquence et de l'impact du goût métallique dans notre cohorte TORCAD seront présentés. Après cet exposé, nous examinerons si la perception métallique est un goût, un arôme ou une saveur et nous présenterons les résultats de GOUTMETALSAIN, une étude auxiliaire que nous avons réalisée sur des volontaires sains. Nous discuterons ensuite des résultats des explorations de toutes les hypothèses causales qui ont été testées, d'une évaluation générale de l'impact des HNC et de leurs traitements sur le goût, d'une synthèse et d'une conclusion.

ETAT DE L'ART

1. Causes et traitements de la dénutrition et des altérations sensorielles chez les patients atteints d'un cancer des voies aérodigestives supérieures

Cet article a été publié dans les carnets de nutrition et de diététique (19). Il présente **un état des lieux des causes de la dénutrition en cancérologie des voies aérodigestives supérieures et évoque le goût métallique**. La traduction anglaise est présentée en [annex 1](#).

Cahiers de nutrition et de diététique 56 (2021) 249–259



Disponible en ligne sur
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com



NUTRITION CLINIQUE

Causes et traitements de la dénutrition et des altérations sensorielles chez les patients atteints d'un cancer des voies aérodigestives supérieures



Causes and treatments for undernutrition and sensory alterations in patients with head and neck cancer

Guillaume Buiet ^{a,b,*}, Ghislain Riffard ^b

^a Service d'ORL et de chirurgie cervicofaciale, centre hospitalier de Valence, 179, boulevard du Maréchal-Juin, 26953 Valence, France

^b Plate-forme de cancérologie, centre hospitalier de Valence, 179, boulevard du Maréchal-Juin, 26953 Valence, France

RESUME

La dénutrition est fréquente et précoce chez les patients atteints d'un cancer des voies aérodigestives supérieures. Elle est d'origine multifactorielle : insuffisance des apports (de loin la plus fréquente et importante), l'augmentation des dépenses énergétiques, les modifications métaboliques liées au cancer. Une des causes de l'insuffisance des apports est l'atteinte des perceptions sensorielles gustatives et olfactives qu'il est difficile de préciser car toutes les variations sont possibles (augmentation/diminution des seuils de perception, perversion des informations perçues). Parmi elles, la perception d'un goût métallique, la torquegueusie, est largement sous-étudiée alors qu'elle est fréquemment rapportée par les patients en pratique clinique courante. Elle mérite une étude plus attentive (incidence, cause(s), traitement(s)).

La prise en charge de la dénutrition doit être précoce (idéalement dès le diagnostic et avant tout traitement), multimodale (orale, entérale, très rarement parentérale) et pluriprofessionnelle (ORL, diététicienne éventuellement aidée d'un médecin nutritionniste, orthophoniste, enseignant d'activité physique adaptée...).

« Points essentiels »

- La dénutrition est fréquente et souvent présente dès le diagnostic de cancer des voies aérodigestives supérieures

- Les causes sont multifactorielles dont les atteintes sensorielles, liées au cancer lui-même et aux traitements
- La prise en charge de la dénutrition doit être précoce et est multidisciplinaire (traitement de la ou des causes, diététique, activité physique adaptée)

INTRODUCTION

Les Carcinomes des Voies AéroDigestives Supérieures (CVADS) sont une entité nosologique. Ils présentent en effet des points communs anatomiques (cavité orale, oropharynx, hypopharynx et larynx) et physiopathologiques (facteurs de risque identiques, carcinome épidermoïde quasiment exclusivement en type anatomopathologique, cancers synchrones fréquents). Leur incidence était évaluée à 14706 nouveaux cas en 2015 (20), les plaçant dans les cancers les plus fréquents en France. Leur traitement est multimodal : chirurgie, radiothérapie, chimiothérapie et depuis 2018 l'immunothérapie. Leur diagnostic est souvent tardif expliquant que leur pronostic reste sombre.

La dénutrition est fréquente chez ces patients. Par exemple, 49% des patients atteints d'un CVADS ont été diagnostiqués comme dénutris dans l'étude transversale des patients hospitalisés d'Hébuterne et al. (21). La dénutrition est précoce (50% des patients ont perdu >5% de leur poids au moment du diagnostic dans l'étude de Langius et al. (22)). Elle semble également être un facteur indépendant de survie globale (22, 23) et de dégradation d'état général (21, 24). Les causes de cette dénutrition sont multiples et souvent associées. Malgré les conseils diététiques, l'enrichissement (25, 26) et/ou la pose d'une gastrostomie prophylactique (27, 28), cette dénutrition s'aggrave habituellement durant le traitement et durablement après la fin du traitement du CVADS (29, 30).

Les troubles de perception des saveurs (informations liées au goût, à l'odorat et à la somesthésie orale) sont une des causes possibles de dénutrition. Les capacités gustatives et olfactives sont modifiées quantitativement et/ou qualitativement, en particulier à cause des traitements proposés. Le diagnostic de ces troubles sensoriels peut être appréhendé par questionnaires ou par tests sensoriels psychophysiques. Cependant il existe des discordances des résultats de ces deux approches (17, 31-33) : une atteinte sensorielle peut souvent être rapportée par les patients avec des tests normaux et inversement (34). Les résultats des tests objectifs psychosensoriels varient également d'une étude à une autre car il n'existe pas de standardisation des tests. Il est donc difficile d'utiliser en pratique courante ces tests psychosensoriels afin d'affiner les signes fonctionnels des patients.

Parmi les dysgueusies, le goût métallique, aussi appelée torquegueusie, est une plainte fréquente, quoique sous-estimée (35). Ni sa fréquence ni sa/ses cause(s) n'est (ne sont) connue(s) précisément.

Nous nous proposons donc dans cet article une mise au point étiologique sur ces différents symptômes en partant des causes générales de la dénutrition chez les patients atteints de CVADS puis celles des troubles gustatifs et olfactifs en terminant par un zoom sur celles de la torquegueusie. Enfin, nous rappellerons les grandes lignes de traitement de la dénutrition et des troubles du goût et de l'odorat.

1. CAUSES DE DENUTRITION DES PATIENTS ATTEINTS D'UN CVADS

Trois grands mécanismes peuvent concourir à la dénutrition dans un CVADS (Figure 1)

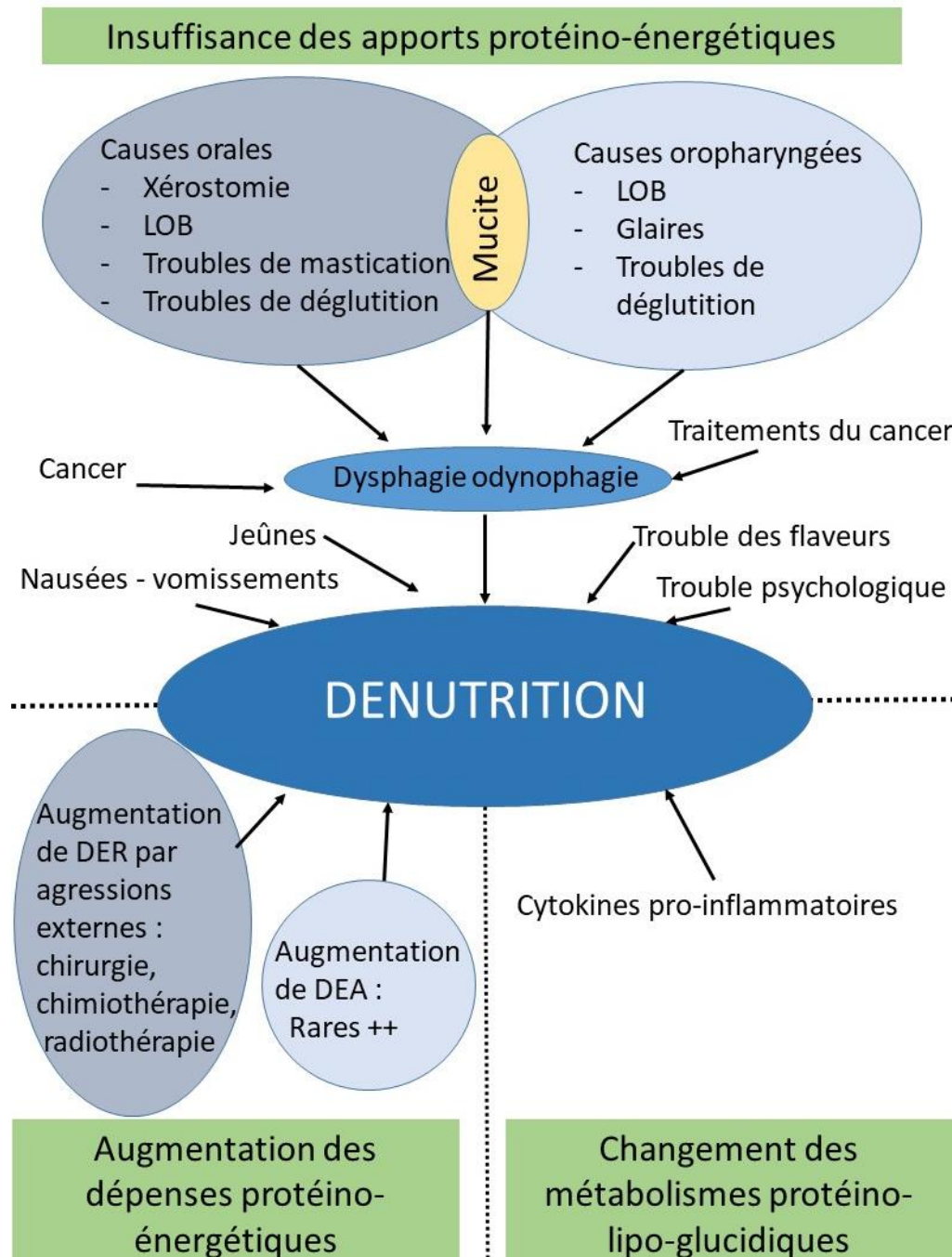


Figure 1 : schématisation des causes de dénutrition chez un patient atteint d'un cancer des voies aérodigestives supérieures

LOB : Limitation d'Ouverture Buccale, DER : Dépenses Energétiques de Repos, DES : Dépenses Energétiques liées à l'Activité

1.1.L'insuffisance des apports énergétiques :

C'est de loin le mécanisme le plus fréquent. Etant donnée la localisation cancéreuse dans le carrefour aérodigestif, un retentissement sur les apports nutritionnels est fréquent et rapide. Différentes causes, schématisées dans la Figure 1, sont possibles, avec parfois des interrelations voire des potentialisations :

- La dysphagie (troubles de la déglutition, blocage, ...) et l'odynophagie sont des signes fréquents de révélation des CVADS. Elles peuvent être causées :
 - par le cancer lui-même (effet de masse tumoral, atteinte nerveuse motrice, sensitive et/ou douloureuse) et/ou par le traitement et peuvent conduire à des aversions alimentaires (36, 37).

- Par une atteinte orale : la xérostomie, la limitation d'ouverture buccale, les troubles de la mastication, le mauvais état dentaire, la nécessité d'avulser des dents dont l'état n'est pas parfait afin d'éviter l'ostéoradionécrose en cas d'irradiation, une denture inadaptée participent également à diminuer les apports caloriques car les patients sont amenés à modifier la texture de leur alimentation et par là-même la quantité des ingesta. Une atteinte de la somesthésie orale peut également limiter les capacités de déglutition (38, 39).
- Par une atteinte oropharyngée : la présence de glaires oropharyngées post-radiques retentit également sur la déglutition. De même la limitation d'ouverture buccale retentit sur les ingesta. Elle peut être liée à la tumeur, l'inflammation qu'elle génère sur les muscles masticateurs, aux traitements (chirurgie d'exérèse, de reconstruction, radiothérapie ou radiochimiothérapie oropharyngée). De même que pour les causes orales, une atteinte de la somesthésie pharyngée peut limiter les capacités de déglutition (40)
- La mucite, cause orale et/ou oropharyngée, mérite une attention particulière. Parfois compliquée d'une candidose, elle est fréquente (jusqu'à 85% des patients en cours de radiothérapie du CVADS (41)) et limite les ingesta par la douleur occasionnée. Elle peut être spontanée par la présence d'un cancer et de l'inflammation péri-tumorale mais est surtout iatrogène, en particulier en cours de radiothérapie, chimiothérapie et a fortiori de la radiochimiothérapie.
- Les nausées-vomissements et l'anorexie, souvent iatrogènes (chimiothérapie, nutrition entérale trop rapide, ...), limitent également les ingesta.
- La réalisation d'examen diagnostiques (panendoscopie sous anesthésie générale, fibroscopie oeso-gastro-duodénale) et/ou thérapeutiques (chirurgie, gastrostomie) impose des jeûnes itératifs.
- Les troubles du goût et/ou de l'odorat (31) sont également fréquents (jusqu'à 100% des patients en cours de radiochimiothérapie du CVADS (3)). Il peut s'agir de modifications des seuils de perception dans un sens (hypergueusie ou hyperosmie) comme dans l'autre (hypogueusie ou hyposmie) ou une distorsion de perception (dysgueusie ou dysosmie). Les troubles du goût et/ou de l'odorat sont parfois déjà présents au moment du diagnostic du CVADS mais sont surtout aggravés par les traitements, en particulier l'irradiation (3, 42, 43). Ils conduisent à une diminution des ingesta par diminution du plaisir de l'alimentation (31, 37, 44). Une personne qui a du plaisir en mangeant mange plus (45). Ce point est abordé dans la suite de l'article
- L'état psychologique des patients (46, 47) peut également limiter les ingesta. Il peut en être la cause (état dépressif, réactionnaire à la maladie et/ou l'état général dans lequel ils se trouvent causant une anorexie) et la conséquence (troubles d'alimentation causant un isolement pour manger, même avec sa propre famille (48)).

1.2.L'augmentation des dépenses énergétiques

La principale cause est iatrogène par les traitements effectués (chirurgie, radiothérapie, chimiothérapie). Ils augmentent le catabolisme de repos.

Les patients augmentent très rarement leur niveau d'activité physique de manière à augmenter le métabolisme d'effort suffisamment pour retentir sur la balance énergétique.

1.3.Les changements métaboliques induits par le cancer

De façon globale, le catabolisme est augmenté et l'anabolisme diminué par les cytokines inflammatoires (TNF- α , IL-6, IL-1) (49, 50). Toutes les voies métaboliques sont touchées :

- Métabolisme protéique : l'anabolisme est réduit, le catabolisme est augmenté, des protéines de l'inflammation sont synthétisées par le foie, aux dépens des protéines de l'anabolisme
- Métabolisme lipidique : augmentation de la lipolyse
- Métabolisme glucidique : activation de la néoglucogenèse et insulino-résistance des tissus périphériques

La dépense énergétique de repos est donc augmentée selon le degré d'inflammation.

2. TROUBLES SPECIFIQUES DU GOUT ET DE L'ODORAT DANS LES CVADS

2.1. Rapide rappel sur la physiologie de perception des saveurs

- i. La perception des saveurs débute avant la stimulation directe des récepteurs sensoriels gustatifs et olfactifs. Lorsqu'on veut manger, la phase préorale (stimulation visuelle, olfactive orthonasale et éventuellement par d'autres sens tels que l'audition, le toucher) permet une mise en appétit et la stimulation de la fabrication de salive. L'exemple-type est le fait que l'on ait souvent faim lorsque l'on va dans une boulangerie ou une pâtisserie car tous les sens y concourent pour une mise en appétit : la vision des pâtisseries bien mises en valeur, l'odeur des viennoiseries toutes chaudes, le bruit d'un pain au chocolat bien feuilleté ou d'un pain que l'on presse... A chacun sa madeleine...
- ii. La phase orale, après dépose de l'aliment dans la bouche, permet la solubilisation des molécules sapides grâce à l'insalivation du bol alimentaire et la mastication. La mastication nécessite une bonne efficacité des joues, lèvres, dents, muscles masticateurs et du voile du palais, des os de la cavité orale afin d'assurer une bonne continence antérieure, postérieure et latérale dans la cavité orale. La langue est particulièrement importante à ce stade car elle distribue les aliments dans la cavité orale et les ramène au niveau des dents, participe à la fragmentation des aliments et homogénéise le bol alimentaire insalivé. Ces molécules vont stimuler les récepteurs gustatifs (sucré, salé, amer, acide, umami) répartis dans toute la cavité orale et de façon plus anecdotique dans l'oropharynx.
- iii. Les molécules vont également s'aérosoliser pour stimuler l'olfaction par voie rétronasale. La surface des récepteurs gustatifs est grande et facile d'accès. La transmission des informations gustatives dépend de plusieurs nerfs : le nerf facial pour les deux tiers antérieurs (en avant du V lingual) de l'hémi-langue homolatérale et le nerf glossopharyngien pour le tiers postérieur (en arrière du V lingual) de l'hémi-langue homolatérale (cf Figure 2). La surface de récepteurs de l'olfaction est petite et difficile d'accès car elle est située tout en haut de l'éthmoïde. La transmission des informations olfactives se fait directement par au cerveau (la première paire de nerf crânien est virtuelle).

2.2. Nosologie des troubles de perception des saveurs

On distingue les troubles des saveurs supra-sensoriels (dégoûts, aversions conditionnées, qui ne seront pas abordés dans ce chapitre), des atteintes directes de la sensorialité.

Les troubles du goût peuvent être quantitatifs (variation du seuil de perception : hypoguesie voire aguesie, hyperguesie) et/ou qualitatifs (variation de la modalité de perception normale du goût ou dysguesie). De même on distingue les troubles quantitatifs de l'olfaction par modification des seuils de perception (hyposmie voire anosmie, hyperosmie) des troubles qualitatifs de l'olfaction (variation de la modalité de perception normale de l'olfaction ou dysosmie). Certaines dysosmies ont des noms particuliers : parosmie (l'odeur perçue n'est pas celle attendue), phantosmie (hallucination olfactive en l'absence de tout stimulus), cacosmie (odeur désagréable perçue par le patient et parfois par l'entourage).

Nous n'aborderons pas les causes générales de troubles du goût et de l'odorat (maladies endocriniennes, neurologiques, psychiatriques...) mais uniquement les causes liées spécifiquement aux CVADS, que nous avons classées selon leur physiopathologie et leur chronologie d'apparition.

2.3. Causes communes des troubles du goût et de l'odorat dans les CVADS

Elles touchent essentiellement la phase orale, avant la stimulation des récepteurs sensoriels. Elles sont schématisées dans le Tableau 1.

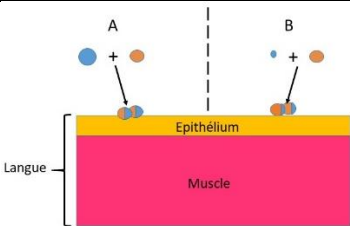
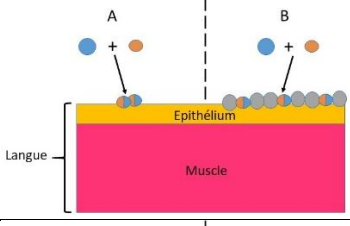
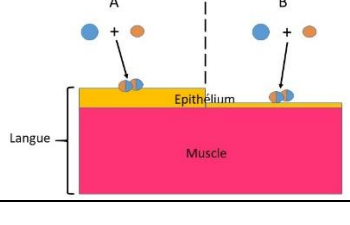
Mécanisme	Causes	Schéma
Salive	Xérostomie Changement de viscosité salivaire	
Molécules de flaveur non apportées par l'alimentation	Nécrose / inflammation Infection orale Médicaments Toxiques	
Atteinte directe de la sensorialité gustative	Réduction du nombre de cellules sensorielles et /ou de récepteurs (chirurgie, radiothérapie, chimiothérapie)	
Atteinte directe de la sensorialité olfactive	Réduction du nombre de cellules sensorielles et /ou de récepteurs (chirurgie, radiothérapie, chimiothérapie)	
Atteinte de la transmission sensorielle	Chirurgie : section nerveuse Radiothérapie – chimiothérapie : polyneuropathie	

Tableau 1: Principaux mécanismes et causes des troubles de perception des flaveurs en cancérologie des voies aérodigestives supérieures

A : situation normale. B : situation pathologique telle que décrite dans la colonne « causes »

● : salive ● : bol alimentaire ● : bol alimentaire insalivé ● : nécrose / inflammation / flore orale / débris cellulaires

i. Atteinte de la mastication

La mastication est essentielle car elle permet de fragmenter l'aliment et d'en libérer les molécules de la flaveur. Toute atteinte de la mastication peut donc retentir sur le goût et l'odorat : troubles de la continence orale (antérieure par atteinte des lèvres, surtout par chirurgie ; postérieure par atteinte du voile du palais, surtout par chirurgie et radiothérapie), troubles de la motricité de la mastication (atteinte de la langue, des joues, des dents, des os mandibulaire et maxillaire par la chirurgie et/ou la radiothérapie).

ii. Salive

La salive est essentielle par la capacité lubrifiante de ses protéines (51), en particulier les mucines, grandes protéines glycosylées lubrifiantes et protectrices de l'épithélium, et pour la perception des flaveurs : les cinq saveurs gustatives (sucré, salé, amer, acide, umami), le gras, la somesthésie orale par le nerf trijumeau, la rétro-olfaction (52).

Elle permet l'insalivation du bol alimentaire facilitant la mastication et l'agglomération des particules (rôle dans la granulométrie du bol alimentaire), la fragmentation des molécules contenant de l'amidon par l' α -amylase, et l'apport aux récepteurs sensoriels des molécules qui doivent les stimuler (la salive liquide apporte les molécules sapides aux récepteurs gustatifs, la salive aérosolisée apporte les molécules olfactives aux récepteurs olfactifs par rétro-olfaction).

La modification de la salive, suite à la radiothérapie et/ou la chirurgie, tant par sa quantité (xérostomie) (53, 54) que par sa qualité (plus visqueuse (55)), diminue la solubilisation des molécules sapides, le cheminement de ces molécules sapides jusqu'aux récepteurs gustatifs et l'aérosolisation des molécules olfactives.

La xérostomie peut aussi imposer de boire pour déglutir (56) et donc de vidanger trop rapidement ces molécules sapides de la cavité orale en plus d'apporter une sensation de satiété précoce.

La modification qualitative de la salive (plus acide, augmentation de l'excrétion de sodium (57)) peut également stimuler directement les récepteurs gustatifs.

iii. Présence d'odeurs et de saveurs (flaveur) non apportées par l'alimentation

Il existe plusieurs situations cliniques de présence de molécules de flaveurs non apportées par l'alimentation :

- La présence du cancer, per se, provoque la présence de nécrose et d'inflammation tumorales et péricancéreuses
- La colonisation orale bactérienne et fongique change (57). Les sécrétions et débris de cette nouvelle flore orale sont également perçus (flaveur).
- La présence d'une mucite orale et/ou oropharyngée, voire d'une candidose (58, 59) : l'inflammation réalise une interposition entre les molécules sapides et les récepteurs situés à la surface des cellules gustatives, en plus des sécrétions et débris cellulaires (cf situation clinique précédente).
- Les traitements médicamenteux : ils peuvent avoir une action directe sur les récepteurs sensoriels (60-63). Le platine de la chimiothérapie (qui est excrété dans la salive après administration intraveineuse), les traitements antifongiques oraux en sont des exemples quotidiens lors des traitements des CVADS. Mais d'autres substances (antimicrobiens, antifongiques, antiviraux, corticostéroïdes, médicaments psychoactifs, ...) altèrent également la perception des flaveurs
- Le tabagisme
- ...

iv. Atteinte des cellules et récepteurs gustatifs

Une chirurgie d'exérèse, en particulier linguale, réduit de facto et définitivement le nombre de cellules gustatives et leurs récepteurs.

De même le nombre de cellules gustatives et leurs récepteurs sont réduits, de façon précoce mais en général transitoire, par l'irradiation (63-68) et/ou la chimiothérapie (63, 65, 69). Cette réduction de la population cellulaire est liée à la fois à l'apoptose radio- et chimio-induite et à l'altération ou le non-renouvellement des cellules sensorielles par atteinte des cellules souches (70).

Les déficits en micronutriments (vitamine A, zinc, niacine) réduisent également la sensibilité des récepteurs gustatifs (70, 71)

v. Atteinte des cellules et récepteurs olfactifs

La majorité des causes de troubles olfactifs isolés est un défaut dans la transmission des substances olfactives jusqu'à l'épithélium olfactif : obstruction des fosses nasales par des croûtes, de la fibrose post-chirurgicale ou radiothérapique, disparition du flux aérien nasal après laryngectomie ...

Une chirurgie d'exérèse touchant l'épithélium olfactif est rarissime du fait de sa difficulté d'accès et de la rareté de son atteinte (esthésioneuroblastome olfactif, hors du champ des CVADS).

Par contre, de manière identique à l'atteinte gustative, le nombre de cellules olfactives et leurs récepteurs peut être réduit par l'irradiation de l'épithélium olfactif (63-68) et/ou la chimiothérapie par neuropathie générale (63, 65, 69).

vi. Atteinte de la transmission de l'information sensorielle

Les nerfs transmetteurs de l'information gustative (nerfs crâniens V, VII, IX, ou X) peuvent être atteints par section nerveuse chirurgicale ou par neuropathie (directe ou par déafférentation) liée à l'irradiation et/ou la chimiothérapie (63).

vii. Retentissement des troubles de perception des flaveurs sur le statut nutritionnel

Il existe de rares études ayant exploré le retentissement des troubles gustatifs sur le statut nutritionnel, en particulier les. McLaughlin (31) a montré dans sa cohorte de 92 patients que la perte de poids était significativement associée à des troubles du goût alors qu'elle ne l'était pas avec le type de traitement, le site de la tumeur et le délai depuis la fin de traitement. Sanchez-Lara et al. (44) ont montré qu'en cas

d'augmentation des seuils de détection du sucre, les apports caloriques, de protéines, de carbohydrates et de zinc sont moindres et la perte de poids plus fréquente. Shi et al. (72) ont montré que l'augmentation des seuils de perception de l'umami était significativement liée à une perte d'appétit, une insatisfaction de l'état actuel, une perte de poids et une atteinte sérieuse de l'hédonisme des repas. On voit donc que les résultats des tests sensoriels divergent (atteinte plutôt du sucré chez Sanchez-Lara (44) et de l'umami chez Shi (72)) mais que les troubles sensoriels gustatifs ont un impact défavorable sur le statut nutritionnel, confirmé par quelques revues de littérature (37, 73, 74).

Toutefois il n'existe pas de données sur le retentissement des troubles olfactifs sur le statut nutritionnel.

viii. Retentissement des troubles de perception des saveurs sur la qualité de vie
Il existe très peu de données (43, 72, 75). De plus, la détermination de qualité de vie par questionnaires intègre les troubles de la perception des saveurs (ex : questions 43 et 44 du questionnaire EORTC HN35, le plus utilisé pour mesurer la qualité de vie en CVADS (76)).

3. TORQUEGUEUSIE EN ORL

La torquegueusie (impression de goût métallique de la bouche) est très largement sous-rapportée dans la littérature. Son incidence chez les patients atteints de CVADS varierait entre 24% (36) et 77% (77) (alors qu'elle toucherait entre 9.7 et 78% dans une population atteinte de cancer toutes localisations confondues (78)). Jusqu'à présent très peu d'études se sont attardées sur la torquegueusie (incidence, causes potentielles, impact sur la qualité de vie, traitements potentiels).

Les causes de la torquegueusie sont mal établies (79) et probablement multiples, de façon concomitante ou séquentielle :

- 3.1. **Mécanismes chimiques** : deux différents mécanismes ont été décrits, selon l'ion métallique
 - Lipopéroxydation orale par Fe^{2+} , avec un rôle important de la voie rétronasale dans la perception du goût métallique (16, 80-82). Le malondialdéhyde est le produit final stable de la péroxydation de la membrane lipidique (83) et contribue à un stimulus olfactif. De plus l'occlusion nasale et/ou l'utilisation de chélateurs tels que l'EDTA et la lactoferrine peut abolir la torquegueusie induite par la lipopéroxydation (82).
 - Perception directe de l'ion métallique (Fe^{3+} , Cu^{2+} , Cu^{3+}) en tant que stimulus gustatif (78, 82).
- 3.2. **Mécanisme électrique** : une stimulation électrique anodique de la langue provoque une torquegueusie probablement par hydrolyse locale et présence d'ion H^+ (16).
- 3.3. **Mécanisme neurologique** (Figure 2) : l'anesthésie de la corde du tympan (une branche du nerf facial responsable de la transmission des afférences gustatives dans les deux tiers antérieurs de la langue homolatérale) cause une agueusie dans le territoire nerveux concerné, l'augmentation de la perception de certaines saveurs sapides dans le territoire du nerf glossopharyngien (responsable de la transmission des afférences gustatives dans le tiers postérieur de la langue homolatérale) et parfois une torquegueusie (13, 14). La réduction ou la suppression des afférences gustatives issues de la corde du tympan provoquerait une levée d'inhibition du nerf facial sur le nerf glossopharyngien : en condition pathologique (diminution des afférences de la corde du tympan), les afférences gustatives médiées par le nerf glossopharyngien serait considérées comme une afférence sensorielle par le système nerveux central alors qu'elles ne le seraient pas en situation physiologique, causant ainsi la torquegueusie (15).

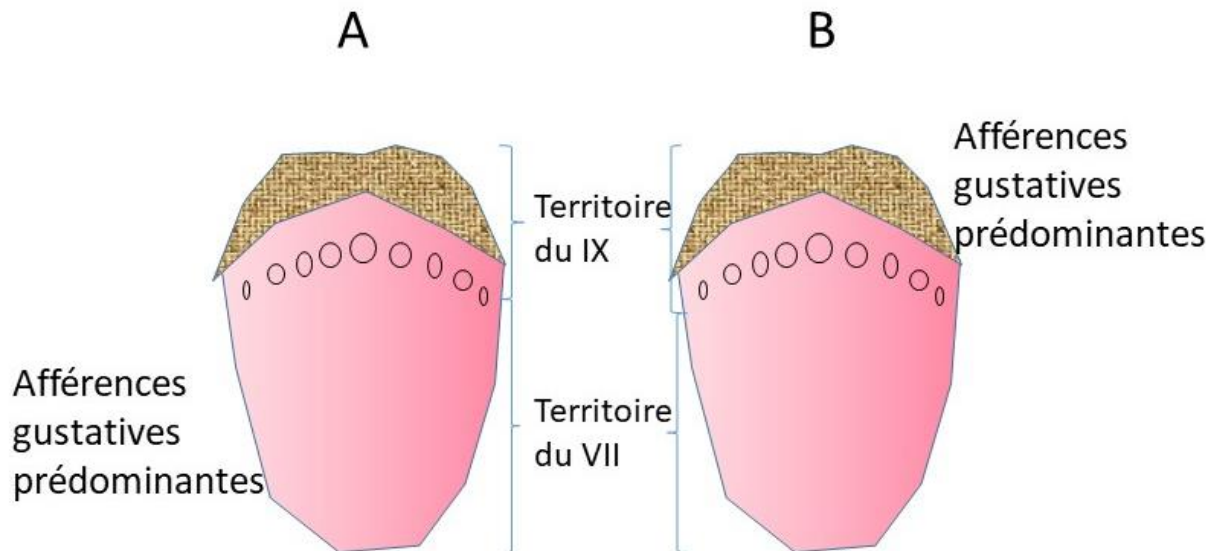


Figure 2: schéma de l'hypothèse de la levée d'inhibition du nerf facial sur le nerf glossopharyngien

A : situation normale. B : levée d'inhibition

VII : nerf facial (médiation de l'information sensorielle par la corde du tympan), IX : nerf glossopharyngien

3.4. Mécanisme protéomique : un lien fort entre hypersensibilité à l'amertume et torquagueusie a été montré (35). De plus, une hyper-expression des gènes des récepteurs à l'amertume de la famille T2R en particulier dans la bouche a été décrite lors d'une radiothérapie (18).

L'étude de cette dysgueusie est donc nécessaire afin d'en établir précisément l'incidence dans les CVADS, la/les cause(s) potentielle(s) et éventuellement un ou des traitements.

4. PRISE EN CHARGE DES TROUBLES DE PERCEPTION DES FLAVEURS ET DE LA DENUTRITION CHEZ LES PATIENTS ATTEINTS D'UN CVADS

4.1. Prévention des troubles de perception des flaveurs

- Une chirurgie d'exérèse linguale la plus « économe » possible permet d'éviter la disparition des organes gustatifs tout en assurant l'exérèse complète du cancer, but premier de la chirurgie
- Concernant la radiothérapie, il faut privilégier la radiothérapie en modulation d'intensité (plus précise, moins dommageable pour les organes gustatifs), minimiser la dose d'irradiation sur la pointe de la langue (zone de densité maximale des organes gustatifs) et dans la cavité buccale de manière générale.
- Eviter les toxiques (tabac, alcool) est également essentiel
- La supplémentation en zinc n'a pas montré d'effet significatif préventif des troubles de perception des flaveurs. (3)
- Prévention et traitement de la mucite : l'utilisation d'un laser basse intensité et énergie, la vérification de l'état buccodentaire avant chaque traitement de radiothérapie et de chimiothérapie, la bonne hygiène des prothèses permettent de prévenir l'apparition des mucites et des candidoses orales. Un traitement spécifique de celles-ci doit être débuté dès leur diagnostic. Il faudra prêter une attention particulière aux antifongiques qui peuvent générer une dysgueusie.

4.2. Conseils

Différents conseils peuvent être donnés (84) : mâcher et avaler lentement pour augmenter l'insalivation et la perception des flaveurs, limiter les odeurs de cuisine, améliorer l'aspect visuel des plats, adapter les assaisonnements et les choix des aliments, augmenter les apports en liquides,

augmenter la production de salive (glaçons ou glace pilée aromatisés ou non, gomme à mâcher, bâtonnets glycélinés neutres, salive artificielle, brumisateur) ...

De nombreuses brochures de conseils culinaires à destination des patients et des professionnels existent.

4.3.Réhabilitation

Différents moyens existent pour traiter les troubles des perceptions des saveurs, de la déglutition et de la dénutrition en général chez les patients atteints d'un CVADS :

- Réhabilitation dentaire / prothétique
- Orthophonie et autorééducation (85, 86). Ces méthodes relèvent du bon sens mais il ne faut pas être dogmatique. En effet, dans l'essai de phase 3 randomisée d'Ahlberg et al. (87), 190 patients atteints de CVADS traités par rééducation durant et 3 mois après leur traitement (orthophonie et kinésithérapie hebdomadaires, exercices à la maison) ont été comparés à 184 patients atteints d'un CVADS contrôles (sans rééducation). Il n'existait pas de différence significative sur le poids ou la survie globale à 2 ans. Il y avait même statistiquement plus de consommation de compléments nutritionnels oraux et moins de retour au travail dans le groupe rééduqué.

4.4.Traitements spécifiques des troubles de perception des saveurs

Peu de traitements permettent de traiter spécifiquement les troubles de perception des saveurs. La supplémentation en zinc semble montrer une diminution de ces troubles (3). Un trouble de l'olfaction peut être traité par corticothérapie nasale +/- générale (en l'absence de contre-indication)

4.5.Augmentation des apports oraux

La prise en charge par une diététicienne est essentielle. Ravasco et al. ont ainsi montré une nette différence de statut nutritionnel entre de simples conseils donnés par les médecins, des compléments nutritionnels oraux prescrits ad libitum par les médecins et un conseil diététique par une diététicienne qui, seul des trois bras, assurait le meilleur état nutritionnel à moyen et long terme chez des patients traités pour un CVADS (26). Idéalement, la prise en charge par une diététicienne doit être faite dès le diagnostic, car les patients sont fréquemment dénutris.

4.6.Nutrition entérale

L'éventualité d'une nutrition entérale à un moment donné du parcours de santé doit être discutée avec le patient et son entourage dès le diagnostic.

L'AFSOS, Association Francophone des Soins Oncologiques de Support, a publié un référentiel interrégional sur la nutrition des patients atteints d'un CVADS (88). Ce référentiel est en libre accès. Un arbre décisionnel, p. 16, reprend les indications de nutrition entérale selon le statut du patient (pas de dénutrition, dénutrition, dénutrition sévère ou critères de gravité) et des apports oraux évalués par l'outil d'Evaluation de la Prise Alimentaire (échelle visuelle analogique) (89).

La SFNCM, Société Française de Nutrition Clinique et Métabolique, recommande des apports quotidiens de 25 à 30 kcal/kg par jour en périopératoire et 30 à 35 kcal/kg par jour en oncologie médicale, et de 1,2 à 1,5 g/kg de protéines par jour (90).

Le tube digestif d'un patient atteint d'un CVADS étant la plupart du temps fonctionnel, il faut éviter la nutrition parentérale. De même, il faut surveiller l'apparition d'un syndrome de renutrition inapproprié (88).

4.7. Activité Physique Adaptée

L'apport de calories par voie orale et/ou entérale (voire parentérale) est une nécessité mais leur assimilation et la synthèse de masse maigre passe par l'APA.

Plusieurs essais randomisés ont montré des résultats statistiquement significatifs sur l'amélioration de la qualité de vie (91-95), la diminution de l'asthénie (93), l'augmentation de masse maigre (91, 92) et des forces musculaires (91, 92)

Capozzi et al. ont réalisé une revue de la littérature de 16 articles sur l'efficacité de l'APA en chez les patients atteints d'un CVADS (96) : bénéfique pour l'activité physique, la masse maigre, la force musculaire, la fatigue, l'absence de dégradation de qualité de vie durant le traitement, augmentation de

qualité de vie. Aucun problème de sécurité n'a été rapporté si l'APA est encadrée par un professionnel formé. L'adhérence semble meilleure si APA est débutée après le traitement. Les auteurs recommandent donc de faire un bilan et une sensibilisation à l'APA avant tout traitement et de débiter l'APA après. Ils ont également émis des recommandations spécifiques sur les conditions de réalisations de l'APA chez les patients atteints d'un CVADS :

- Plutôt en salle car on peut facilement moduler le temps (endurance) et l'intensité (force) (97, 98)
- Régularité : 3 fois / semaine (97-100)
- Progressivité : augmentation progressive de charge (20% au début, jusqu'à 60-70% de force maximale) (97-99)
- Exercices globaux (étirements, exercices posturaux, exercices de renforcements musculaires avec poids légers, et bandes élastiques) et spécifiques (épaules) (98-100)
- Supervision surtout si âgé, IMC >30 et traitement par radiothérapie (101). Capozzi et al. ont ainsi recueilli une adhérence de 66 à 88% si l'APA était supervisée, 95 à 97% si l'APA était supervisée et à la maison, et 53% uniquement à la maison (96)
- Faire un bilan et une information initiale et débiter l'APA après les traitements du cancer (96)

CONCLUSION

Les CVADS sont l'archétype de la dénutrition induite par le cancer de façon générale, des troubles du goût et de l'odorat de par leur localisation et les traitements utilisés et encore plus particulièrement de la torquagueusie. Plusieurs champs de recherche, tant clinique que fondamentale, sont actuellement en cours : types de nutrition entérale, intérêt ou non de la pose prophylactique d'une voie d'abord de nutrition entérale, intérêt de l'APA chez les patients atteints d'un CVADS, ...

Toutefois, il manque nettement de données sur l'impact des troubles de perception des saveurs sur le statut nutritionnel et la qualité de vie en CVADS. Enfin, les causes de la torquagueusie devront également être identifiées et un ou des traitement(s) éventuel(s) évalué(s). La prise en charge de la dénutrition doit être précoce et pluriprofessionnelle.

2. Perception du goût métallique

Auteurs : G Buiret, G. Feron

Soumis à l'Encyclopédie des Sciences (chapitre 7). Ce chapitre évoque **l'état de l'art sur la perception du goût métallique, avant la réalisation de nos propres études sur le sujet, en particulier l'étude ancillaire GOUTMETALSAIN.**

La traduction anglaise est présentée en [Annex 2](#).

INTRODUCTION

La flaveur est l'ensemble des sensations olfactives, gustatives et chemesthésique (tactiles, thermique, (astringence, menthol, molécules produisant une sensation de piquant)) perçues lors de la consommation d'un aliment. C'est un mécanisme important sur le plan de l'évolution car elle contribue à la survie des organismes vivants. Elle permet en effet la détection des nutriments indispensables à leurs besoins physiologiques et leur bien-être ainsi que la reconnaissance de leur environnement. Elle est aussi essentielle pour l'identification de composés de l'aliment qui pourraient être potentiellement toxiques pour eux et donc devant être évités et ne pas être consommés. Cinq saveurs de base sont décrites, i.e. le salé, le sucré, l'acide, l'amer et l'umami (102), pour certaines desquelles les papilles gustatives de la langue contiennent des récepteurs moléculaires spécifiques. Il est suggéré cependant que d'autres saveurs existent mais mal caractérisées comme le gras ou le goût métallique. Pour Omur-Ozbek (82), le goût métallique est une flaveur, pas un goût (rôle du goût, de la voie rétronasale, de la sensibilité trigémينية avec l'astringence). Néanmoins, dans la mesure où le terme « goût métallique » est consacré dans la population générale, nous continuerons de l'utiliser. Ce chapitre se propose de présenter les différentes causes responsables du goût métallique chez l'homme avec une attention particulière pour les raisons physiologiques et pathologiques.

1. La formation du goût métallique dans les aliments

Le goût métallique est couramment signalé comme un défaut dans de nombreux aliments contenant des matières grasses polyinsaturées tels que les huiles, les produits céréaliers et les produits laitiers mais aussi dans l'eau minérale. Le goût métallique provient, entre autres, du contact avec les emballages métalliques des aliments et les équipements de transformation (80).

Par exemple, dans le cas de la bière conditionnée en canette, l'utilisation de champs électriques pulsés afin d'en améliorer la qualité microbiologique a pour conséquence de libérer des ions ferreux dans le breuvage et ainsi d'induire un goût métallique peu apprécié (103). De même, l'utilisation d'ultra-sons à haute intensité dans différents procédés alimentaires renforce le goût métallique de produits alimentaires contenant des matières grasses cristallisées comme les margarines, les glaces ou les mayonnaises (104) mais aussi de la viande (105). Dans le cas de chips de maïs, le procédé de fabrication ainsi que la qualité de l'huile végétale jouent un rôle important dans la formation du goût métallique et donc l'acceptabilité par le consommateur (106).

Le goût métallique est aussi fréquemment rapporté dans le cas d'utilisation d'édulcorant comme la saccharine, l'aspartame, le sorbitol, le sucralose ou la stevia par exemple (107-109), ce qui limite leur utilisation dans les aliments transformés. Il en est de même pour le chlorure de potassium (KCl) utilisé comme substitut du sel et dont le goût métallique interfère avec les qualités organoleptiques des aliments comme cela a été montré pour les sauces tomates (110).

L'origine de ce goût métallique est souvent liée à la présence d'ions métalliques, et en particulier du fer, soit rajouté dans l'aliment soit par transfert de métaux de l'emballage vers le contenu. Les ions métalliques peuvent entraîner, par oxydation de la matière grasse présente dans l'aliment, la formation de composés volatils responsables du goût métallique ou alors directement en bouche par lipopéroxydation orale (voir §7.5 et Figure 5). La formation de ces notes métalliques peuvent affecter la qualité organoleptique de produits alimentaires fortifiés en fer mais aussi enrichis en acides gras polyinsaturés comme cela a été montré pour le lait en poudre (111) ou des biscuits à base de manioc (112). Cependant, l'utilisation de lactoferrine comme antioxydant dans ces aliments peut limiter

sensiblement ce problème d'oxydation. En effet, la lactoferrine (Figure 6 **Erreur ! Source du renvoi introuvable.**), une protéine du lait liant le fer, réduit la production de sous-produits de l'oxydation des lipides et il a été démontré qu'elle réduit la formation des saveurs désagréables.

2. Causes de la perception métallique

Plusieurs hypothèses s'affrontent pour expliquer la perception métallique. Aucune d'elles n'est actuellement pleinement validée. Selon les circonstances de déclenchement de la perception métallique (iatrogène après une chirurgie de l'oreille moyenne, lors du traitement d'un cancer, ...), une ou plusieurs de ces causes pourraient l'expliquer, de façon séquentielle ou concomitante.

2.1. Hypothèse d'une perception par des récepteurs sensoriels spécifiques.

2.1.1. Existe-t-il donc des récepteurs spécifiques au goût métallique ?

Stevens et al. (113) ont appliqué au sulfate de fer leur concept des échelles multidimensionnelles du goût (localisation d'un goût parmi les cinq saveurs habituelles et l'aluminium) chez 47 volontaires sains. Le goût métallique n'était pas groupé avec les saveurs sapides traditionnelles. Nez ouvert, les saveurs les plus proches du sulfate de fer étaient l'acidité et le sucré ; nez occlus, l'amertume et l'umami, alors que l'acidité et le salé étaient les plus lointaines. La perception du sulfate de fer était plutôt localisée avec l'aluminium, témoin de l'astringence, trigéminée. Existe-t-il des récepteurs spécifiques au goût métallique ?

Certains auteurs le pensent et attribuent la perception du goût métallique aux familles de récepteurs T2R (79) ou TRPV1 (79, 107). D'autres auteurs au contraire, plus nombreux, réfutent cette hypothèse. En effet, il existe une diminution nette de la perception du fer en cas d'élimination de la voie rétro-olfactive (12, 114-116), sans modification de perception des autres saveurs, soulignant l'importance de la voie rétronasale (113). Pour eux, la perception du goût métallique est surtout rétro-olfactive et liée à la lipopéroxydation orale (cf infra).

2.1.2. Circonstances physiologiques de perception du goût métallique

Si l'hypothèse d'une stimulation directe de récepteurs spécifiques n'est pas confirmée, une perception métallique peut avoir lieu dans des circonstances physiologiques :

- la présence de sels métalliques dans la bouche (ceux à base de fer principalement : chlorure, gluconate, sulfate ferreux mais aussi cuivre, zinc, magnésium, platine), de sels de calcium (16, 82). L'ion ferrique (Fe^{3+}) n'est pas détecté (82)
- des médicaments (117), des édulcorants comme décrit au §7.2 (16)

2.1.3. Hypothèse des modifications protéomiques des récepteurs sensoriels

Cette hypothèse est surtout évoquée en cancérologie, particulièrement en oncologie médicale. De façon schématique, les chimiothérapies agissent sur les cellules à renouvellement rapide et induisent leur apoptose. Outre la neuropathie induite par la chimiothérapie, les sensorialités olfactives et gustatives sont directement modifiées :

- le nombre de cellules sensorielles varient. Ces cellules se renouvellent mais de façon différente selon leurs précurseurs, induisant des modifications des ratios de cellules sensorielles entre elles
- le nombre de récepteurs à la surface de ces cellules sensorielles varie. Hirai et al. (18), par exemple, ont montré des variations de l'expression de récepteurs T2R de l'amertume lors des chimiothérapies dont trois avaient un taux d'expression significativement différents (TAS2R42, TAS2R43, T2R3)

Ces modifications induisent des modifications de l'olfaction et de la gustation, soit dans leurs seuils de détection (hyper-hypogouesie / hyper-hyposmie), soit une perversion de leur perception (paragouesie, parosmie).

Deux hypothèses ont été ainsi émises pour expliquer le goût métallique : i. un ligand présent dans la bouche se lie avec un récepteur surreprésenté ; ii. un goût métallique est perçu en l'absence de ligand dans la bouche (liaison spontanée entre le récepteur et une protéine G). Dans tous les cas, il existe une

diminution du seuil de sensibilité au métal et donc une propension accrue à percevoir un goût métallique (78).

2.2. Hypothèse d'un galvanisme entre couronnes dentaires

Une stimulation électrique de la langue (12, 16), en particulier anodique plutôt que cathodique (118), provoque une perception métallique, probablement par hydrolyse locale et production d'ions H⁺. Ayres a montré qu'un courant spontané, d'une intensité maximum de 100µA, peut parfois être spontanément enregistré (119). Sutow et al. (120) ont également montré la présence d'un pic d'intensité puis d'une décroissance exponentielle par contact électrique entre des couronnes dentaires opposées dans la bouche.

Cependant, cette hypothèse du galvanisme dentaire pour expliquer la perception métallique est peu probable. En effet, Bergman et al. n'ont pas montré de différence significative de différence de polarité chez 16 personnes se plaignant de galvanisme oral (dont quatre se plaignaient d'un goût métallique) comparés à neuf patients contrôle (121) (même si le nombre de sujet est faible et l'étude peu robuste scientifiquement). De plus, de nombreuses personnes se plaignant d'un goût métallique n'ont pas d'amalgame ou de couronnes dentaires. Enfin, dans les diagrammes tridimensionnels de Stevens et al. (113), l'acidité (excès d'ions H⁺) et la perception métallique du sulfate de fer étaient proches nez ouvert mais éloignées nez occlus. Ces variations sont difficilement explicables par l'hypothèse du galvanisme.

2.3. Hypothèse d'une atteinte neuronale

Une atteinte de la corde du tympan (branche végétative et sensorielle du nerf facial s'anastomosant avec le nerf lingual, Figure 3) lors d'une chirurgie otologique est une circonstance connue et ancienne de déclenchement de la perception d'un goût métallique. Une atteinte neurologique sans atteinte orale peut donc provoquer un goût métallique. Cette atteinte est la plupart du temps d'origine iatrogène (chirurgicale avec section d'un nerf, neuropathie induite par une chimiothérapie, une radiothérapie, un autre médicament).

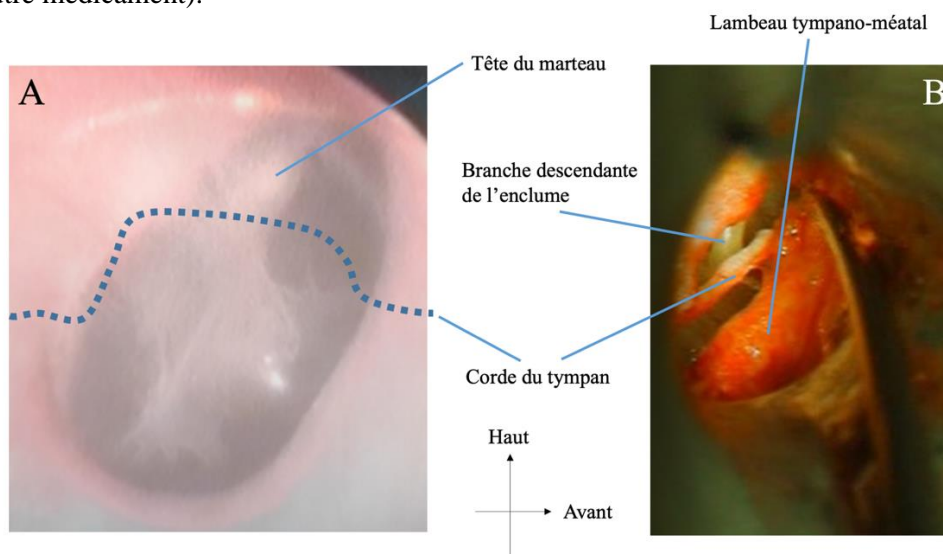


Figure 3: Rapports de la corde du tympan et du tympan

A : projection du trajet de la corde du tympan sur l'otoscopie droite. B : vue de la corde du tympan lors de l'abord chirurgical de l'oreille moyenne

2.3.1. Neuropathie et goût métallique

La stimulation directe ou la section de la corde du tympan (Figure 3) et la glossodynie (névralgie linguale) sont connues pour déclencher un goût métallique (16).

Le goût métallique peut aussi être rencontré durant la grossesse (16). Il s'agit alors d'une phantoguesie, hallucination sensorielle.

2.3.2. Levée d'inhibition du nerf facial sur le nerf glossopharyngien

Pour rappel :

- la corde du tympan transmet les informations gustatives issues des deux tiers antérieurs de la langue homolatérale
- le nerf grand pétreux superficiel, branche du nerf facial, transmet les informations gustatives issues de l'hémi-palais homolatéral
- Le nerf glossopharyngien, nerf essentiellement moteur et sensitif du pharynx, transmet les informations sensorielles gustatives issues de la base de langue.

Kveton et al. (13) ont étudié l'impact sensoriel de l'atteinte d'une corde du tympan après chirurgie de schwannome vestibulaire (la chirurgie du nerf cochléovestibulaire dans le conduit auditif interne induit une atteinte du nerf facial sans atteinte du nerf glossopharyngien), en réalisant des tests supraliminaires d'intensité des quatre saveurs à différents endroits de la cavité orale, dépendant de la corde du tympan ou du nerf glossopharyngien (Figure 4). Ils ont montré une agueusie ou une hypoguesie dans le territoire de la corde du tympan et, en miroir, une hyperguesie dans les territoires du glossopharyngien. Yanagisawa et al. ont procédé aux mêmes constatations en cas d'anesthésie d'une corde du tympan chez des volontaires sains (15). Pour eux, l'activité sensorielle du nerf glossopharyngien, normalement inhibée par celle de la corde du tympan, est considérée comme une afférence prédominante par le système nerveux central en cas d'atteinte de la sensorialité issue de la corde du tympan.

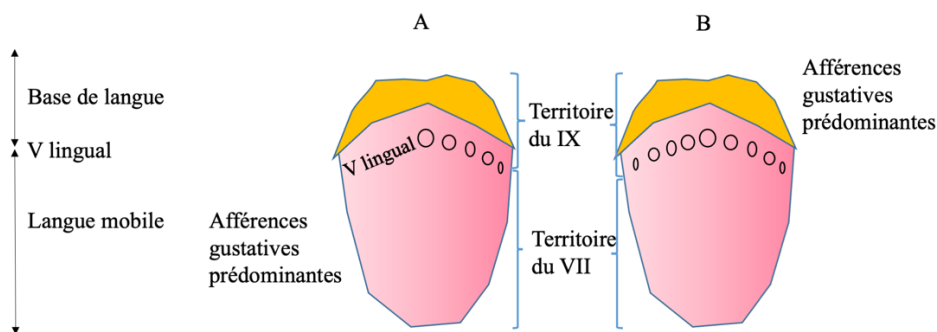


Figure 4: Schéma de la levée d'inhibition

D'après (19) :

A : situation normale, B : levée d'inhibition du VII sur le IX. VII : nerf facial, IX : nerf glossopharyngien

Une atteinte neuronale peut donc provoquer une inhibition de la perception d'une saveur amplifiant la perception des autres (14, 122, 123). Cette constatation est la base de l'hypothèse de la levée d'inhibition du nerf facial sur le nerf glossopharyngien dans la genèse du goût métallique (79). En effet, les effets neuropathiques des traitements anticancéreux sont très complexes (diminution du nombre de récepteurs sensoriels, altération de la structure cellulaire ou changement de la surface du récepteur, interruption dans le codage neural, renouvellement des récepteurs gustatifs et des cellules gustatives avec connexions synaptiques irrégulières entre la cellule sensorielle et sa fibre nerveuse (65)). Les seuils de détection des saveurs ont en général tendance à augmenter (124). Un travail est en cours pour confirmer ou infirmer cette hypothèse.

2.4.Hypothèse de la lipopéroxydation orale

L'oxydation de lipides non saturés dans l'alimentation ou dans la bouche génère un goût métallique avec une participation importante de la voie rétronasale (78, 122, 125) même si le volet gustatif ou trigéminal ne peut pas être complètement exclu pour certains ions, cuivre en particulier (126). En particulier, le fer mais aussi le cuivre en solution peuvent causer le plus d'oxydation lipidique orale, dont le marqueur est le malondialdéhyde, elle-même génératrice de goût métallique. Des études portant sur la perception du goût métallique ont mis en évidence de manière claire que ce goût correspondait principalement à la formation instantanée de composés volatils lors d'une stimulation gustative par une solution de sulfate de fer ou de cuivre. Ces composés volatils sont générés dans la cavité orale et passent dans la cavité nasale pour être perçus (perception rétronasale). En effet, Epke et Lawless (127), par des études sur les seuils de perception de solution de fer et de cuivre, ont montré

que ces seuils étaient significativement abaissés en absence de pince-nez par rapport à un test avec pince-nez, où la perception rétronasale est abolie (Tableau 2).

Composé	Seuil de détection (μM) (min, max)	
	Nez ouvert	Nez fermé
Sulfate de fer	29.9 (7.5, 120)	161 (60.1, 429)
Chlorure de fer	64.0 (21.2, 193)	227 (99.8, 515)
Sulfate de cuivre	7.8 (3.2, 19.0)	24.6 (11.1, 54.4)
Chlorure de cuivre	8.2 (3.4, 19.5)	15.6 (6.5, 37.5)

Tableau 2: Seuils de détection par voie rétronasale (nez ouvert) ou gustative (nez fermé) de solution de fer et de cuivre

Adapté d'Epke et Lawless, 2007 (127).

Si les réactions en bouche à l'origine de la formation de composés volatils à note "métallique" ne sont pas clairement identifiées, de nombreux auteurs suggèrent que cette genèse soit issue de l'oxydation instantanée d'acides gras poly-insaturés contenus dans la salive ou au niveau des muqueuses (82). Cette synthèse entraînerait la formation de composés volatils à très faible seuil de détection comme certains aldéhydes, mais aussi des cétones (Figure 5). Ces composés volatils générés en bouche contribuent à altérer le goût de l'aliment consommé mais n'ont pas tous une note métallique spécifique. Cependant certaines cétones comme la 1-octen-3-one et la 1-nonen-3-one et aldéhydes comme le trans-4,5-epoxy-2(E)-décénal sont très caractéristiques de ce goût métallique. Elles ont de plus un seuil de détection très bas de l'ordre du ppb (partie par billion) (128). Cette hypothèse s'est trouvée renforcée au travers des travaux de Glindemann et al. (129) qui ont montré que l'arôme métallique qui se formait au contact de fer sur la peau humaine était dû, entre autres, à l'oxydation des lipides de la peau et à la formation de ces composés volatils.

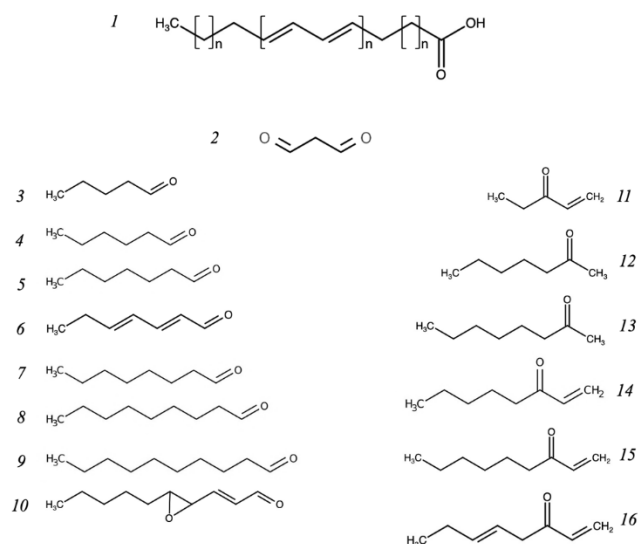


Figure 5: composés volatils

(à l'exception du malondialdéhyde) susceptibles d'être formés, soit dans l'aliment soit dans la cavité orale en cours de consommation, par lipopéroxydation à partir d'acide gras polyinsaturés contenus dans la matrice alimentaire, la salive ou au niveau des muqueuses orales. 1: acide gras polyinsaturés présentant deux doubles liaisons conjuguées; 2: malondialdéhyde; 3: pentanal 4:hexanal; 5:heptanal; 6: trans,trans-2,4-heptadienal; 7: octanal; 8: nonanal; : 9: decanal; 10: trans-4,5-epoxy-2(E)-décénal; 11: 1-penten-3-one; 12: 2-heptanone; 13: 2-nonanone; 14: 1-octen-3-one; 15: 1-nonen-3-one; 16: cis-1,5-octadien-3-one

Une autre hypothèse serait que les molécules odorantes responsables du goût métallique se formeraient directement à partir des acides gras polyinsaturés constitutifs de lipides contenus dans

l'aliment avant qu'il soit consommé (voir le paragraphe 1). Lors de la mise en bouche de l'aliment, les composés d'arômes formés deviendraient plus volatils à cause de l'augmentation de la température (130). Il a été aussi montré que, contrairement à l'or ou à l'inox l'utilisation de couverts en zinc ou en cuivre pouvait entraîner aussi un goût métallique en bouche (131), ceci lié au contact du métal avec les muqueuses orales.

3. Incidence et retentissement du goût métallique

L'incidence du goût métallique a été très peu étudiée, et exclusivement en cancérologie. Nous avons identifié 22 études rapportant l'incidence du goût métallique, estimée entre 5.2% et 80% des patients. Une méta-analyse (11) a retrouvé une incidence moyenne chez 29% des patients traités pour un cancer. Pour rappel, l'Institut National du Cancer a estimé à 382 000 nouveaux le nombre de nouveaux cancers par an en France métropolitaine en 2018. La prévalence du goût métallique est donc loin d'être négligeable.

De même, le retentissement du goût métallique n'a été « étudié » qu'en cancérologie. Seulement cinq études ont rapporté des changements de poids dans leur population de patients cancéreux (8, 132-135), en lien avec des modifications du goût et de l'odorat chez des patients atteints de cancer mais aucune n'était dédiée au goût métallique. Par conséquent il n'est pas possible d'évaluer l'impact spécifique du goût métallique sur le statut nutritionnel. De même deux études ont noté une altération de la qualité de vie à cause de troubles du goût et de l'odorat dont le goût métallique (77, 136) mais aucune d'elles n'était spécifique au goût métallique.

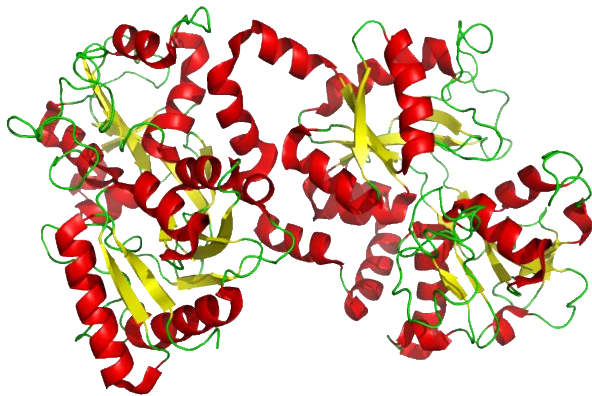


Figure 6: Structure de la lactoferrine

Pour ces populations, une supplémentation en lactoferrine permet de réduire l'importance du goût métallique de manière significative. En effet, la lactoferrine (Figure 6) est une protéine présente dans beaucoup de fluide biologique (lait, salive, larmes) et sert à contrôler le niveau de fer dans ces fluides. Dans son utilisation en supplémentation, elle permet donc de complexer le fer et donc de réduire sa concentration dans la salive et donc de limiter les réactions indésirables d'oxydation buccale (137).

3. Problematics on metallic taste

Several factors are known to enhance the perception of a metallic taste. The presence of metallic tastants, such as metallic salts—especially those containing iron like chloride, gluconate, and sulfate (12, 16, 78, 82, 107, 122)—can contribute to this phenomenon. Other compounds like calcium and magnesium salts (16), certain sweeteners (16, 107), and antifungal treatments are also implicated. Changes in chemosensory receptors (proteomic hypothesis), including a modification of the number of chemosensory receptors and/or alteration of their functioning that could lead to a decrease in the threshold of sensitivity to metal and thus an increased propensity to perceive a metallic (78, 79), may enhance the metallic sensation. This might be due to the direct stimulation of receptors possibly specific to metallic taste. There are three proteomic hypotheses (18): ligand binds with over-represented receptor, such as those in the T2R family or TRPV1 (79); abnormal taste perception without ligand; salivary proteome/peptidome analysis for candidate peptides. Additionally, lipoperoxidation (12, 79, 82) and alterations in saliva composition (78, 79) can intensify this sensation. External factors like electric anodic stimulation (12, 16) such as spontaneous galvanism between dental amalgams (119), and the inhibition release (79) by sectioning or stimulating the chorda tympani (16, 122) also play a role. Neurological conditions like glossodynia (burning mouth syndrome), neuropathy, and phantogeusia during pregnancy (16) have been observed to contribute to metallic taste perception. Based on these observations, potential causes have been identified. From these constatations, we determined some potential causes.

Concerning our hypotheses, after considering the above-mentioned causes, we didn't retain:

- the hypothesis of a tastant because MT can occur spontaneously in HNC pathway, not necessarily related to the eating period.
- the proteomic hypothesis was not included in TORCAD because it is not mature enough and needs for further research
- the hypothesis of galvanism because MT can occur in patients whose teeth were completely removed before any radiotherapy, in order to prevent osteoradionecrosis. Furthermore, measurement of intra-oral electrical currents was inconclusive (121, 138).

We focused on the two following hypotheses, that were more deeply explained in the previous chapter (chapter 7 of the Encyclopédie des Sciences):

- **Lipoperoxidation**

Oral lipoperoxidation may be modified by the disease and/or treatments and may lead to the production of MDA, which could activate the olfactory system by retro-olfaction and generate MT perception. It was therefore chosen as the primary objective of TORCAD study as, in our experience, MT occurs during the radiotherapy, a moment of intense oxidation (139) desired to enhance the apoptosis of cancerous cells. TORCAD study focused on salivary malondialdehyde concentration as a primary endpoint.

- **Glossopharyngeal and Facial Nerve Interaction**

As radiotherapy impacts facial and glossopharyngeal nerves, increasing detection thresholds (123), the hypothesis of release of inhibition of the facial nerve on the glossopharyngeal nerve was considered as a secondary objective and TORCAD study included taste tests on different tongue areas as a secondary endpoint.

MATERIEL ET METHODES

1. Partie réglementaire

Pour atteindre nos objectifs, nous avons mené deux études.

L'étude sur la population atteinte d'un cancer des voies aérodigestives supérieures (TORCAD) a été validée par le CPP Est I le 08 mars 2018 (autorisation dossier ID RCB 2017-A03641-52).

Tous les patients diagnostiqués pour un cancer des voies aérodigestives supérieures entre avril 2021 et mars 2023 s'étaient vus proposer d'intégrer l'étude TORCAD sur le goût métallique avant, pendant et après le cancer des voies aérodigestives supérieures. Peu de patients avaient accepté car le recueil des données était long et un peu fastidieux.

Les critères d'inclusion étaient d'être majeur et de présenter un cancer des voies aérodigestives supérieures avec une espérance de vie de plus d'une année. Les critères de non-inclusion étaient de ne pas se voir proposer une laryngectomie totale (l'odorat par voie orthonasale devant être conservé), ne pas avoir déjà eu un cancer, CVADS ou non (le traitement précédent pourrait avoir modifié les capacités gustatives des individus), ne pas être enceinte (la grossesse perturbe le goût).

Les critères d'exclusion étaient la récurrence du cancer durant l'année de suivi post-thérapeutique. Dans ce cas, les données acquises jusqu'à la récurrence étaient gardées. De même, en cas de retrait du consentement, les données acquises jusqu'au retrait du consentement étaient gardées.

Le traitement proposé faisait l'objet d'une réunion de concertation pluridisciplinaire.

Les patients avaient plusieurs évaluations : T1 avant tout traitement ; T2 après chirurgie avant radiothérapie le cas échéant ; T3 au milieu de la radiothérapie le cas échéant ; T4 : à la fin de la radiothérapie le cas échéant ; T5 : trois mois après la fin du traitement ; T6 : six mois après la fin du traitement ; T7 : neuf mois après la fin du traitement ; T8 : 12 mois après la fin du traitement.

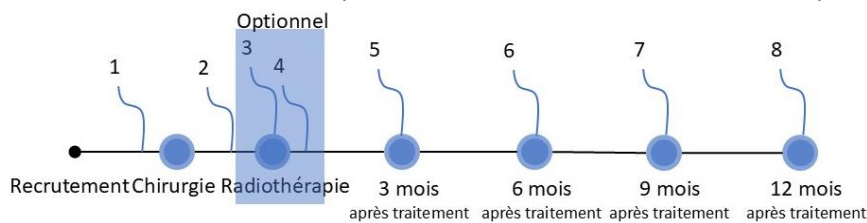


Figure 7: Temporalité des tests

A chaque moment d'évaluation, des tests sensoriels de reconnaissance et d'intensité étaient réalisés et des questionnaires portant sur la qualité de vie et le goût métallique étaient remplis.

L'étude de la perception du goût métallique chez les volontaires sains (GOU METALSAIN) a été validée par le CPP Est IV le 12 octobre 2021 (autorisation dossier ID RCB 2021-A02149-32).

Les patients de la salle d'attente de la consultation ORL se sont vus proposer de participer à des tests supraliminaire d'intensité de perception du sulfate de fer. Les patients adultes (≥ 18 ans) volontaires ont été inclus, tandis que les critères de non-inclusion étaient d'avoir un antécédents de cancer des voies aérodigestives supérieure, de consulter pour troubles du goût et de l'odorat, de refuser de participer, d'être mineur ou majeur sous tutelle, ou de maîtriser insuffisamment la langue française pour répondre aux questions. Aucun critère d'exclusion n'a été appliqué.

Les tests ont été réalisés sans occlusion nasale pour les 60 premiers participants (50%) et avec occlusion nasale pour les 60 derniers (50%) afin d'éviter les flux d'air rétro-olfactifs.

2. Tests sensoriels

La partie préparatoire a été réalisée au Centre des Sciences du Goût et de l'Alimentation par Mme Justine Parise, étudiante en M1, encadrée par Mme Chantal Septier, ingénieure d'étude.

2.1. Sélection des composés sapides et de leur concentration pour les tests

Cette phase a consisté à sélectionner cinq produits pour chacune des saveurs retenues (sucré, salé, amer, acide et goût métallique), présenté dans le Tableau 3.

	Fournisseur	Grade	Numéro CAS	Référence	Masse molaire
Chlorure de sodium	Supelco	ACS, ISO, Reag. Ph Eur	7647-14-5	106404	58,44 g/mol
Sucrose	Sigma-Aldrich	Ph Eur, BP, NF	57-50-1	16104	342,30 g/mol
Caféine anhydre	Sigma-Aldrich	FCC, FG	58-08-2	W222402	194,19 g/mol
Acide citrique	Sigma-Aldrich	FCC, FG	77-92-9	W230618	192,12 g/mol
Sulfate ferreux	Supelco	ACS, ISO, Reag. Ph Eur	7782-63-0	1.03965	278,01 g/mol

Tableau 3 : substances retenues pour les tests gustatifs

Après avoir vérifié leur solubilité, des tests de perception de deux différentes concentrations ont ensuite été réalisés chez des volontaires sains (personnel du laboratoire), présentés dans le Tableau 4 et Tableau 5.

Saveur	C1 (g/L)	Masse composé (g)	Volume eau (mL)
Amer	20	0,5	24
Acide	50	1,25	24
Salé	75	1,875	24
Sucré	400	10	23
Métallique	5	0,125	25

Tableau 4 : premier essai de concentration des composés sapides

Saveur	C2 (g/L)	Masse composé (g)	Volume eau (mL)
Amer	50	1,25	24
Acide	100	2,5	24
Salé	200	5	23
Sucré	850	21,25	12
Métallique	10	0,25	25

Tableau 5 : deuxième essai de concentration des composés sapides, avec des concentrations supérieures

Finalement, les concentrations surlignées en jaune ont été retenues car elles étaient identifiées correctement par 100% des volontaires sains.

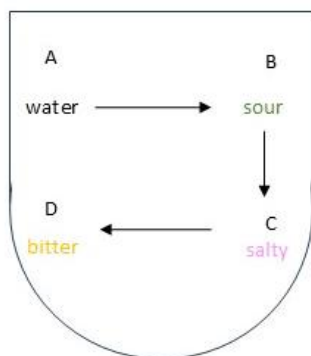
2.2. Modalités de réalisation des tests gustatifs

Différents moyens de déposer la solution sapide sur la langue ont été essayés : disque, pipette, coton-tige classique, coton-tige pour bébé (partie en coton plus grosse). Finalement le coton-tige pour bébé permettait d'apporter suffisamment de liquide sapide tout en étant précis sur la localisation à stimuler. Il a donc été choisi.

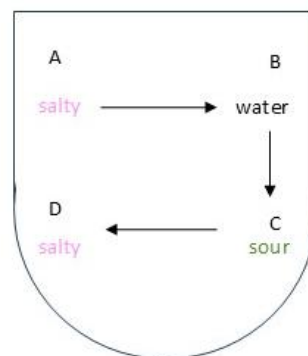
Ces cotons-tiges étaient imbibés de solution gustative et appliqués sur différentes régions de la langue : la base de langue droite (A), la base de langue gauche (B), la pointe de langue gauche (C) et la pointe de langue droite (D).

L'ordre et la localisation d'application des composés sapides est présenté dans la Figure 8 en version anglaise, la version française est dans l'[annexe 3](#).

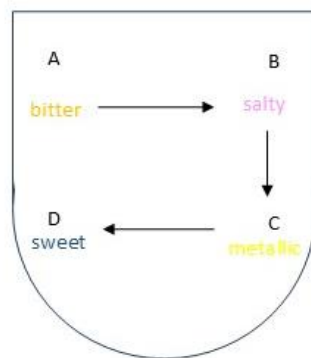
1



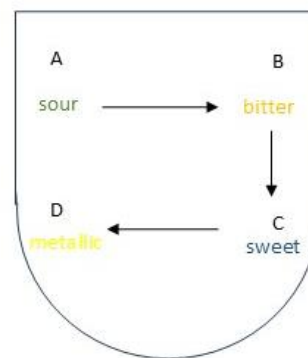
2



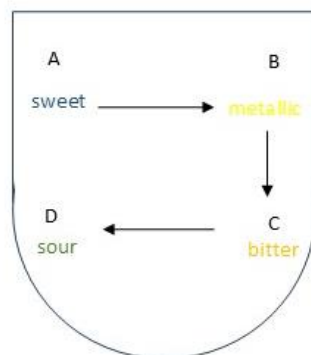
3



4



5



6

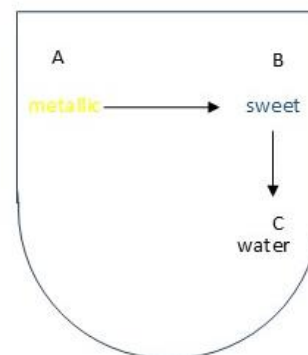


Figure 8: order and location of application of sapid compounds on the tongue

La Figure 9 représente une illustration d'un test sensoriel.



Figure 9: Illustration d'un test sensoriel

L'ordre des saveurs et la localisation de l'application du coton-tige étaient fixés à l'avance dans le protocole et identiques pour tous les individus. Toutefois la multiplicité des tests (quatre saveurs appliquées sur les quatre localisations de la langue) et plusieurs semaines entre chaque test ne permettaient pas aux personnes d'anticiper les réponses des tests.

Un mode opératoire a été rédigé, fourni en [annexe 3](#).

3. Dosages salivaires du malondialdéhyde

Des prélèvements salivaires non stimulés, avec recueil de la salive pendant 10 minutes ont été réalisés en tout début de chaque consultation. Le but de ce dosage est de tester l'hypothèse de la lipopéroxydation comme cause du goût métallique.

Le flacon de prélèvement était immédiatement pesé puis le contenu était transféré dans un microtube et entreposé à -80°C. L'ensemble de la méthodologie est détaillée dans l'article ci-dessous.

ARTICLE TITLE

Determination of the Optimal Method for Measuring Malondialdehyde in Human Saliva

Article soumis le 06/08/2024 à MethodsX

AUTHORS

Brignot H ¹, Rayot C ¹, Buiet G MD ^{1,2} *, Thomas-Danguin T PhD ¹, Feron G PhD ¹

AFFILIATIONS

1. Centre des Sciences du Goût et de l'Alimentation, CNRS, INRAE, Institut Agro, Université de Bourgogne, F-21000 Dijon, France

2. Centre Hospitalier de Valence, 179 Boulevard du Maréchal Juin, 26953 Valence, France

KEYWORDS

Lipoperoxydation; oxidative stress; MDA; TBARS; fluorescence, saliva

ABSTRACT

While few methodological studies have been published on the salivary measurement of malondialdehyde (MDA), none have precisely detailed the analytical method. This work presents the development of an analytical method for MDA measurement in microvolumes of saliva samples from healthy individuals, using thiobarbituric acid derivatization and fluorescence reading of the formed compound.

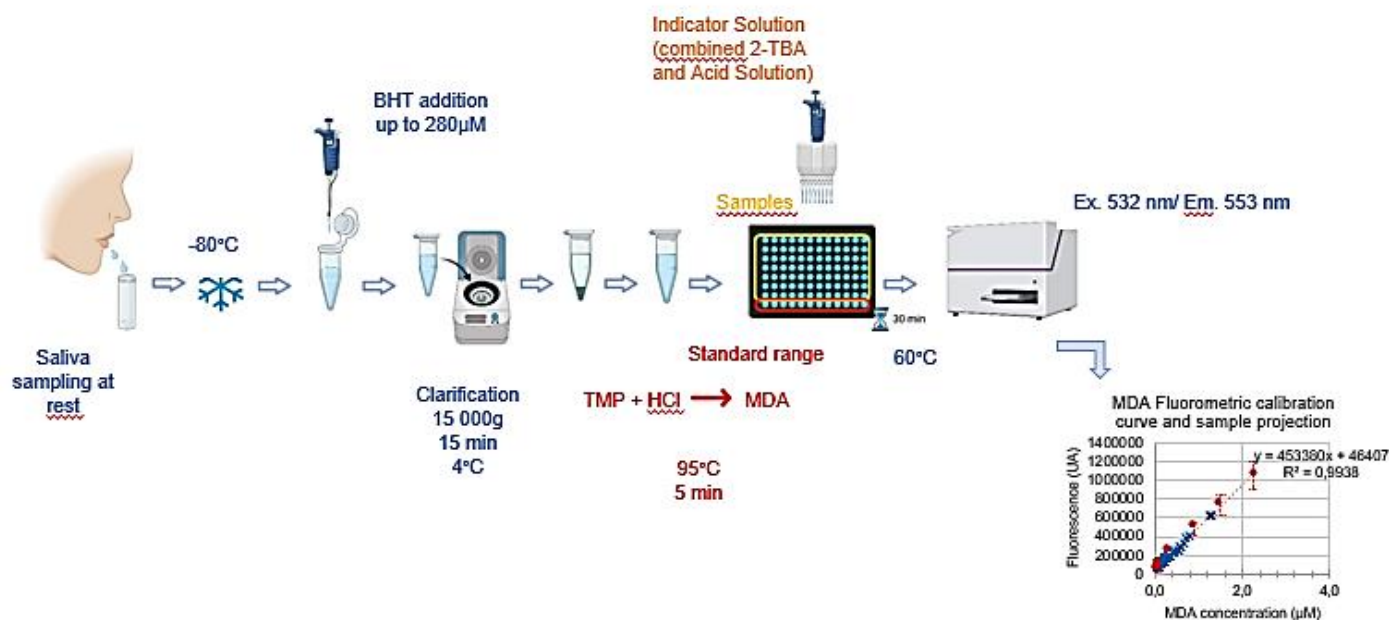
This method was progressively designed to meet specific constraints such as the limited sample volume available, cost-effectiveness of each assay, and time required for analysis. After studying the impact of various parameters, quantitative validation of this method was conducted using the accuracy profile.

The study demonstrated that the fluorimetric analysis method for salivary MDA accurately quantifies the concentration of this molecule within a validity range from 0.031 μM to 0.3 μM . Additionally, the accuracy profile allowed estimation of measurement uncertainty for each concentration level. It was determined that the calibration method using raw data was most appropriate as it introduced the least uncertainty in the obtained results.

Bullet points:

- This protocol is valuable for obtaining an accurate estimation of MDA levels in saliva
- This protocol is well suited for small sample sizes and for important number of samples in term of time consumed and cost
- This protocol can serve as a tool to monitor oral lipid peroxidation in cancer patients

GRAPHICAL ABSTRACT



Created with BioRender.com

Figure 10: graphical abstract

Subject area	Biochemistry, Genetics and Molecular Biology
More specific subject area	Analytical biochemistry, Human Body Fluid, Sample Handling

Name of your protocol	Evaluation of malondialdehyde (MDA) levels in salivary human samples
Reagents/tools	<p>Analytical balance sensitive to 0.1mg Fluorometric plate reader capable of exciting the sample at 532 nm and reading at 585 nm. Polypropylene microcentrifuge tubes Deionized Water (dH₂O) Adjustable monochannel and multichannel micropipettes (10 – 1,000 µL) and tips Heated bain-marie Magnetic stirrer and magnetic bars Classic laboratory glassware 100 mL Reagent reservoirs Non-treated 370µL working volume 96-well flat bottom black polypropylene wells Chemicals: TCA : SIGMA ALDRICH T9159-100G NaOH : FLUKA 71692 HCl : CARLO ERBA 403871 Ethanol RPE : CARLO ERBA 4146072 TBA : SIGMA ALDRICH T5500-25G BHT : SIGMA B1378-100G TMP : SIGMA 108383-100mL</p>
Experimental design	<p>This paper describes an iterative method for the development of a protocol aiming at evaluating MDA levels in salivary human samples considering specific constraints (e.g. size of the sample). The following steps were conducted:</p> <ul style="list-style-type: none"> - literature survey & choice of a fluorometric methodological approach - development of the protocol: choice of the wavelength, incubation time and temperature, reagent orders and concentrations, reagent stability, conditions for measurement - salivary sample handling (clarification, deproteinization) - characterization and validation using the accuracy profile
Trial registration	NA
Ethics	As our work involved human subjects, all the relevant informed consent was obtained from the volunteers
Value of the Protocol	<ul style="list-style-type: none"> ● This protocol is valuable for obtaining an accurate estimation of MDA levels in saliva ● This protocol is well suited for small sample size and for important number of samples in term of time consumed and cost ● This protocol can serve as a tool to monitor oral lipid peroxidation in cancer patients

Tableau 6: Specifications table

BACKGROUND

The perception of a metallic taste is a frequent occurrence in oncology, affecting 21% of patients in a recent meta-analysis (11). There are several possible explanations for the metallic taste, all of which may occur at the same time: lipoperoxidation, inhibition of the facial nerve on the glossopharyngeal nerve, presence of sweeteners, calcium and magnesium salts, anodal stimulation of the tongue, sectioning of the tympanic cord, direct stimulation of the tympanic cord, phantoguesia during

pregnancy, burning mouth syndrome, metals in the mouth (iron, copper, zinc), etc. (16, 79). Among these potential causes, the peroxidation of lipid membranes of the oral cavity cells and salivary lipids, also called lipoperoxidation, could be a principal cause of the perception of this metallic flavor and discomfort in the mouth (12, 82, 129). Lipoperoxidation refers to the oxidation of lipids in the presence of oxygen. It is one of the main biomarkers of oxidative stress (83, 140-144). It concerns lipids with at least two carbon-carbon double bonds (145, 146). Polyunsaturated lipids are the preferred targets of free radicals due to the high vulnerability of the methylene group (-CH₂), positioned between the double bonds.

The most studied class of molecules resulting from lipid peroxidation are α,β -unsaturated aldehydes. The decomposition of hydroperoxides results in the formation of carbonyls including malondialdehyde (MDA) and 4-HNE (4-hydroxy-2-nonenal), MDA being the major and stable final compound of lipid peroxidation (140).

The aim of this report was to develop a reproducible MDA analysis methodology, adapted to salivary samples. This methodology will be used in a study currently being carried out aiming at investigating the association between the presence of a metallic flavor and the amount of salivary MDA in cancer patients. On the basis of a literature search, a protocol was developed. Tests were carried out to vary the assay conditions, adapting to the constraints of sample micro-volume, the time allocated to the assay for use in future analysis campaigns, and the cost of the method.

1. Literature review

Thirty-seven articles were identified (82, 147-182), describing a methodology for MDA level analysis. MDA levels specific to salivary medium were determined in only two studies (181, 182).

MDA was usually determined by derivatization, i.e. MDA forms an adduct with the derivatization molecule, which is then determined by various methods. Of the various derivatization molecules, thiobarbituric acid (TBA) was the most commonly used (23 out of 41 articles). Assay can be performed using a chromatographic separation method, or optionally using a traditional extraction method such as liquid-liquid extraction. The easiest of these methods use direct detection by spectrophotometry and fluorescence. As TBA is used as the derivatization molecule, they are therefore called TBARS (thiobarbituric acid reactive substances) methods. The hot acid condensation reaction between TBA and MDA leads to the formation of a pink pigment by Knoevenagel-type condensation. Fluorescence reading is possible on this molecule because it has several fluorophore groups: 2 conjugated aromatic rings and several π -bonds (double bonds).

Chromatographic analyses make the method more specific for MDA, as they enable the latter to be specifically selected from a number of end products formed during the free-radical-mediated decomposition of polyunsaturated fatty acids. Furthermore, saliva contains few other oxidation products that would react with TBAs, compared with blood products, for example. However these methods are more expensive than microplate-reader assays, due to the cost of the associated specific equipment, are more time-consuming and often require a high sample volume, ill-suited to saliva samples. In addition, pre-analytical liquid-liquid extractions consume a lot of sample amount and are likely to modify the initial content of oxidation products, so this type of preparation was not chosen.

Finally, detection by fluorescence is the most sensitive compared to detection by ultraviolet and visible spectrophotometry (169, 170). It was therefore decided to develop a method, which would enable us to assess lipid peroxidation in saliva and on oral cavity surfaces.

2. Development of the fluorimetric assay method

All the assays described in the following paragraphs were carried out in black-walled and black-bottomed 96-well microplates (Thermo Fisher Scientific, Waltham, MA, USA), due to the constraints of micro-volume and fluorescence usage respectively. Fluorescence intensity data were acquired using the EnSight® plate reader (Perkin-Elmer, Waltham, MA, USA).

Parts 2.1 to 2.9 of the protocol development were carried out on model solutions composed of pure MDA, produced extemporaneously. Parts 2.10 to 2.12 were carried on human saliva from 10 healthy volunteers.

2.1. Initial version of the protocol

The initial version of the protocol was as follows: an MDA calibration range was established from the acid hydrolysis of a 1,1,3,3-Tetramethoxypropane solution. A reagent solution containing a 1.25% solution of TBA, a 50% solution of trichloroacetic acid (TCA) and a 3.5 mM solution of the antioxidant butylated hydroxytoluene (BHT), identified as the most efficient in this reaction (183), was added as a reaction mixture. The various concentrations of the range points were prepared directly in the plate, then the reaction mixture was added. The plate was filmed and incubated for 30 minutes at 60°C in an oven. To stop the reaction, the plate was then cooled in crushed ice. It was confirmed that the reaction in the absence of TBA did not generate an artifact.

2.2. Choice of emission wavelength and control of signal origin

The emission wavelength where fluorescence intensity was maximal was determined by plotting the emission spectrum of the TBA:MDA adduct with a known excitation wavelength, $\lambda_{exc} = 532$ nm and a range of emission wavelengths studied from $\lambda_{em} = 550$ to 580 nm with 1-nm-steps.

2.3. Study of the effect of incubation time and temperature

In the literature, incubation times ranged from 15 to 60 min and temperature ranged from 60 to 100°C. Studies also highlighted that there was a tendency to avoid over-oxidation phenomena during the assay reaction at 60°C compared to higher temperatures. Based on this, the incubation time was varied at 30 minutes and 60 minutes and the incubation temperature at 60°C and 80°C. The effect was estimated via a change on the calibration curve parameters.

2.4. Study of the effect of reagent introduction order

The aim was to study the order of introduction of reagents on the right-hand equation of a calibration range to control the effect of compound auto-oxidation.

In the initial protocol (§2.1), the reagent was prepared by mixing TBA, TCA and BHT together. In an alternative protocol, the calibration range was established by first mixing the MDA standard solution with BHT and TCA in the same proportions as in the initial protocol reagent. The plate was then incubated for 15 minutes at 60°C. The TBA solution was then added and the plate incubated again for 30 minutes at 60°C.

2.5. Study of the effect of BHT concentration

In the initial protocol, the BHT concentration in the reagent was set to 5%. We varied this BHT concentration to 0.5% and 10% to assess the effect on the equation of the straight line of a calibration range.

2.6. Determining the acid to use for the reagent solution

The reaction is only possible in an acidic environment (167). In the literature, around ten different acids have been used (169, 184), most often the same as those used for the preliminary extraction of the analyte. We have decided to compare only the two most frequently cited and common laboratory acids: TCA and acetic acid, whose pH has been reduced to 3.5 using NaOH.

2.7. Study of the effect of TCA concentration in the reaction medium

As the pH of the reaction medium had to be between 2 and 4 (167), it was necessary to control pH to ensure that the reaction takes place under the best possible conditions. In the initial protocol (§2.1), a 50% TCA solution was chosen in accordance with the literature describing the same assay applied to mitochondrial-type samples (173). During our experiments, a reaction pH of 0.35 before incubation and 0.12 after reaction was measured. To achieve the correct pH range which has to be between 2.5 and 4.5, the concentration of the TCA solution used for the reagent was lowered to 9.8% (183, 184).

2.8. Reagent stability

Assessment of reagent stability enabled us to evaluate the degradation of solutions over time, and to optimize the time allocated to each assay, by knowing which solution could be prepared in advance and which could not. To this end, we focused on TBA only and not on BHT. Indeed, the function of BHT is to prevent further oxidation when the reaction medium is heated and the choice was to not take any methodological risk regarding this preventing effect. For TBA solution, dosing was performed with the same solution from D to D+5.

2.9. Checking the MDA ranges of measurement

The initial version (§2.1) was based on a protocol conducted on mitochondrial suspension samples (173). However, in our study, the saliva used to develop the method came from 10 healthy subjects (with no pathologies or damage to the oral cavity) and the amount of MDA was supposed to be very low. For this reason, it was necessary to work with different concentration ranges. To best meet the assay objectives, 2 MDA ranges of measurement were studied and are presented below:

- Range from 0 to 0.09 nmol/well

The range was set between 0 and 0.09 nmol MDA/well, corresponding to MDA concentrations ranging from 0 to 0.3 μM .

- Range from 0 to 0.18 nmol/well

Subjects treated for cancer have higher salivary MDA concentrations than healthy subjects, $1.0 \pm 0.21 \mu\text{mol/l}$ vs $0.08 \pm 0.07 \mu\text{mol/l}$ respectively (141). Although our method was developed on salivary samples from healthy subjects, it will be used for assaying samples from cancer patients. The study by Kaur et al (185) showed that the salivary MDA concentration of a cancer patient is equal to 1 μM , corresponding to an amount of 0.3 nmol/well, the test being conducted on 150 μL of saliva.

The range was thus set between 0 and 0.18 nmol/well, corresponding to MDA concentrations ranging from 0 to 0.6 μM . The aim was to ensure that the method's results were linear at concentrations close to the target concentration, and to enable modeling of the accuracy and uncertainty of results at these concentrations.

- In addition, measurements were performed at concentrations between 0 and 0.006 nmol/well, to test the sensitivity of the assay.

2.10. Sample pretreatment and optimization

The overall aim of the project was to find an assay method that would use a minimum of sample amount, be easy to implement and not time-consuming.

Parts 2.10.1 to 2.10.4 were carried out using a saliva pool consisting of a mixture of saliva samples collected from 10 individuals. Each condition was carried out over five replicates, due to the limited amount of saliva available. Parts 2.10.5 to 2.10.7 used samples from 3 other individuals to estimate the inter-individual effect of pre-treatments. In these sections, to monitor the fate of MDA during the preparative stage and evaluate a recovery yield, a sample corresponding to high concentration of MDA (1 μM) was mimicked.

Each salivary sample was collected from an in-house panel of healthy subjects in accordance with biosafety regulations for studies involving human subjects. Saliva was collected at rest in a collection jar placed on ice to preserve sample from inherent degradation. All samples were mixed, clarified or deproteinized, aliquoted in 2 mL cryotubes and frozen at -80°C for less than 1 month. The required volume was thawed just before each assay.

2.10.1. Study of the effect of the amount of sample used for the assay and the degree of sample concentration

From the literature review, results obtained on the amount of native salivary MDA from non-pathological patients are very heterogeneous. Thus the issue regarding the amount of saliva that has to be introduced into the wells for MDA detection was addressed by testing several volumes of saliva, i.e. 150 μL / 40 μL / 20 μL / 10 μL / 4 μL .

Moreover, saliva was concentrated by 2 and by 4 using a centrifugal evaporator. A volume of 40 μL of these concentrated saliva samples was introduced into the wells.

2.10.2. Study of the effect of sample clarification

Clarification is a gravimetric centrifugation method in which large particles are removed. The supernatant, homogeneous and free of debris and large salivary proteins such as mucins, is analyzed.

We assessed the effect of clarification versus no treatment on the determination of native MDA in saliva. Saliva was centrifuged for 15 min at 15,000 g at 4°C . 40 μL of centrifuged or uncentrifuged saliva were deposited in each well, 135 μL of reagent and water qsp 300 μL . The rest of the protocol was the same as described in § 2.1.

2.10.3. Study of the effect of a detergent

Sodium dodecyl sulfate (SDS) is an anionic surfactant. It can form micelles due to its amphiphilic properties. It can act thus as a detergent, breaking the covalent bonds of macro-molecules (proteins, lipids) and making the analyte (MDA) available for the assay reaction.

400 μL of saliva was added to 400 μL of 10% SDS solution. The mixture was vortexed for 5 minutes. The comparison was carried out on a non-centrifuged saliva sample. 80 μL of the saliva/SDS mixture were deposited per well. The rest of the protocol was the same as in § 2.1.

2.10.4. Sample deproteinization study

Proteins are the main constituents of saliva (1 mg/mL), and can cause interference, notably due to the presence of bound sialic acid/N-acetyl-neuraminic acid (166, 184). Indeed, if present in the reaction medium, these compounds may fluoresce, hindering assay interpretation. TCA is often used to

precipitate proteins from biological fluids. In the chemical method used in our protocol proteins are removed from the sample using a 10% TCA solution. The mixture was vortexed for 5 minutes, frozen for 12 hours and centrifuged the following day for 1 hour at 15,000 g at 4°C, with only the supernatant retained. 150 µL of the extract was inserted into the wells. The rest of the protocol was the same as in § 2.1.

2.10.5. Comparison of clarification versus sample deproteinization

MDA (+/-) BHT qsp 3.5mM was introduced into the sample and the 2 preparative methods were compared. The addition of BHT was tested after preparation before freezing if it had not been added with MDA, in order to assess the importance of its protective antioxidant role towards MDA in each separation method.

2.10.6. Study of the antioxidant effect of BHT

BHT was added at 3.5mM to samples before or after clarification (§2.10.2). A control in the absence of BHT was carried out.

2.10.7. Study of the effect of sample freeze/thaw cycles

Once the samples had been prepared, they were assayed before freezing and after a freeze/thaw cycle. To ensure that the preparation conditions tested corresponded to those used in future studies, the MDA concentration in samples that had undergone 2 freeze/thaw cycles was determined.

2.11. Verification of the dosing method using the dose-response approach

To ensure that the method doses MDA and no other interacting product, it was necessary to implement a dose-response approach during development. This quantitative analysis technique was used to control and minimize matrix effects that interfere with analyte measurement signals. It involved adding known quantities of a standard solution of MDA to identical samples. This method was very time-consuming for salivary samples, but was essential when developing methods for biological samples where matrix effects are present.

To implement this method, 150 µL of deproteinized saliva, known quantities of standard MDA solution, 135 µL of reagent solution and 300 µL qsp of ultrapure water were added to each well. Due to the small amount of saliva available, a 6-point dosing range was established with the following quantities added per well: 0; 0.006; 0.018; 0.03; 0.06; 0.09 nmol of MDA, with 2 replicates per quantity added.

This method was used throughout the development of the salivary sample pre-treatment method.

2.12. Choice of data processing method

Throughout the salivary sample tests, calculations were carried out using 3 different methods:

- Using the equation of the regression line of the calibration range (pure MDA) from the raw data
- Using the equation of the regression line of the calibration range (pure MDA) from data corrected for the blank value (solution without MDA)
- Using the equation of the regression line of the range of MDA additions into a saliva sample (pool of saliva from different individuals) used for the dose-response approach compared to a direct method.

3. Characterization and validation using the accuracy profile

3.1. Validation data collection

The aim of the validation experimental design was to estimate the method's performance under conditions similar to those of its routine application. This technique enables us to determine how the method will perform when used on a regular basis. The tests had to be carried out using the method as it will be used routinely.

To achieve the plan, were carried out:

- 5 series of measurements ($1 \leq i \leq 5$) (i.e. 5 different assays)
- For each series, there are 2 repetitions ($1 \leq j \leq 2$) (due to the low volume of saliva available)
- 7 concentration levels ($1 \leq k \leq 7$) for the calibration range and 6 concentration levels for the saliva samples ($1 \leq k \leq 6$) covering the range of application of the method.

3.2. Accuracy profile

The accuracy profile has been implemented in accordance with AFNOR NF-V03-110 and Feinberg et al. (186). The interval that contains on average a defined proportion (in %) of future measurements, obtained according to a given procedure and for a given concentration has a so-called tolerance limit, β . The so-called acceptability interval specifies the required performance for the method, expressed as an

acceptable relative deviation (in %), called λ , around the reference value. Preliminary tests have established: $\beta = 80\%$ and $\lambda = 30\%$. The range of validity of the method is determined by the zone of the accuracy profile in which the method provides a proportion of acceptable results at least equal to β . It is limited by a lower bound which is equivalent to the lower quantification threshold, determined by the intersection between the acceptability limit and the tolerance limit. The upper bound will be chosen as the highest concentration in the range. As soon as the tolerance interval falls outside the acceptability interval, it can be concluded that the method is no longer capable of providing sufficient so-called acceptable results with the choices made for λ and β . This graph also provides other indications: the variation in accuracy (with recovery) as a function of concentration. In addition, this approach makes it possible to model accuracy and uncertainty over a concentration interval.

The accuracy profile was implemented:

On one hand, on a pure MDA concentration range

On the other hand, on a concentration range of MDA added to 4 salivary samples corresponding to 4 different individuals for whom at least 3 mL of saliva had been collected.

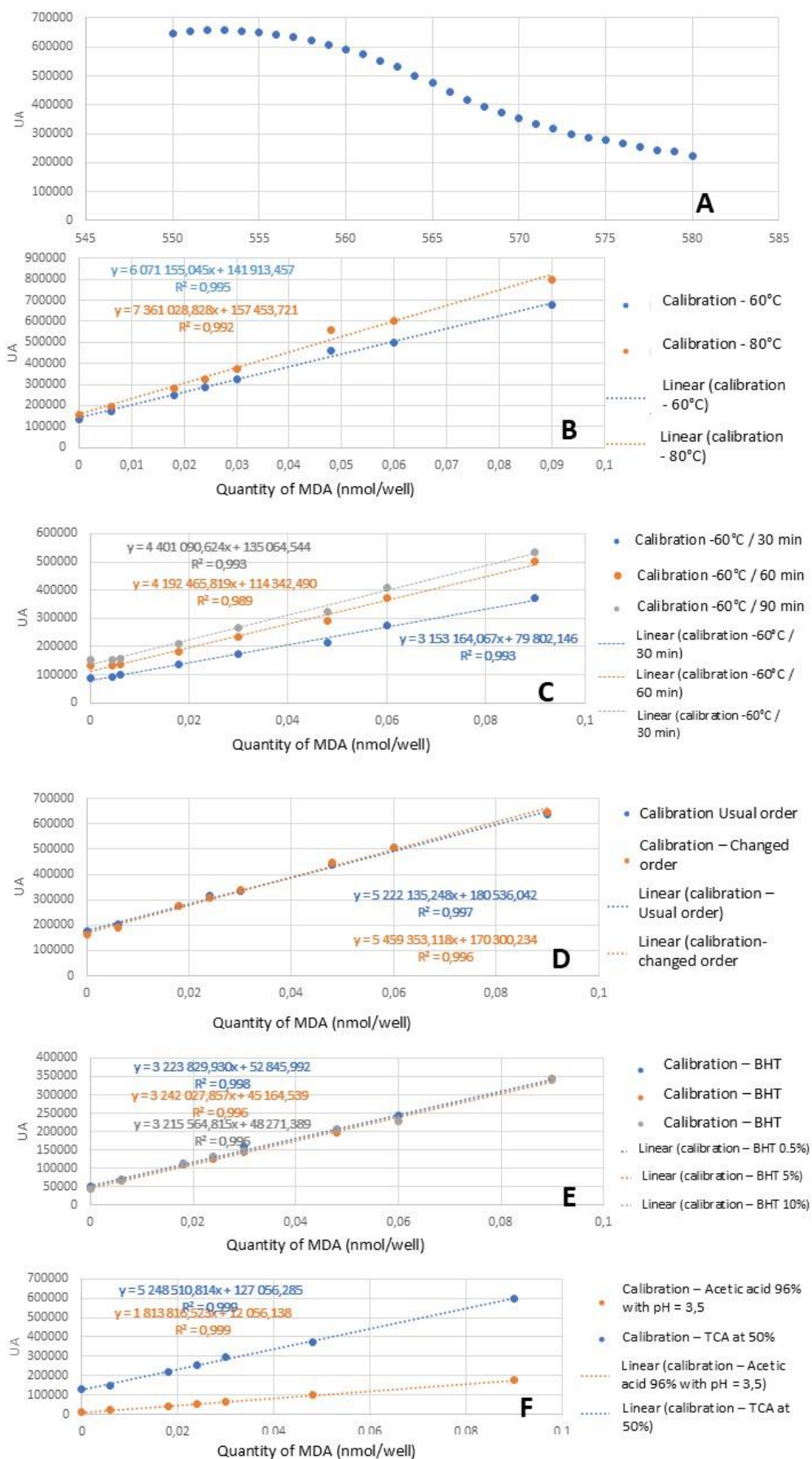


Figure 11: protocol validation (1/3)

in all graph the measured values are represented by the dots and the regression line by the dotted line.

A: spectrum of the TBA:MDA adduct with $\lambda_{exc} = 532$ nm

B: calibration lines $UA=f(QtyMDA)$ with comparison of incubation temperature

C: calibration lines $UA=f(QtyMDA)$ with comparison of incubation time

D: calibration lines $UA=f(QtyMDA)$ with comparison of order of reagent introduction : incubate BHT + TCA + TBA all together (blue) or premix BHT + TCA + sample incubated 15 min and then add TBA and incubate (orange)

E: calibration lines $UA=f(QtyMDA)$ with comparison of BHT concentration

F: calibration lines $UA=f(QtyMDA)$ with comparison of acid used in reagent

4. Procedure for developing the fluorimetric method

Figure 11A shows the evolution of the emission spectrum of the TBA:MDA adduct with $\lambda_{exc} = 532$ nm.

The maximum fluorescence intensity is at a wavelength $\lambda_{em} = 553$ nm.

For the rest of the protocol development, we therefore chose $\lambda_{em} = 553$ nm in line with the literature (167).

The reaction in the absence of TBA adduction shows a negligible signal (Tableau 7) representing 1.6% of the fluorescence intensity value of the signal in the presence of TBA in the reaction medium.

replicates	Fluorescence intensity (AU) if [MDA] = 0 μ M	
	without TBA	With TBA
1	721	51429
2	856	50100
3	764	47859
Mean	780	49796
ratio	1,6%	

Tableau 7: Fluorescence values for reaction mixtures with and without TBA.

5. Study of the effect of incubation time and temperature

Figure 11B shows the calibration lines $UA=f(QtyMDA)$ as a function of incubation temperature.

The linear regression modeling the reaction at 80°C has a directrix 1.2 times greater than that of the reaction at 60°C. This proportion was not very high, which validates the use of the 60°C assay. Indeed, the aim is to work with biological samples and over-oxidation of salivary compounds must be avoided. Thus the lowest possible temperatures allowing the TBA:MDA adduction reaction to take place must be used.

6. Incubation time

Figure 11C shows the calibration lines $UA=f(MDAQty)$ as a function of incubation time.

It has been noted that the directing coefficient of each linear regression increased when incubation time increase. It means that higher the incubation temperature is, higher is the sensitivity of the assay for pure MDA. An incubation time of 30 min versus 60 min reduced reactivity by 25%. This reduction was judged as acceptable for further experimentation, the main aim of the protocol being to minimize oxidation of salivary components during the measurement. An incubation time of 30 min was therefore chosen for further experimentation. However, the y-intercept (corresponding to fluorescence intensity of sample-free wells) also increase when incubation time increase and is due to natural evolution of the reactional mixture. Ainsi, cela souligne l'importance de respecter des conditions d'incubation fixes (30 min at 60°C as it was chosen).

7. Study of the effect of reagent introduction order

Figure 11D shows the calibration lines $UA=f(QtyMDA)$ with comparison of the order of reagent introduction. The order of introduction of the reagents has no effect on the directing coefficient, nor on the y-intercept of the calibration line, since the equations of the calculated lines are identical.

In the following development, the reagent solution is carried out as in the initial protocol (§2.1), i.e. TBA, TCA and BHT added at the same time, followed by the incubation step.

8. Study of the effect of BHT concentration

Figure 11E shows the calibration lines $UA=f(QtyMDA)$ comparing the use of BHT solutions at 3 different concentrations. There was no effect of BHT concentration on the directrix or y-intercept of the calibration line.

For further development, we decided to keep the BHT concentration used in the initial protocol, i.e. 5% in the reagent.

9. Test of the acid to be used in the reagent solution

Figure 11F shows the calibration lines $UA=f(QtyMDA)$ as a function of the acid used in the reagent. The directrix of the calibration line with acetic acid reduced to pH 3.5 was 3 times lower, but also with a 10-fold lower intercept. The reaction mixture with TCA showed much better reactivity, and it was decided to retain it for further development.

10. Study of the effect of TCA concentration

The background noise (Figure 11F) was lower with acetic acid, indicating that the presence of TCA at a high final concentration (around 800mM) induces a background noise that should be reduced by lowering the TCA concentration. The TCA solution was thus reduced to 9.8% instead of 50% in the preparation protocol, which corresponded to 150mM in the final reaction medium, five times less than initially. By decreasing this concentration, the pH increased by nearly 2.5 pH units both before and after the reaction (Tableau 8). The drop in pH after incubation, in both situations, proved that a reaction indeed took place in each well

	pH before incubation	pH after incubation	Blank value (UA)
TCA at 50%	0,35	-0,12	153 621
TCA at 9,8%	2,8	2,5	46 248

Tableau 8: Presentation of blank values and pH before and after incubation

Résultats : Prévalence du goût métallique en oncologie

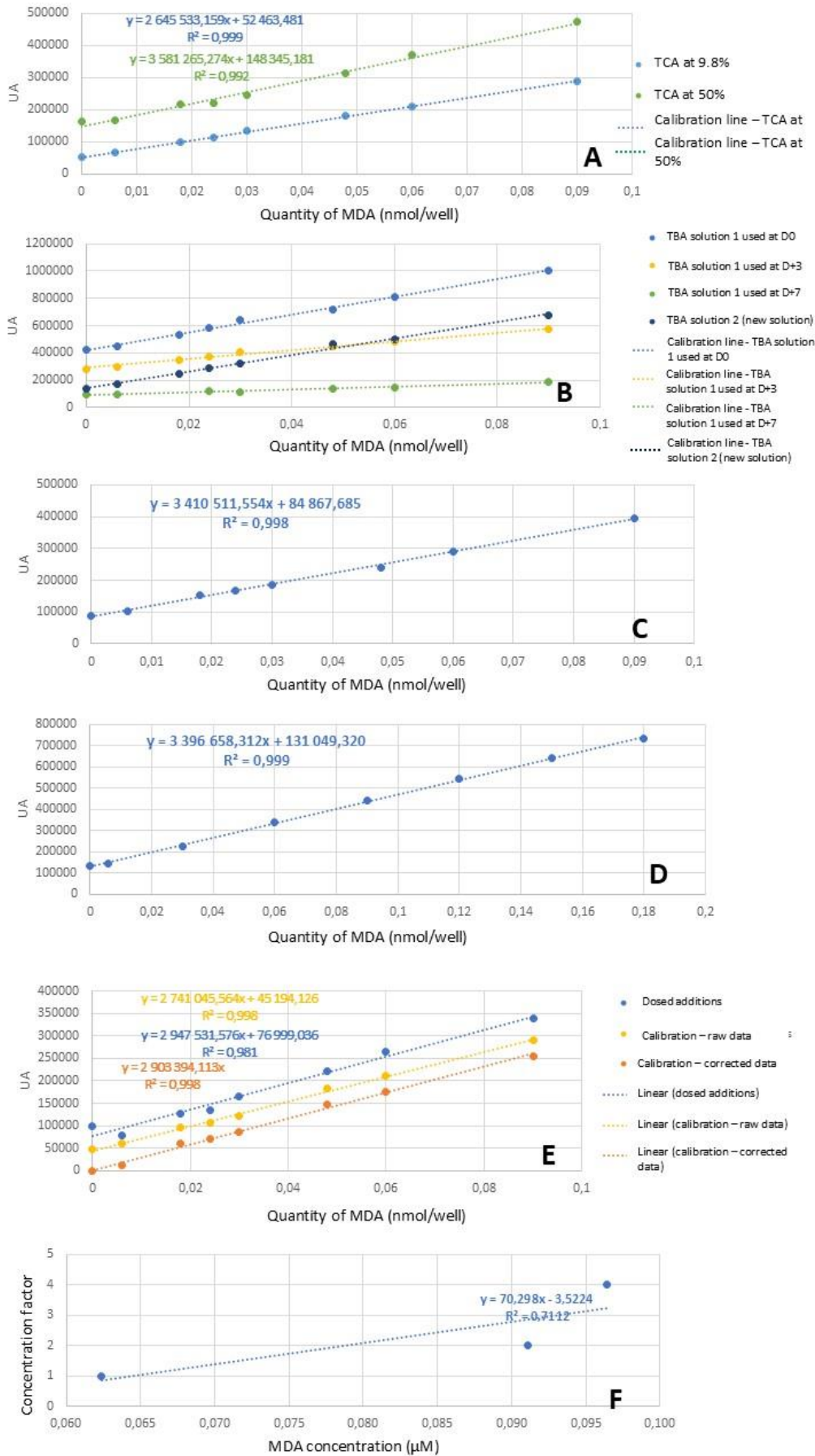


Figure 12: protocol validation (2/3)

In all graph the measured values are represented by the dots and the regression line by the dotted line.

A: calibration lines $UA=f(QtyMDA)$ with comparison of the TCA concentration used in the reagent

B: calibration lines (yellow and orange) $UA=f(QtyMDA)$ and dosed addition line (blue) $UA=f(QtyMDAadded)$ for assay n°1

C: calibration lines (yellow and orange) $UA=f(QteMDA)$ and dosed addition line (blue) $UA=f(QteMDAajoutée)$ for assay n°2

D: calibration line $UA=f(QtyMDA)$ ranging from 0 to 0.09 nmol MDA/well

E: calibration line $UA=f(MDAQty)$ ranging from 0 to 0.18 nmol MDA/well.

F: calibration lines (blue and green) $UA=f(QtyMDA)$ and dosed addition lines (yellow) $UA=f(QtyMDAadded)$.

Figure 12A presents the calibration curves of $UA=f(MDA \text{ amount})$ in each reaction medium with the two TCA concentrations used to prepare the reagent. Additionally, by using a lower concentration of TCA, it was possible to reduce the y-intercept value by nearly 30%, thus decreasing the background noise. Since the obtained curves were parallel, no variation in reactivity with the change in TCA concentration was observed. Only the blank value decreased with the concentration reduction, proving that TCA induced fluorescence in the reaction medium.

For further development, we therefore decided to use a 9.8% TCA solution in the reagent.

11. Stability of reagents

Figure 12B presents the calibration curves of $UA=f(MDA \text{ amount})$ based on TBA solutions used at different time of storage. The TBA solution degraded over time as a decrease of the directing coefficient of each linear regression over the days (D+0, D+3, D+7) was observed. With TBA solution 2 was freshly prepared, the directing coefficient of linear regression is the same than solution 1 at D+0 ;

It was decided that the TBA solution should be stored for a maximum of 48 hours in the refrigerator.

Figure 13 summarizes the step-by-step approach.

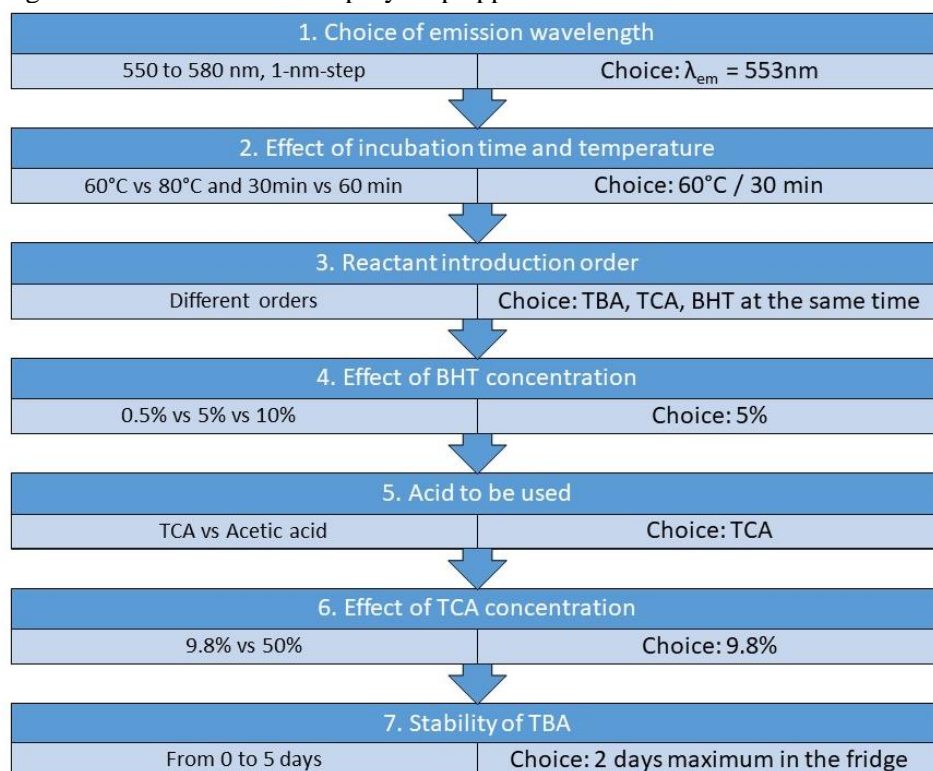


Figure 13 : step-by-step approach of the analytical method.

12. Verification of the measurement range

12.1. Range from 0 to 0.09 nmol/well

Figure 12C presents the calibration curve of $UA=f(MDA \text{ amount})$ ranging from 0 to 0.09 nmol of MDA/well.

The linear correlation coefficient R^2 was greater than 0.99. Amounts of MDA and corresponding fluorometric responses are highly correlated.

12.2. Range from 0 to 0.18 nmol/well

Figure 12D presents the calibration curve of $UA=f(\text{MDA amount})$ ranging from 0 to 0.18 nmol of MDA/well.

The linear correlation coefficient R^2 is greater than 0.98. Amounts of MDA and corresponding fluorometric responses are highly correlated.

The method can therefore be used for the assay of saliva samples collected from pathological subjects.

13. Optimization of Sample Pretreatment

When the expression 'unusable results' is used, it means that the fluorescence intensity was equal to that of the blank for the saliva samples. This indicates that MDA was not detected in the sample.

13.1. Study of the Effect of Dilution and Concentration of Saliva (without Deproteinization)

Figure 12F presents the results obtained with the modified protocol with the following parameters: reagent prepared with 9.8% TCA and deproteinization of the saliva sample (section M&M 2.9.4). There was no proportionality between the MDA concentration of the saliva sample; however, there was a trend towards linearity ($R^2=0.71$). Indeed, concentration by evaporation changed the physical properties of the saliva. The fluid thickened, and the concentration increased the turbidity, which is incompatible with the chosen detection mode during the assay. Similarly, a diluted sample did not have the same refractive index as an undiluted sample, making it impossible to compare the measurements. Thus, with a set of samples having a wide range of MDA content, highly concentrated saliva samples could not be diluted to be included in the calibration range. The calibration range will need to be extended with higher concentrations.

13.2. Study of the Effect of Clarification

This study is not presented because the results could not be exploited.

13.3. Study of the Effect of a Detergent: SDS (without Deproteinization)

This study is not presented because the results could not be exploited.

13.4. Study of Sample Deproteinization

Figure 12E presents the calibration curves (yellow and orange) $UA=f(\text{MDA amount})$ and the spiked addition curve (blue) $UA=fB(\text{added MDA amount})$. The spiked addition method allows checking that the linear regression curve after spiked additions of MDA in a saliva sample had a slope equivalent to the regression curve of a pure MDA solution calibration range. The smaller the difference between these two slopes, the better the assay accuracy and analyte extraction during the sample pretreatment. In this case, the slope of the spiked addition curve was 1.07 times greater than that of the calibration curve, considering the raw data, and 1.01 times greater when considering the corrected data. The deproteinization of saliva proved to be effective and was retained for the preparation of saliva samples.

13.5. Comparison of Clarification versus Deproteinization of the Sample

The four experiments showed that the introduced MDA concentration was recovered after clarification only. After protein depletion with TCA, only 4 to 25% of the initial MDA concentration was recovered (3 intermediate preparative experiment conducted on 3 different days, 2 replicates, $n=3$ subjects). One possible interpretation is that MDA binds to and/or is trapped by the sample proteins to buffer this oxidative excess. Although the results of these two methods were usable, we chose to proceed with clarification to implement the analytical method, considering the practical constraints of the studies while being aware of the potential interactants, which are the salivary proteins.

13.6. Study of the Antioxidant

Effect of BHT: If BHT was not added, the amount of MDA recovered in the sample after clarification varied from 1 to 100% of the introduced amount (one experiment day, 2 replicates, $n=3$ subjects) meaning that the recovery percentage depended on the sample and was not reliable. If BHT was

introduced after clarification, the amount of MDA presented in the sample was 50 to 75% of the initially introduced amount (one experiment day, 2 replicates, n = 3 subjects) even though the recovery was not total it is similar and repeatable among the samples. BHT seemed to play an antioxidant role, especially when introduced quickly into the samples. Therefore, BHT had to be introduced before clarification to maximize the amount of MDA recovered in the sample.

13.7. Study of the Effect of Freeze-Thaw Cycles on Samples

72 to 80% of the added amount is recovered in samples that underwent a freeze-thaw cycle compared to those that did not (one experiment day, 2 replicates, n = 3 subjects). If the preparation and assay were done on samples that had already undergone a freeze-thaw cycle (2 experiment days, 2 replicates, n = 3 subjects), the initially introduced MDA amount was also recovered.

14. Conclusions on preparation

The practice of saliva collection during clinical and sensory studies does not allow for immediate preparation and assay. Therefore, BHT must be added, and the samples must be clarified after undergoing a freeze-thaw cycle, then refrozen in microplates, and thawed again to perform all analyses simultaneously.

Analysis is designed to be performed in 96 wells microplates. Two Internal Quality Control in replicate were added into each one. It consisted of a saliva sample that had undergone the same preparatory steps as the study samples and is placed in each microplate to control a bias between plates.

The sample preparation protocol that optimized MDA yield considering the study conditions and context is presented in Figure 14.

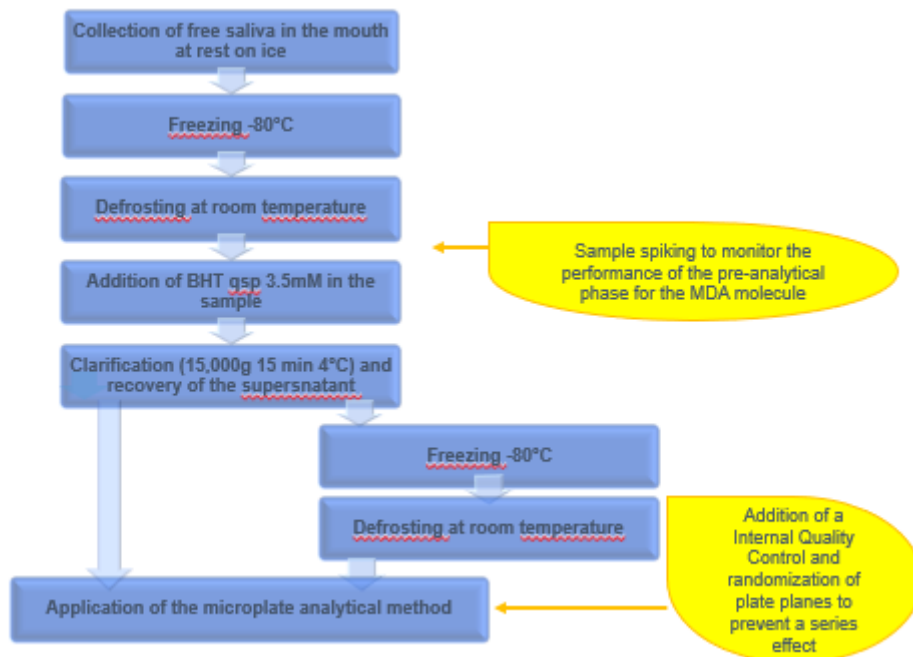


Figure 14: optimized sample MDA yield considering the study conditions and context

15. Verification of the Concentration of an Average Saliva Sample by the Spiked Addition Method

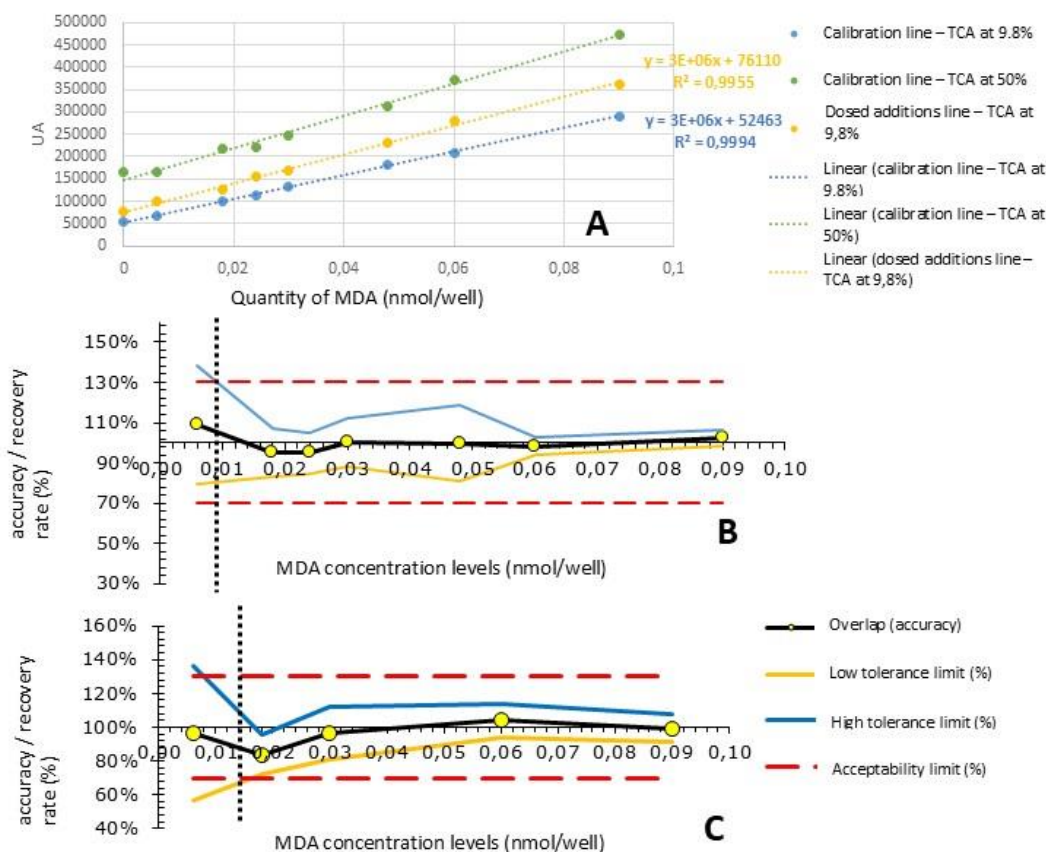


Figure 15: protocol validation (3/3) :

A: $UA=f(QtyMDA)$ calibration lines as a function of different TCA solutions. The measured values are represented by the dots and the regression line by the dotted line.
 B: Accuracy profile obtained for the crude calibration method
 C: Accuracy profile obtained for a saliva sample from an individual spiked with MDA at 5 concentration levels.

Figure 15A presents the calibration curves (blue and green) $UA=f(MDA \text{ amount})$ and the spiked addition curve (yellow) $UA=f(\text{added MDA amount})$.
 As previously noted, it was necessary for the spiked addition curve to be above the calibration curve to obtain usable results. Using a solution highly concentrated in TCA in the reagent caused too high background noise (sections 6 and 9 of the Results) and made it impossible to use the spiked addition method, as it was then below the calibration curve on the graph. By reducing the TCA concentration from 50% to 9.8%, the blank value decreased, and graphically, the spiked addition curve passed above the calibration curve, making the results exploitable.
 Plotting the spiked addition curve ensured that it had a slope very close to that of the pure analyte calibration curve (3.106 UA/nmol). The difference in the y-intercept was explained by the presence of MDA in the saliva sample.
 The regression coefficient R^2 was > 0.99 , indicating excellent regression quality, matching the quality level of a pure analyte calibration curve.

16. Determination of the Quantification Limit and Characterization by Accuracy Profile

Thanks to the accuracy profile representation, the quantification limit was determined as the intersection of the vertical dashed line with the x-axis. It was equal to 0.0092 nmol/well, corresponding to an MDA concentration of 0.031 μM .

The direct method using corrected data (see section 2.12.) presented a higher quantification limit (0.016 nmol/well, or 0.053 μM) than the one using raw data. It was decided to proceed with direct calibration using raw data because this method provided a lower quantification limit compared to the method using data corrected for the blank value

Figure 15B shows the accuracy profile obtained at different levels of the pure analyte concentration in solution using the direct method with raw data. The determination of the quantification limit with spiked Addition Method was carried out as previously described. It was 0.014 nmol/well, corresponding to an MDA concentration of 0.046 μ M. It was better compared to direct method but the sensitivity is close. Figure 15C shows the accuracy profile obtained for a saliva sample from an individual spiked with MDA at 5 concentration levels.

17. Application of the Spiked Addition Method compared to a direct method

The Spiked Addition Method is not suitable for measuring saliva samples in microvolumes for a research project. Indeed, the method as implemented during development (6 concentrations and 2 replicates) required a volume of saliva of 1.8 mL per sample. This volume may be difficult or impossible to obtain in the case of pathological subjects for example. In case of the direct method only 150 μ L of prepared supernatant is required by replicate and is convenient for low volume saliva samples.

FINAL PROTOCOL

The final protocol is presented in [annex 4](#).

CONCLUSION

The iterative approach implemented for designing the most efficient protocol for the evaluation of MDA levels in saliva was successful regarding the different constraints, i.e. size of the salivary samples, costs, and time consumed and despite some limitations described above. It will be used for investigating MDA levels in a clinical study conducted on cancer patients. However, this protocol could be also used for other studies with same aims.

4. Questionnaires

Les questionnaires de qualité de vie EORTC QLQ 30 (187) et HN35 (76) étaient remplis pendant que l'infirmière traitait le prélèvement salivaire. Le questionnaire du goût métallique (35) était rempli entre deux séries de tests gustatifs. Les trois questionnaires sont présentés en [annex 5](#).

RESULTATS

1. Metallic taste prevalence in patients treated for cancer: A systematic literature review and meta-analysis

Ce travail a été présenté en poster au congrès de la Société Française d'ORL à Paris en octobre 2021 et à celui de l'Association Francophone des Soins Oncologiques de Support à Lille en octobre 2022. Il s'agit d'une **revue systématique de la littérature sur l'incidence du goût métallique en oncologie**. Il a été publié dans *Supportive Care in Cancer* (11).

Supportive Care in Cancer
<https://doi.org/10.1007/s00520-022-06904-y>

REVIEW ARTICLE



Metallic taste prevalence in patients treated for cancer: a systematic literature review and meta-analysis

Guillaume Buiret¹ · Thierry Thomas-Danguin² · Gilles Feron²

Received: 1 June 2021 / Accepted: 9 February 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

ABSTRACT

Background: Metallic taste (MT) is a taste abnormality often reported by cancer patients. The aim of this systematic review was to exhaustively report MT incidences in cancer patients and to evaluate the risk of bias in the pertinent studies in accordance with a meta-analysis approach.

Methods: The research objective was to determine the prevalence of MT in patients treated for cancer. A literature search was conducted using PubMed, Web of Science, and Embase. The authors each screened articles and evaluated the eligibility and individual risk of bias for each article. Then, all of the results were compared. A meta-analysis was conducted on studies that specifically focused on MT evaluation.

Results: Very few articles have been published on the incidence of MT among taste and smell abnormalities in cancerology (22 of 1674, 1.3%), and the quality of the reports on MT was often low. The most common bias was the methodology used for MT evaluation. Pooling the results of the 22 studies led to an estimated MT incidence in the cancer patient population of 29% (95% CI [0.21;0.39]) with high and significant heterogeneity observed among the studies. A heterogeneity analysis was performed to identify the causal factors of this heterogeneity. The specific impact of MT on nutritional status (two) and quality of life (five) studies were reported, respectively, and without a specific evaluation of MT. There was no mention of oral health in any of the studies.

Conclusion: Although in clinical practice cancer patients often report MT, its incidence has only been reported in 22 studies, most of which have a moderate to severe risk of bias. Considering the rather high prevalence of MT, more research should be conducted in this field to better identify its causes and mechanisms.

Keywords: metallic taste, incidence, cancer, treatment, meta-analysis

INTRODUCTION

Taste and smell abnormalities (TSAs) are frequent in oncology regardless of the type of cancer (any solid or hematological type (2-6)) and are often considered caused by the treatments administered. For instance, dysgeusia affects 50% to 75% of patients depending on the administered treatment(s) (chemotherapy, radiotherapy, or radiochemotherapy) (3, 188). Perception thresholds can be modified (increased or decreased), perceptions can be distorted (parageusia or parosmia) and/or patients may have perceptions of flavors that do not exist (phantogeusia or phantosmia). TSAs can also impact nutritional status by limiting oral intake (7, 8) and subsequently decreasing quality of life (3, 9, 10).

Metallic taste (MT) is an unusual form of parageusia. Patients describe MT as a spontaneous, unpleasant, persistent metallic sensation in the mouth. Very little is known about the trigger mechanisms for this sensation, possibly due to oncologists' inconsistent reporting of this symptom, one illustration of which being the large difference in MT incidence reported by patients and their oncologists (189) in which oncologists believed that only 9.9% of their patients suffered from MT, whereas 32.2% of patients reported MT. A few conditions associated with MT are well known, such as oral candidiasis and/or dry mouth (69, 190). However, the specific mechanisms underlying MT sensation are poorly understood, although several hypotheses have been suggested. The origin of MT may be (i) biochemical, through oxidation between Fe²⁺ and oral lipids in the mouth (12); (ii) electrical, induced by local hydrolysis and a spontaneous electrical current between dental amalgams (16); (iii) neurological, due to the removal of inhibition of the facial nerve on the glossopharyngeal nerve (13-15); and/or (iv) proteomic in origin linked to the overexpression of bitter receptors on the tongue (17, 18).

Little is known about the impact of TSAs on cancer patients' food behavior and nutritional status. However, several studies have reported correlations between chemical sensory alterations and the food behavior of patients while receiving chemotherapy. A recent review (191) noted that the occurrence of chemosensory disorders was associated with a decrease in the oral intake of patients diagnosed with cancer and the evolution of patients' food preferences and food behavior while receiving chemotherapy. Moreover, among TSAs, the sensation of MT is likely one of the most negatively influential factors on food behavior (133).

To gain further insights into the impact of MT we performed a systematic review and meta-analysis of the literature to delineate the prevalence of MT in cancer patients.

METHODS

The research objective was to determine the prevalence of metallic taste in patients treated for cancer.

PICO question:

Population: patients treated for cancer.

Intervention: chemotherapy, radiotherapy, surgery, other (target and hormonal therapies)

Comparison: N/A

Outcomes: the prevalence of MT

Data sources, search strategy

An initial search was performed in September 2019 in PubMed, Web of Science (WoS), and Embase. Two updates were performed in January 2020 and November 2020. The search terms used are given below.

- EMBASE: 'neoplasm'/exp AND ('taste disorder' OR 'metallic taste')

- PubMed: "neoplasms"[MeSH] AND ("taste disorders"[MeSH] OR ("metallic"[All Fields] AND "taste"[All Fields]) OR "metallic taste"[All Fields])

- Web of Science: ALL=(neoplasm* OR tumor* OR cancer*) AND (ALL=(taste* OR ageusia OR hypogeusia* OR gustat*)) AND (ALL=(disorder* OR alteration* OR metal*))

This systematic review was conducted following the PRISMA 2020 guidelines (192), which are described in the supplementary material (Tables S1 and S2). The study protocol was registered in November 2019 on PROSPERO (an international database of prospectively registered systematic reviews) under the number CRD42020157450. Several changes to the initial protocol were made: (i) nutritional and quality of life aspects could not be assessed because very few articles have been published on MT and nutritional and/or quality-of-life issues and (ii) a meta-analysis on MT prevalence was performed that was not part of the initial plan.

Study selection

Screening step

Screening was conducted by the three investigators (GB, GF, and TTD). GB and GF screened all of the identified titles and abstracts, and discrepancies were resolved by the third investigator (TTD). The inclusion criteria for the screening steps were (a) title or abstract describing any taste disorder in cancer patients, not necessarily MT, or (b) full papers mentioning MT and its prevalence. When only the title (without an abstract) was accessible in the first step and there was doubt concerning eligibility, the corresponding record was considered for the second step. No restrictions were placed on language or

publication dates. Abstracts and full papers were included. Case reports, reviews, meta-analyses, commentaries, and editorials were excluded.

Determination of eligibility

Eligibility was assessed by all three investigators. GB and GF screened all the retrieved articles. Discrepancies were resolved by the third investigator (TTD). The eligibility criteria consisted of a specific description of metallic taste (MT), including data on prevalence (number and/or % of patients). The screening and eligibility steps were performed using the Rayyan web app for systematic reviews (193).

Data extraction

The three investigators extracted the following data for each study: author names, year, title, journal, study design, sample size, sex, age (mean, SD, and range), country, type of cancer, type of treatment, prevalence of MT (n/N and/or %), date of measurement (in months), and methodology for MT evaluation. When detailed data on MT prevalence were not provided in a paper, the respective authors were contacted.

Individual risk of bias

A bias analysis was performed for each study using the ROBINS-I tool following the Cochrane guidelines for nonrandomized studies and the RoB2 tool for randomized studies (194, 195).

The main confounding domain was used to evaluate MT based on three criteria, namely, the date of MT measurement after treatment, MT awareness of participant, and specificity of MT evaluation. Other identified confounding variables were age, sex, toxicity factors (smoking and alcohol consumption), and oral health.

Outcome

The outcome was the prevalence of MT in cancer patients.

Data analysis

Prevalence was expressed as the number of patients reporting MT divided by the total number of patients. MT prevalence was estimated using a random effects meta-analysis model. The random effects model was chosen assuming that clinical and methodological heterogeneity were likely to exist and affect the results. The DerSimonian and Laird method was used to pool the studies (196). The Clopper-Pearson interval, also called the 'exact' binomial interval, was used to calculate confidence intervals (197, 198).

Heterogeneity was assessed by the I^2 statistic (199). Contour-enhanced funnel plots were used to investigate small study effects (200). Funnel plot asymmetry was tested using the weighted linear regression method (201). Leave-one-out analysis was performed on the included studies. The analyses were conducted with R software (v3.5.3) using the package Meta and the function Metaprop for meta-analysis of single proportions (the R code is provided in Doc S1).

Registration of the review: This review was not previously registered.

RESULTS

Search results

The PRISMA flowchart for article selection is presented in Figure 16.

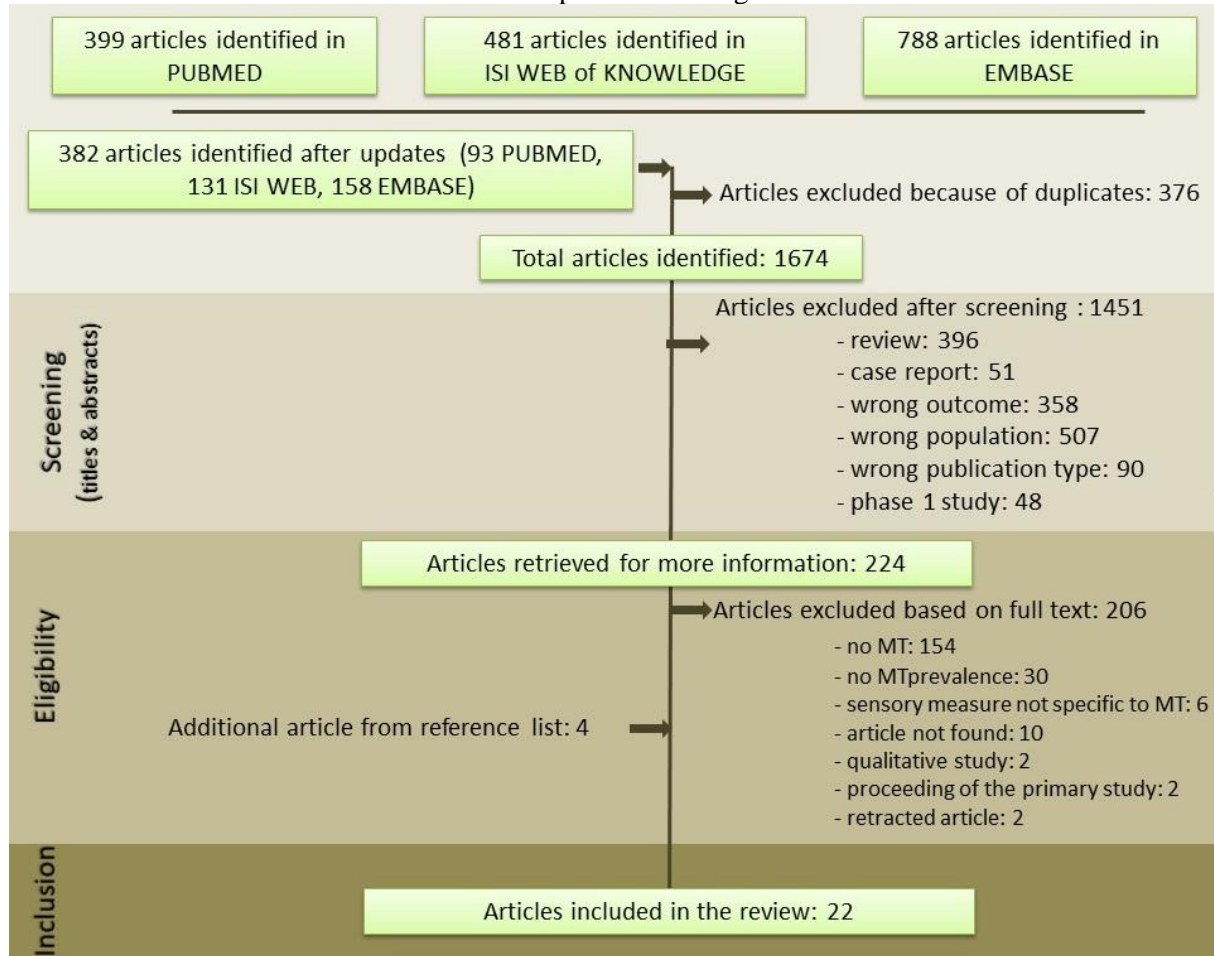


Figure 16: PRISMA flowchart.

The search of the three databases identified 2050 records in the cancer area mentioning dysgeusia; 1678 articles remained after removing duplicates. A further 1451 articles were excluded through title and abstract screening because the selection criteria were not met. Specifically, 507 articles dealt with an irrelevant population, 358 articles dealt with an unrelated outcome, and 396 articles were reviews. Of the 224 remaining articles, 206 were excluded after full-text review principally because the text did not mention MT (n=154) or MT prevalence (n=30). Despite our efforts to access all the references, including contacting the authors directly, 10 articles remained inaccessible. Of these 10 articles, seven were published before 2000, only titles were available for six, and six were in a language other than English and published in local journals. Finally, 22 articles were included in the meta-analysis (8, 35, 77, 122, 132, 134, 135, 189, 202-215).

Characteristics of included studies

The characteristics of the included studies are presented in Tableau 9. Among the 22 included articles, there were 11 cross-sectional studies, three randomized controlled trials (RCTs), four cohort studies, one observational retrospective study, one study in which variables were controlled before and after the study was performed, one case-control study, and one quasi-randomized control study. The studies were categorized in terms of the type of cancer as follows: 13 dealt with any solid or hematological cancer, three with head and neck cancer, one with breast cancer, two with lung cancer, one with colorectal cancer, one with testicular cancer, and in one study the cancer location was not specified. In this last study, the treatments were chemotherapy (platinum-based, taxan-based, and others), hormonal therapies, target therapies (tyrosine-kinase inhibitors, others), and concomitant radiochemotherapy (35).

Assessment of MT

Most of the studies used a questionnaire to evaluate MT in cancer patients. However, there were significant variations regarding questionnaire implementation. Only 6 studies reported the time at which MT was evaluated after treatment; 8 studies explicitly included training patients for MT recognition; and 4 studies used an evaluation methodology specific to MT. These variations led to a significant risk of bias for MT evaluation, as presented below and in Figures S1A and S1B , annex 6.

Risk of bias

The risk of bias analyses for the 3 RCTs and the 19 non-RCTs are presented in the supplementary material (Table S3, annex 6). The distribution of the risk of bias for the 3 RCTs and 19 non-RCTs are presented in Figure 17 A and B, respectively.

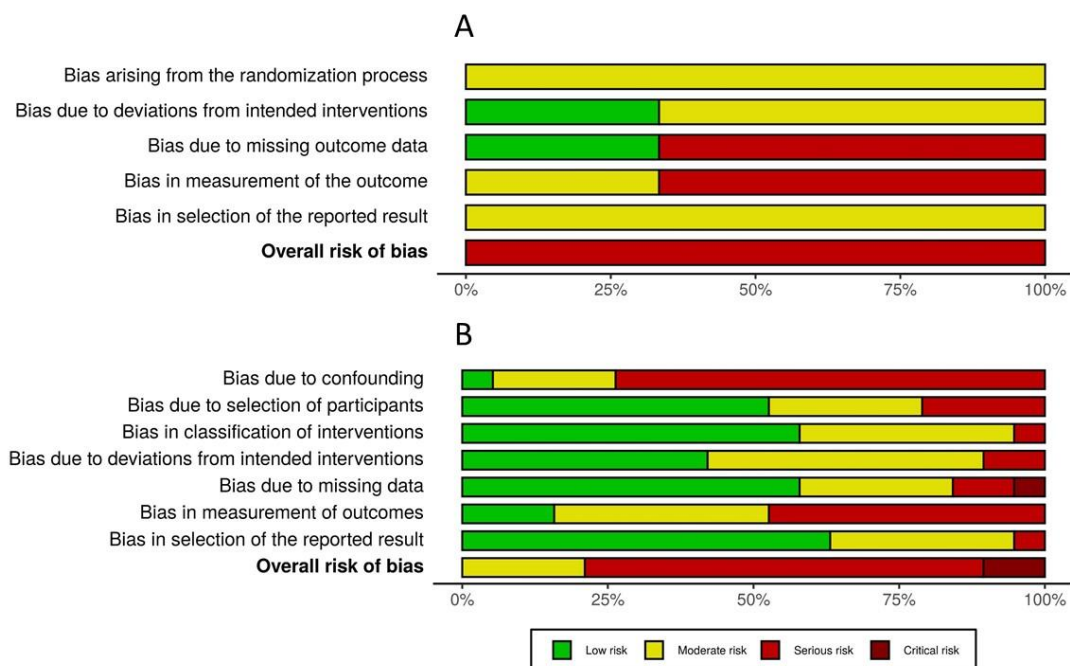


Figure 17: Review authors' judgments of the risk of bias for each item, presented as percentages across all included studies.

None of the included non-RCTs had a low risk of bias regarding MT evaluation. The risk of bias in the non-RCT studies was moderate for four (21%), serious for 13 (68%), and critical for 2 (11%) of the studies. The 3 available RCTs were judged to have a high risk of bias (100%) for MT evaluation. The main issue encountered was a risk of bias for the MT evaluation confounding domain, especially the absence of precision in assessing oral health status (mucositis, candidiasis, and dry mouth) or tobacco consumption. Twelve studies showed a high risk of bias; we had concerns regarding 6 studies; and only two studies were evaluated as having a low risk of bias (see Figures S1A and S1B , annex 6).

MT incidence

A forest plot is presented in Figure 18.

Résultats : Prévalence du goût métallique en oncologie

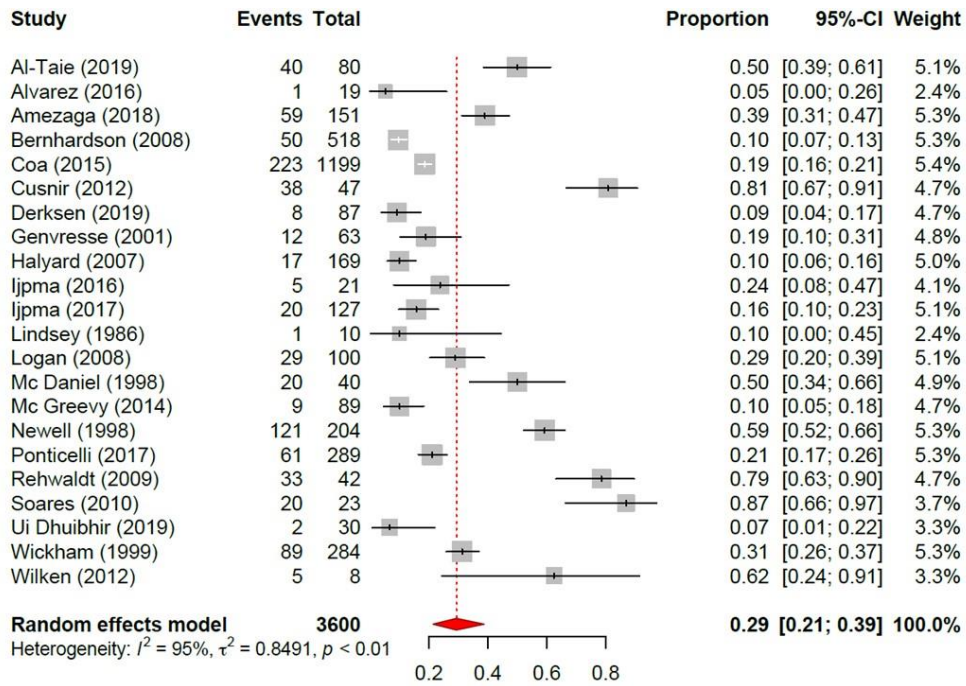


Figure 18: Random effect meta-analysis of MT prevalence in cancer patients.

The red dotted line represents the estimated pooled random effects.

The incidence of MT (with random effects) was estimated at 29% in the general cancer population (95% CI [0.21;0.39]).

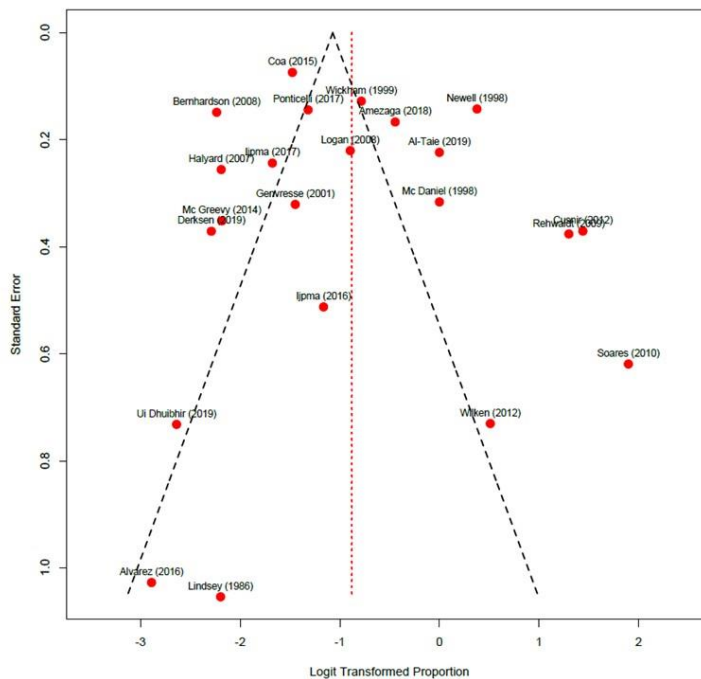


Figure 19: Funnel plot for random effects of meta-analysis of MT prevalence rate versus standard error

The dotted black triangle line represents the 95% confidence interval. The red dotted line represents the pooled random effects estimate

The heterogeneity among studies was high and significant ($I^2=97\%$, $\tau=0.85$, $p<0.01$, Figure 19) which prompted us to perform subgroup analyses in an attempt to explain this heterogeneity and the test results are presented in the supplementary material. Many factors were considered to account for the heterogeneity: the cancer type (Figure S2, annex 6), treatment type (Figure S3, annex 6), overall risk of bias (Figure S4, annex 6), and risk of bias of the sensory evaluation method (Figure S5, annex 6). The heterogeneity remained close to 1 and significant for every factor considered, except lung cancer. However, only two studies (134, 211) on lung cancer were included, making it difficult to draw sound conclusions. Finally, leave-one-out analysis (Figure S6, annex 6) confirmed that no single study substantially influenced the MT prevalence estimation. The funnel plot analysis (Figure S7, annex 6) showed that several of the studies ($n=16$) lay outside the 95% confidence interval, which confirmed the heterogeneity among the studies. However, the funnel plot did not provide any evidence indicating that the results of studies with few patients were systematically different from those with a larger number of patients (test of funnel asymmetry: $t = 0.87$, $p \text{ value} = 0.40$).

DISCUSSION

We carried out a systematic literature review and meta-analysis of the prevalence of MT in cancer patients. Five main results can be highlighted. First, very few articles reported MT incidence among TSAs in oncology (22 out of 1678 (1.3%)). Then, the pooled results led to an estimated MT incidence of 29% in the general cancer population (95% CI [0.21;0.39]). The specific impact of MT on nutritional status and quality of life has rarely been evaluated. Only five studies reported MT and nutritional status, and only two studies mentioned MT and the quality of life of patients, but without specific evaluation. Furthermore, the quality of the reports concerning MT was often low (Figure 17, Figure 18 and Figure 19) and we did not identify any studies with a low risk of bias; only 18% of the studies had a moderate risk of bias. Finally, there was no mention of oral health in any study.

Most of the included studies were observational cross-sectional studies. Three RCTs and three longitudinal cohort studies were included. The primary outcome of the latter was the incidence of TSAs, but not MT specifically.

Exploration of heterogeneity

A heterogeneity test assesses whether the results of studies can be considered to be similar (homogeneity hypothesis); if all the results are similar, the studies can be considered together. If the heterogeneity is significant, then there is at least one study for which the results cannot be considered identical to those of the others, making it inappropriate to pool the studies. Significant heterogeneity can usually be resolved by subgroup analyses (216). Considering the significant and high heterogeneity (0.97, $p<0.01$) encountered in our meta-analysis, several subgroup analyses were carried out (see the supplementary material). However, the heterogeneity remained high and significant according to the cancer type (except for the two studies on lung cancer (134, 211)), the treatment type, the global risk of bias and the risk of bias for sensory evaluation. Several hypotheses can be proposed as a possible explanation of this heterogeneity. First, the treatments in the 22 included articles were mainly chemotherapies using many different drugs and administration schemes (depending on the cancer type). A subgroup analysis by drug type could not be performed because individual data was not available. Second, a large variability in the time of MT measurement was observed (e.g., immediately after chemotherapy or many months after the end of the treatment), which might account for a large part of the observed heterogeneity. Third, several confounding variables, such as tobacco use, alcohol consumption or oral health status, were not controlled. Indeed, it has been shown that chemotherapy can cause oral mucosal lesions, oral candidiasis, and an increased abundance of acidophilic oral microflora, which may induce the measured taste disturbances (190, 217).

Two studies (135, 206) reported a high MT incidence but were based on the same cohort; only the population size varied between these two studies. None of the included studies reported MT incidence in the noncancer population, and we found no corresponding data in the literature. However, one of the coauthors of Logan's paper (122) (Prof. L. Bartoshuk) shared data for the control group with us, in which the MT incidence was 8%. Many factors were considered in the analysis of MT sensation for the general population (16, 35, 79, 122): high intense sweetener consumption, anodic stimulation of the tongue, chorda tympani damage or direct stimulation, pregnancy, burning mouth syndrome, the presence

of metals (iron, copper, zinc, platinum) or salts (calcium, magnesium) in the mouth, and medical treatments. However, no clear mechanism explaining MT has been reported.

Impact of MT on nutritional status and quality of life of cancer patients

There was a presumed impact of MT on weight and/or quality of life in several of the included studies, however, only five studies reported weight changes in the investigated population (8, 132-135). Although every included study reported TSAs in cancer patients, none of the studies were dedicated to MT. Therefore, we were not able to evaluate the specific impact of MT on nutritional status and weight in patients treated for cancer. Similarly, neither of the two studies in which TSAs were mentioned as affecting quality of life (207, 208) reported a specific impact of MT on quality of life.

Quality of evidence

The quality of evidence for MT prevalence in the cancer patient population was considered to be low for different reasons. First, the substantial heterogeneity observed among studies could reflect the influence of the different types of cancer and/or of the different types of treatments considered in the included studies. Moreover, for several studies, the absence of a description of the location and dose used for the treatment might also contribute to the discrepancies among studies. Second, most of the included studies were unclear regarding MT evaluation or had a high risk of bias in the MT evaluation. These findings highlight the necessity of using a more rigorous and/or standardized methodology for MT evaluation.

The perceptual status of MT

This systematic review addresses the question of MT as a symptom of cancer patient treatments and highlight the difficulty to evaluate this symptom in an adequate manner. Indeed, it is likely that MT is not a basic taste since its perception vanished when retro-olfaction was suppressed in many studies (12, 16, 114, 115, 118, 125, 127) ; MT is thus more in line with the concept of flavor that could involve the activation of several chemosensory systems (218). Therefore we suggest the need to introduce an appropriate testing methodology of MT as a flavor. We decided to explore, in healthy volunteers, if there was a significant difference of intensity in the perception of MT when a ferrous sulfate solution is specifically applied by swabs to the anterior portion (facial nerve) or to the posterior portion (glossopharyngeal nerve) of the tongue. Another prospective line of study is currently led by our team, the TORCAD program (NCT03558789), and tries to better objectify the MT in head and neck cancers by a study of saliva composition, basic tastes and MT testing (132) as well as employing serial quality of life (76, 187) questionnaires at specific times of treatments.

Limitations

Bias in the selection phase cannot be excluded. First, all relevant studies may not have been indexed in the three searched databases (WoS, PubMed, Embase). Second, the search was based on a specific list of terms related to the subject of this review. The possibility that additional articles may have been identified using other terms cannot be excluded, although the search was intended to be as extensive as possible.

Another limitation is the systematic absence of evaluations of oral health status and *Candida* in the selected articles. These health factors may not affect the incidence of MT; although they could be critically related to the etiology of MT. To clarify this, they should be considered in future MT studies.

CONCLUSION

This systematic review and meta-analysis revealed that approximately one-third of cancer patients suffer from MT disorder. However, these results should be interpreted with caution due to the low quality of the included studies which evaluated MT. The findings of this review highlight that a standardized procedure needs be employed to evaluate MT in order to obtain high-quality results. Moreover, sensory and/or survey protocols should be followed to maximally reduce confounding MT with other tastes, particularly the bitter taste. The timing of MT evaluation should also be systematically reported to reduce the heterogeneity among studies. One of the initial objectives of this review was to evaluate the relationship between MT prevalence and the nutritional and quality-of-life status of cancer patients. However, insufficient information was available in the literature to accomplish this objective, revealing a gaping lack of knowledge in this field. This analysis clearly demonstrated that taste disorders, particularly MT, are considered independently of quality of life and

nutritional issues in prospective clinical studies. Therefore, it appears to be necessary to (i) promote rigorous evaluation of both nutritional status and quality of life in prospective clinical and biological studies, (ii) consider oral health and treatment in cases of mucositis or candidiasis, and (iii) conduct high-quality controlled trials to account for MT as well as other taste and smell disorders to improve the nutritional status and quality of life of patients treated for cancer. To this end, we are currently conducting a prospective study implementing these recommendations in patients with head and neck neoplasms. We expect that the results will provide better insights into the causes and possible treatments of MT.

Résultats : Prévalence du goût métallique en oncologie

Reference	Study design	Sample size (women, men)	Mean age ± SD [range] (years)	Country	Type of cancer	Type of treatment	Prevalence of MT (specified the dates in months) n/N (%)	Methodology for measurement
Al-Taie et al. (202)	Cross-sectional	80 (25, 55)	58.4 ± 14.8 [60-92]	Turkey	Any type (lung, breast, brain, skin, colorectal, prostate, pelvis)	RT	40/80 (50%)	Not specified
Alvarez-Camacho et al. (203)	Cross-sectional	19 (2, 17)	Less physical symptom burden group: 58 [56-65]; greater physical symptom burden group: 57 [50-58]	Canada	Head & Neck	RT, CT + RT, Surgery + RT, Surgery+ CT + RT, RT + target therapy	1/19 (5.2%)	Questionnaire TSS (216)
Amezaga et al. (204)	Cross-sectional	151(96, 55)	60.3 ± 11.3 (28-87)	Spain	Any type (breast, colon, lung, prostate, ovarian, lymphoma, others)	CT	59/151(39.1%)	Questionnaire
Bernhardson et al. (205)	Cross-sectional	518 (398, 120)	58.71 ± 10.77 [24;85]	Sweden	Any type of solid cancers (breast, GI, gynecological, others)	CT	50/518 (9.7%)	Questionnaire
Coa et al. (8)	Cross-sectional	1199 (717, 450)	Not specified	USA	Any type (breast, gastro-intestinal, lung cancer, other solid cancers, hematologic, or unknown malignancies)	CT, RT, surgery, hormone therapy, transplant	223/1199 (18.6%)	Questionnaire
Cusnir et al. (206)	RCT with cross-over	47 (25, 22)	61	USA	Any type (gastro-intestinal, breast, other)	CT	38/47(80%) at baseline	Questionnaire Wickham (77)
Derksen et al. (207)	Cohort (prospective)	87 (26, 61)	64 ± 7.6	Netherlands	Colon	Adjuvant CT	8/45(18%)	Questionnaire
Genvresse et al. (208)	Observational retrospective	63 (34, 29)	Group 1: median age = 75.5 [70-87]; group 2: median age = 64 [42-69]	Germany	Any type (non-Hodgkin's lymphoma, pancreatic, breast, esophageal, lung)	CT	12/63(19%)	Not described
Halyard et al. (136)	RCT	169 (51, 118)	median 59 [31-88] for treatment; 58 [38-95] for placebo	USA	Head & Neck	RT	10%	Questionnaire

Résultats : Prévalence du goût métallique en oncologie

I Ijpma et al. (132)	Cohort (prospective)	21 (0, 21)	Median 32 [27-36]	Netherlands	Testicular	CT	5/21 (24%) at baseline, 3/21 (14%) at 14 days, 2/21 (9.5%) at 1 months after CT	Questionnaire
I Ijpma et al. (35)	Cross-sectional	127 (73, 54)	56.8 ± 13,7	Netherlands	Any type (not precised)	CT, CT + RT, hormonal therapy, target therapies	20/127 (15.7%)	Questionnaire
Lindsey et al. (134)	Cohort (prospective)	10 (0,10)	55.5 (43-67)	USA	Small cell lung carcinoma	CT + prophylactic cranial RT	1/10 (10%) at 3 months	Questionnaire
Logan et al. (122)	Case-control	100 (28, 72) in case and 101(29, 79) in control	64.9 ± 10.9 in case and 64.1 ± 10.7 in control; [32-90]	USA	Head & Neck	RT, CT + RT, RT & surgery, RT + CT + surgery	Patients: 29/98 (29%) responding (100 in total) ; Controls: 8/100 (8%) responding (101 in total)	Questionnaire
McDaniel et al. (210)	Cross-sectional	40 (40, 0)	[18-68]	USA	Breast	CT	20/40 (50%)	Interview
McGreevy et al. (211)	Cross-sectional	89 (49, 40)	67 ± 9	Sweden	Lung	Surgery, CT, RT, target therapies, alone or in combination	9/89 (10.1%)	Questionnaire TSS (216)
Newell et al. (189)	Cross-sectional	204 (126, 78)	57 [20;86]	Australia	Any type (colorectal, breast, Head & Neck, stomach, lung, others)	CT	121 (59.3%): 32.2% declared by patients and 9.9% by oncologists	Questionnaire
Ponticelli et al. (212)	Cross-sectional	289 (154, 155)	61 ± 13.07	Italy	Any type (lymphoma, myeloma, leukemia, breast, colorectal, lung, stomach, Head & Neck, other)	CT in combination with RT or target therapies	61/289(21%)	Questionnaire
Rehwaldt et al. (213)	Before & after (uncontrolled)	42 (35, 7)	56 ± 14.7	USA	Any type (lymphoma, breast, lung, ovarian)	CT	33 (78%)	Questionnaire TSS (216)
Soares et al. (135)	RCT with cross-over	23(11, 12)	63	USA	Any types (gastro-intestinal, breast, other)	CT	20/23(87%)	Questionnaire Wickham (77)
Ui Dhuibhir et al. (214)	Cross-sectional	30 (26, 4)	53.7 ± 13.6	Ireland	Any type (gynecological, breast, colorectal)	Surgery, CT, RT	2/30 (6.7%)	Questionnaire
Wickham et al. (77)	Cross-sectional	284(179, 105)	58 ± 15	USA	Any type (colon/rectum, breast, lung, lymphoma, ovarian, other)	CT	89/284 (41%)	Questionnaire

Wilken et al (215)	Quasi-randomized (cross-over, non-randomized order)	8 (6,1,1 not specified)	60.8 ± 8.9	USA	Any type (breast, ovarian, pancreatic, endometrial, carcinamatosis of unknown origin, lymphoma, sarcoma of the thigh)	CT	5/8 (62%)	Questionnaire / Interview
-----------------------	--	-------------------------	------------	-----	---	----	-----------	---------------------------

Tableau 9: list of the 22 selected articles

CT: chemotherapy, RCT: randomized controlled trials, RT: radiotherapy, TSS= Taste and Smell Survey

2. The Nutritional Impact of Metallic Taste in Head and Neck Cancer Patients: Explorations and Clinical Implications

Travail présenté au congrès de l'International Symposium on Olfaction and Taste, Reykjavik, juin 2024 et la Multinational Association for Supportive Care in Cancer, Lille, juin 2024.

Article publié dans Supportive Care in Cancer, il présente l'impact du goût métallique sur l'état nutritionnel chez les patients de l'étude TORCAD.

Supportive Care in Cancer (2024) 32:651
<https://doi.org/10.1007/s00520-024-08854-z>

RESEARCH

The nutritional impact of metallic taste in head and neck cancer patients: explorations and clinical implications

Authors: Guillaume Buiret^{1,2,3} MD, BRIGNOT Hélène³, SEPTIER Chantal³, THOMAS-DANGUIN Thierry PhD³, FERON Gilles PhD³

Affiliations:

Service ORL et Chirurgie Cervicofaciale, Centre Hospitalier de Valence, 179 Boulevard du Maréchal Juin, 26953 Valence, France

Université de Bourgogne Franche Comté, Esplanade Erasme, 21000 Dijon France

Centre des Sciences du Goût et de l'Alimentation, 9^E Boulevard Jeanne d'Arc, France

ABSTRACT

PURPOSE: Metallic taste (MT) is frequently observed during head and neck cancer treatments, but very little is known about its impact on nutritional status. The aim of this study was to explore the impact of MT on the quality of life and nutritional status in patients with head and neck cancer expressing MT.

METHODS: Questionnaires on quality of life, MT, weight, and food intake were filled out by 44 patients with head and neck cancer before, during, and up to one year after their treatment. Patients were divided into two groups based on their reported experience of MT.

RESULTS: MT was commonly observed (n=12, 27.2%), always during the treatment phase and mostly linked with radiotherapy or radiochemotherapy. Reported MT intensity was moderate (n=6, 40%) to high (n=4, 26.7%). MT had a significant negative impact on quality of life linked to dysgeusia (p=0.025). The negative impacts of MT on food intake and on weight were not significant, possibly due to a combination of sample size, dropouts, and duration of observation. Further research in this area could provide additional insights into how to better address the issue related to MT and enhance the quality of care provided to this patient population.

CONCLUSION: Metallic taste, experienced by 27.2% of the 44 patients with head and neck cancer, contributes to dysgeusia and results in a significant decline in quality of life associated with dysgeusia.

ClinicalTrials.gov trial registration number: NCT03558789

Keywords: metallic taste, head and neck cancer, quality of life, dysgeusia, impact

INTRODUCTION

Head and Neck Cancers (HNC) are one of the most common forms of cancer worldwide, ranking as the fifth most prevalent cancer type in France (219). Apart from the medical challenges posed by this disease, patients with HNC face a unique set of symptoms and treatment-related complications, among which taste alterations hold significant importance. Their impact on nutritional status, food intake, and weight is important (26, 31, 36, 43, 44, 220).

Among these taste alterations, Metallic Taste (MT) proves to be a disruptive symptom, affecting patients' quality of life and their ability to maintain adequate nutrition. However little is reported on this symptom, including its incidence and impact.

This taste alteration, characterized by a sensation of metal in the mouth and an unpleasant, persistent metal flavor, is frequently reported by HNC patients (31), slightly more than in the general cancerology population (11) despite there is no comparative study according to the type of cancer. The impact of MT extends beyond a mere sensory nuisance; it can have substantial consequences on food intake, oral hygiene, medication adherence, and, consequently, on disease progression and overall patient quality of life (78). Yet, despite its prevalence and clinical implications, MT remains relatively understudied and poorly understood in the context of HNC. This knowledge gap hinders the development of effective, targeted management strategies to alleviate this symptom, improve treatment compliance, and optimize the nutritional quality of patients.

This study aims to address this gap by delving more deeply into the impact of MT in HNC patients through a descriptive manner, offering a comprehensive analysis and understanding of its implications and clinical significance.

METHODS

Study population:

The prospective study involved a cohort of 44 patients diagnosed with HNC between April 2021 and March 2023 in a regional teaching hospital. These patients were included at the onset of their cancer, before the occurrence of MT, and were followed up for one year, unless a recurrence or another cancer occurred. Treatment was not considered at the inclusion but was decided according to the Tumor and Node stage and the pathological results after surgery. It could be surgery, radiotherapy and/or chemotherapy (cisplatin). In case of radiotherapy or radiochemotherapy, a prophylactic gastrostomy tube was systematically put in place to allow enteral nutrition. At the end of the study, patients were categorized into two distinct groups based on the presence or absence of MT as reported by the patients.

Testing:

Population's characteristics were assessed at the beginning of the study. Several questionnaires were systematically administered to each patient (whether MT was perceived or not) at regular intervals: before any treatment; after surgery if any; midway and upon completion of radio(chemo)therapy if any; and at 3-6-9 and 12 months. These assessments encompassed the EORTC QLQ30 (187) and HN35 (76) questionnaires to evaluate Quality of Life (QoL), another specific questionnaire to assess MT impact (35) in patients complaining about MT (provided in the annex 5), measurement of weight, a Visual Analog Scale (VAS) to quantify food intake (221) (ranging from 0 for total eating abstention to 10 for normal eating). The MT intensity was an item rated by the patients in the specific questionnaire to assess MT impact (35) (question 5 of EORTC HN35 in the annex 5).

Data Analysis:

The threshold for statistical significance in this study was a p-value of <0.05 for all conducted tests. Population characteristics differences based on MT status were assessed using chi-squared tests for qualitative data and t-tests for continuous data when the distribution was normal; non-parametric tests (Wilcoxon Mann Whitney tests) if not. According to Neyman and Pearson (222), non-crossing confidence intervals were indicative of statistical significance. ANOVA on repeated data weren't

performed on the weight, the VAS data for food intake, and question #44 of the EORTC HN35 (“During the last week, did you have trouble with taste?”) concerning MT status. Due to the small sample size and the fact that normality assumptions were not met which led to retain all the data and to not violate normality assumption.

Linear and non-linear regressions were conducted on.

The statistical tests were performed using R software (www.theproject.com) v4.3.1. The packages prettyR, nlme, gplots, ggplot2, and patchwork were used.

Ethical Considerations:

This study was performed in line with the principles of the Declaration of Helsinki and adhered strictly to the French ethical guidelines and principles, with all participating patients providing informed consent prior to their involvement. Approval for the study was granted by the CPP Est I, Dijon, France (#2017-A03641-52).

Additionally, the study is registered on ClinicalTrials.gov (NCT03558789).

RESULTS

44 patients were included and followed up for one year. The flowchart of the study is presented in Figure 20.

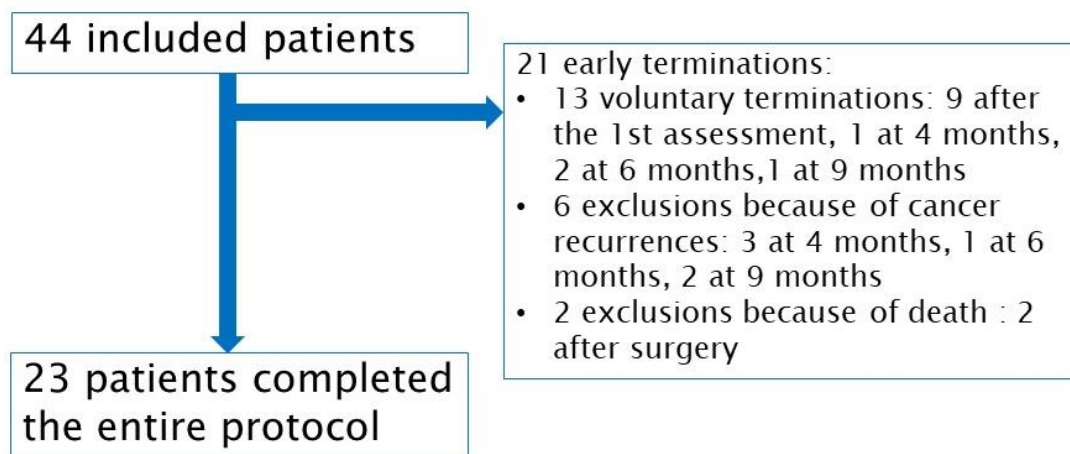


Figure 20: Study flowchart

Tableau 10 displays the demographic characteristics of the study population at inclusion according to the MT status. Patients were predominantly male, of middle age, and with advanced Tumor and Node stages. The treatment modalities were predominantly multimodal.

	MT	No MT	p-value
Sex: males n(%) ; females n(%)	9 (75%), 3 (25%)	25 (78.1%), 7 (21.9%)	1
Mean age at diagnosis ± SD (year)	61.7 ± 10.2	63.6 ± 9.8	0.58
Location n (%):			0.57
Hypopharynx	0 (0%)	3 (9.4%)	
Larynx	1 (8.3%)	7 (21.9%)	
Oropharynx	4 (33.3%)	10 (31.2%)	
Oral Cavity	5 (41.7%)	9 (28.1%)	
Unknown primary	2 (16.7%)	3 (9.4%)	
T stage n (%):			0.69
1	3 (25%)	6 (18.7%)	
2	6 (50%)	12 (37.5%)	
3	1 (8.3%)	7 (21.9%)	
4	0 (0%)	4 (12.5%)	
x	2 (16.7%)	3 (8.8%)	
N stage n (%):			0.77

Résultats : prévalence du goût métallique et impact sur la qualité de vie

0	4 (33.3%)	16 (50%)	
1	4 (33.3%)	6 (18.7%)	
2a	1 (8.3%)	3 (9.4%)	
2b	2 (16.7%)	3 (9.4%)	
2c	0 (0%)	2 (6.2%)	
3a	1 (8.3%)	2 (6.2%)	
Smoking at inclusion n (%):			0.11
Yes	3 (25%)	17 (50%)	
No	3 (25%)	2 (6.2%)	
Quit	6 (50%)	13 (40.6%)	
Treatment			0.54
Withdrawal of consent before treatment	0 (0%)	5 (15.6%)	
Chemotherapy, surgery, radiotherapy	0 (0%)	1 (3.1%)	
Surgery	2 (16.7%)	12 (37.5%)	
Surgery, radiotherapy	7 (58.3%)	5 (15.6%)	
Surgery, radiochemotherapy	3 (25%)	9 (28.1%)	

Tableau 10: studied population characteristics at inclusion according to metallic taste status.

MT = metallic taste

Among the included patients, 12 complained of MT (27.2%), always during the treatment phase (periods 2 to 5, with one patient still complaining at period 6). There were no significant demographic differences at inclusion, between MT and no MT status.

Tableau 11 reports the most intense responses to the metallic taste-specific questionnaire (provided in annex 5), exclusively among patients who reported experiencing metallic taste (n=12).

The MT is intense n (%)	
Not at all	2 (13.3%)
A little	3 (20%)
Moderately	4 (26.7%)
A lot	6 (40%)
I don't know	0
The MT bothers me with food in general n (%)	
Not at all	4 (26.7%)
A little	0 (0%)
Moderately	4 (26.7%)
A lot	7 (46.7%)
I don't know	0 (0%)
The MT bothers me with only a few foods n (%)	
Not at all	4 (28.6%)
A little	3 (21.4%)
Moderately	2 (14.3%)
A lot	4 (28.6%)
I don't know	1 (7.1%)
The MT gets stronger when I eat n (%)	
Not at all	8 (53.3%)
A little	0 (0%)
Moderately	3 (20%)
A lot	4 (26.7%)
I don't know	0 (0%)
The MT is present all day long n (%)	
Not at all	5 (33.3%)
A little	4 (26.7%)
Moderately	0 (0%)
A lot	6 (40%)

I don't know	0 (0%)
Do you consider this MT to be the most negative aspect of your taste changes? n (%)	
No	4 (28.6%)
Yes	8 (57.1%)
Don't know	2 (14.3%)
How long have you had this metallic taste in your mouth? n (%)	
Less than a week	2 (13.3%)
Between one week and one month	5 (33.3%)
Between one and three months	6 (40%)
More than three months	1 (6.7%)
I can't answer this question	1 (6.7%)

Tableau 11: Impact of Metallic Taste as assessed by the specific metallic taste questionnaire.

MT = metallic taste

Figure 21 presents the evolution of MT intensity according to the patients who experienced it.

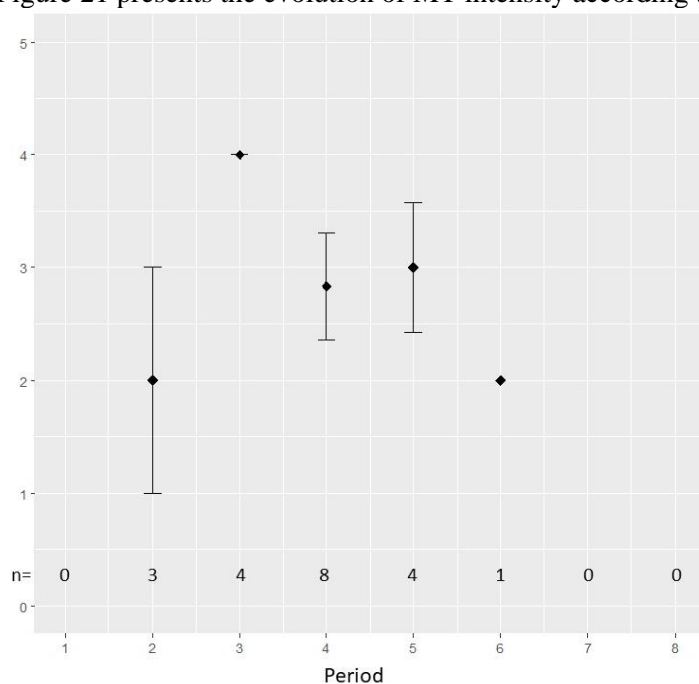


Figure 21: evolution of MT intensity according to the patients who experienced it.

Periods: 1: before any treatment; 2: after surgery if any; 3: in the middle of radiotherapy (if any); 4: after radiotherapy (if any); 5: 3 months after treatment completion; 6: 6 months after treatment completion; 7: 9 months after treatment completion; 8: 12 months after treatment completion. No MT was reported after Period 6.

Figure 22 illustrates the evolution of weight over time, according to the presence or not of a MT (Figure 22A), a gastrostomy (Figure 22B), mucositis (Figure 22C), or an oral candidiasis (Figure 22D). On average, weight decreased by 0.68kg per Period ($p < 0.0001$). However, The MT status did not significantly influence the evolution of weight (-0.41kg per Period in MT compared to non-MT patients, $p = 0.54$).

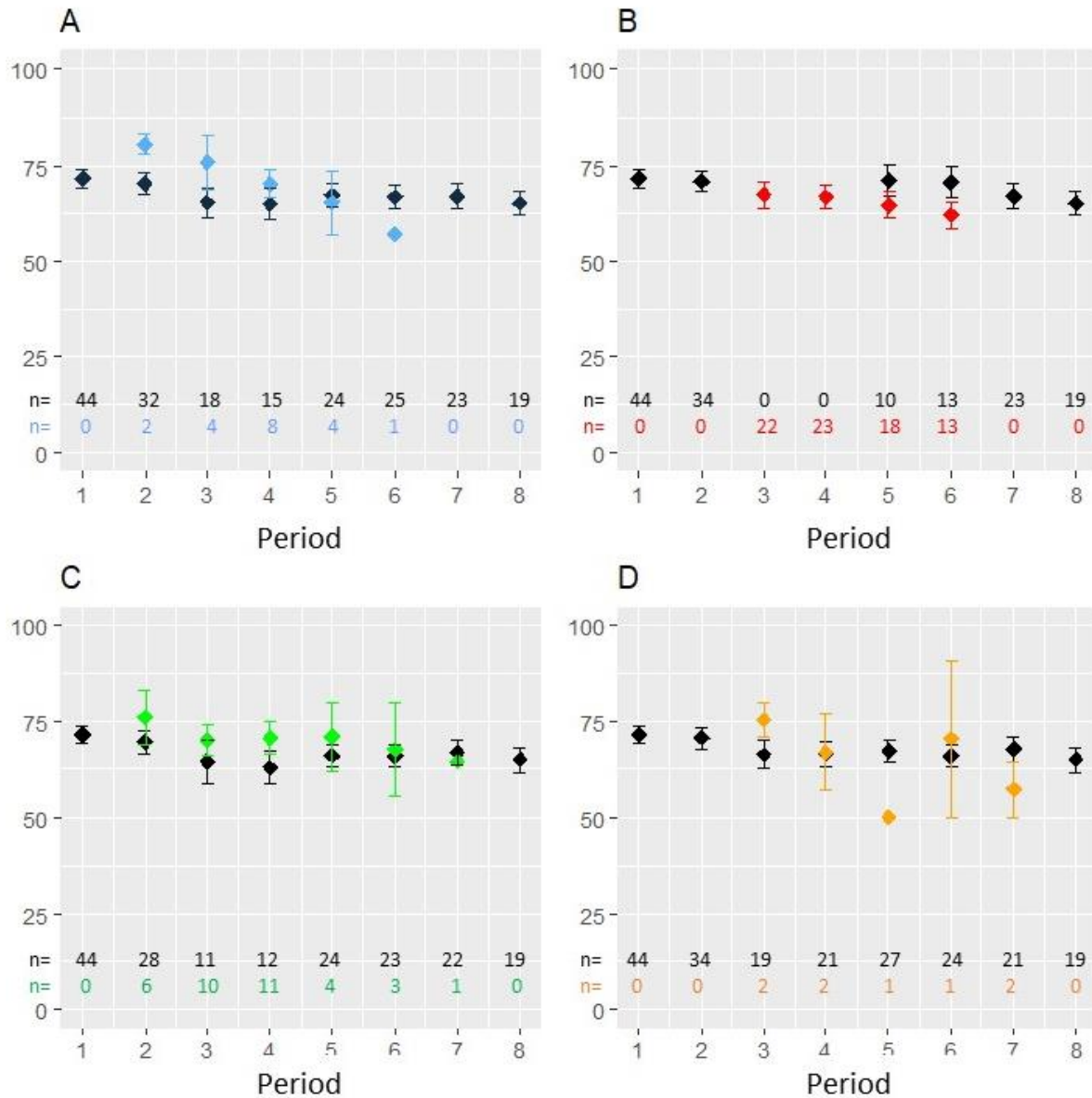


Figure 22: weight (kg) evolution

Changes in weight (kg) according to the presence or not of a Metallic Taste (Figure 3A, blue = yes, black = no), a gastrostomy (Figure 3B, red = yes, black = no), mucositis (Figure 3C, orange = yes, black = no), oral candidiasis (Figure 3D, green = yes, black = no), and time point. Periods: 1: before any treatment; 2: after surgery if any; 3: in the middle of radiotherapy (if any); 4: after radiotherapy (if any); 5: 3 months after treatment completion; 6: 6 months after treatment completion; 7: 9 months after treatment completion; 8: 12 months after treatment completion

In the the EORTC questionnaire, the questions 29 (“How would you rate your overall health during the past week?”) and 30 (“How would you rate your overall quality of life during the past week?”) were assessed with a VAS from 0 to 7. The mean evaluations of question 29 were 4.21/7 ±0.71 in patients with MT and 4.78/7 ±1.16 in patients without MT (p=0.0045). The mean evaluations of question 30 were 4.47/7 ±0.96 in patients with MT and 4.93/7 ±1.3 in patients without MT (p=0.0066).

The Figure 23 represents the evolution of Visual Analog Scale scores for food intake over time, according to the presence or not of a MT (as noted in the Figure 23A), a gastrostomy (as noted in the Figure 23B), a mucositis (as noted in the Figure 23C), and an oral candidiasis (as noted in the Figure 23D). The impact of MT status on VAS scores was not significant (+0.3/10 per Period, p=0.41).

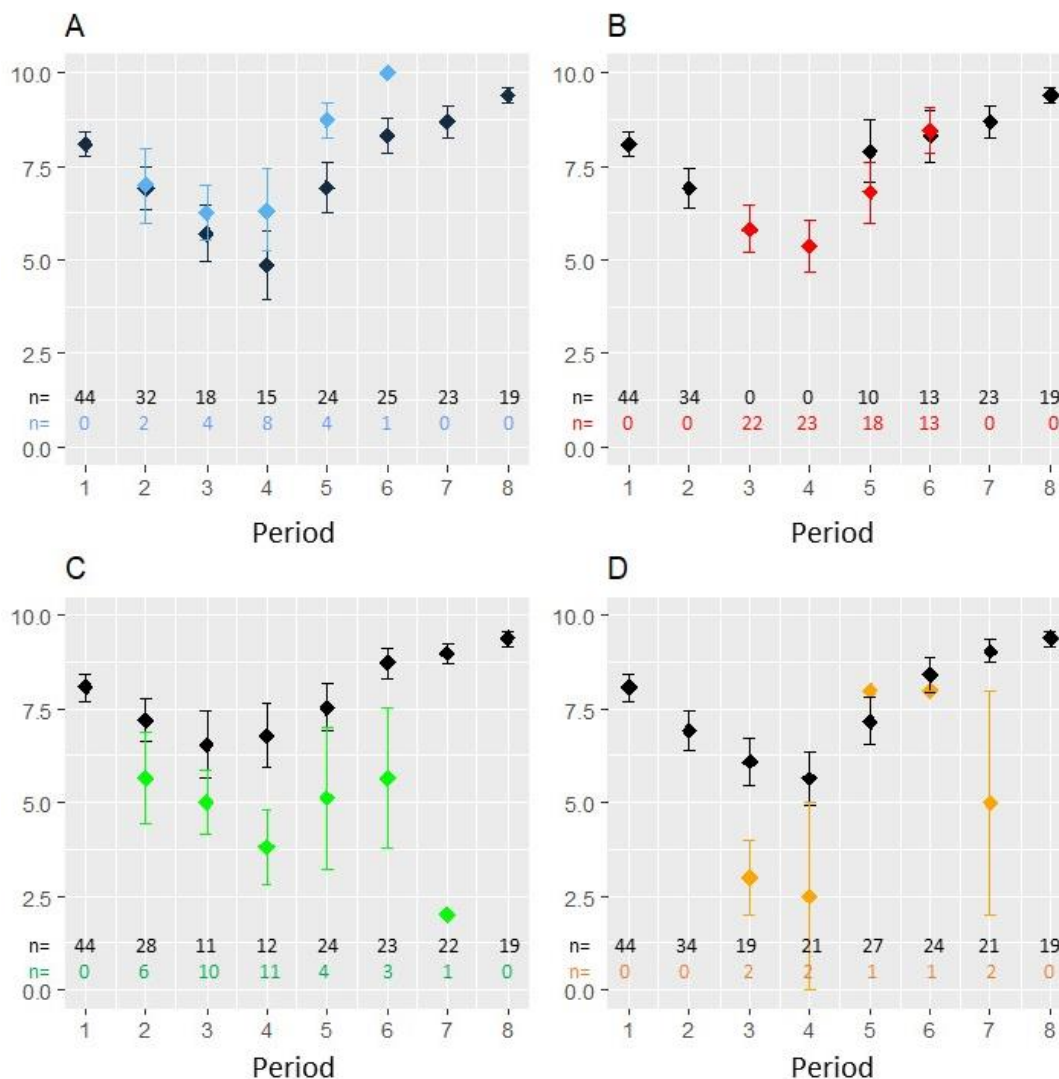


Figure 23: Changes in Visual Analog Scale scores for food intake according to Time Point.

Changes in Visual Analog Scale for food intake according to the presence or not of a Metallic Taste (Figure 4A, blue = yes, black = no), a gastrostomy (Figure 4B, red = yes, black = no), mucositis (Figure 4C, orange = yes, black = no), oral candidiasis (Figure 4D, green = yes, black = no), and time point.

Periods: 1: before any treatment; 2: after surgery if any; 3: in the middle of radiotherapy (if any); 4: after radiotherapy (if any); 5: 3 months after treatment completion; 6: 6 months after treatment completion; 7: 9 months after treatment completion; 8: 12 months after treatment completion.

Figure 24 displays the evolution of responses to question#44 of the EORTC HN35 (“During the last week, did you have trouble with taste?”) over time according to MT status. Linear regression analysis revealed a significant increase in dysgeusia as a function of time period (0.05/Period, $p=0.046$). Furthermore, the impact of MT status on dysgeusia was significant ($p=0.025$).

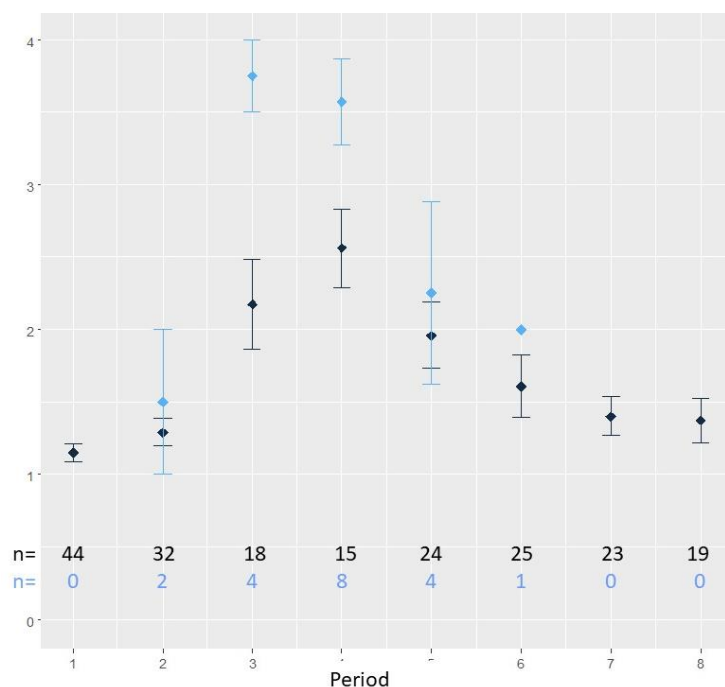


Figure 24: Changes in EORTC HN35 question #44 (“During the last week, did you have trouble with taste?”) according to Metallic Taste status and time point.

Blue = metallic taste, black = no metallic taste.

Periods: 1: before any treatment; 2: after surgery if any; 3: in the middle of radiotherapy (if any); 4: after radiotherapy (if any); 5: 3 months after treatment completion; 6: 6 months after treatment completion; 7: 9 months after treatment completion; 8: 12 months after treatment completion.

DISCUSSION

The 27.2%-incidence of MT in our study closely aligns with the findings of McLaughlin’s series (31), which reported a 24% incidence, and our systematic literature review on the incidence of MT in the general population with cancer (11). Although there was no correlation between self-questionnaires and sensory tests, Mc Laughlin (31) also followed-up 92 patients in whom weight loss was significantly associated with taste disorders, but not with type of treatment, tumor site, or time since the end of treatment. The taste disorders often became chronic. Additionally, Mc Laughlin (31) was retrospective, with data captured up to several years after treatment. Our study noted that HNC and their treatments have a general impact on nutritional status, as evidenced by weight loss and worsened VAS scores for food intake. These findings are consistent with previous reports (26, 31, 36, 43, 44, 220) and highlight the detrimental effect of HNC and its treatments on dysgeusia and quality of life (QoL). Interestingly, there was a discrepancy between the occurrence of MT and its impact. The occurrence of MT, assessed through self-reported intensity rating (Figure 21), and in the EORTC HN35 question #44 (“During the last week, did you have trouble with taste?”, Figure 23A), was significantly more important in the MT group and there were no significant differences in VAS scores for food intake (Figure 23A) or weight. Food intake (VAS) recovered significantly more rapidly in the MT group despite no significant effect on nutritional indicators (food intake (VAS), weight), MT significantly affected overall health as indicated by question #29 of EORTC QLQ30. This emphasizes the specific and impact of MT on the sensory experience of taste, distinct from its effects on other aspects of nutritional status. This can be attributed to a contrast effect, where a higher intensity and earlier onset of metallic taste (MT) was associated with a more rapid recovery, and/or to coping strategies in patients with MT.

Our study also highlighted a temporal evolution of side effects, with a peak during the irradiation phase followed by a gradual recovery, a pattern commonly reported in the literature for various parameters including taste intensity tests (31, 223-225), salivary flows (53), and QoL (226). Lilja et al. found a statistically significant correlation between taste disorders and QoL only at 3 months (75), i.e. at the end of the treatments, corresponding with the Period 5 of our study.

The impact of HNC and their treatments on dysgeusia, and especially MT, is frequently underestimated in clinical practice, despite its strong and long-term effects (73). Nevertheless, patients often perceive these effects as "acceptable". In a qualitative study by Ganzer et al. (227), patients reported that they enjoyed eating, with eating becoming easier over time, despite minimizing the impact on their overall QoL as "the price to pay". Similarly, in an observational study examining the priorities and regrets of patients with HNC (n=30), caregivers (n=30), and carers (n=25) and their concordance, Gill et al. (228) reported that the top four priorities for all three groups were to be cured of cancer, to live as long as possible, to be pain-free, and to swallow normally. Notably, normal taste and smell ranked lowest among the concerns for the three groups. These findings are in line with the report of List et al. (229).

Despite the valuable insights provided by our study, we acknowledge its limitations, which primarily stem from the small population sample size (n=44). In our study, motivating patients to participate in these long sessions proved difficult, leading to a high attrition rate. This limitation underscores the necessity in including these parameters in larger clinical trials to confirm our findings and further elucidate the complex interplay between MT, treatment outcomes, and patient-reported QoL in HNC populations. Our study also focused on a specific patient population with advanced tumor and node stages, predominantly undergoing multimodal treatments. While this focus allows for a detailed examination of MT in a high-risk group, it may limit the applicability of our findings to other HNC patients and future studies should consider including a more diverse patient population to explore the broader applicability of the findings.

Understanding the nuances of MT and its impact on patients' lives is essential for improving clinical management and patient care. Despite its often underappreciated impact, MT significantly affects patients' sensory experiences and overall health, as indicated by the EORTC QLQ30 questionnaire question ("During the last week, did you have trouble with taste?"). Clinicians should be aware of the potential for MT to influence patients' quality of life and consider incorporating strategies to manage this symptom effectively.

Given the limitations of our study, future studies should aim to include objective measures alongside self-reported data to provide a more comprehensive understanding of MT. Investigating the long-term effects of MT and its evolution over time is another important area for future research. Additionally, exploring the underlying mechanisms of MT in HNC patients could offer valuable insights into potential therapeutic targets. Understanding the biological basis of MT may lead to the development of targeted interventions to alleviate this symptom. Longitudinal studies could provide a deeper understanding of how MT impacts patients throughout their treatment journey and recovery, informing strategies to support patients more effectively.

CONCLUSION

Our study highlights the prevalence of metallic taste (MT) in head and neck cancer (HNC) patients, with an incidence of 27.2%, always observed during the treatment phase (Periods 2 to 5 i.e. after surgery to after radiotherapy or radiochemotherapy except one patient still 3 months after the radiochemotherapy). Importantly, MT significantly impacted the quality of life (QoL) associated with dysgeusia. While the impact of MT on food intake and weight loss was not statistically significant, this may be attributed to the small size of the population followed to one year after treatment. Future research efforts in this area are warranted to further investigate these findings and elucidate optimal strategies for addressing the challenges posed by MT in HNC patients. Such insights are crucial for enhancing the quality of care provided to this patient population.

3. Is metallic perception a taste, an aroma or a flavor?

Article soumis à J Sens Stud le 15/10/2024, il présente les résultats de l'étude GOUTMETALSAIN, sur la perception nez ouvert ou occlus de la solution de sulfate de fer appliquée à différentes localisations de la langue.

Authors : Guillaume Buiret^{1,2,3} MD, THOMAS-DANGUIN Thierry PhD³, FERON Gilles PhD³

Affiliations :

1. Service ORL et Chirurgie Cervicofaciale, Centre Hospitalier de Valence, 179 Boulevard du Maréchal Juin, 26953 Valence, France
2. Université de Bourgogne Franche Comté, Esplanade Erasme, 21000 Dijon France
3. Centre des Sciences du Goût et de l'Alimentation, 9^E Boulevard Jeanne d'Arc, France

ABSTRACT

AIM: Metallic taste is known to vanish with nasal occlusion, suggesting it should be termed metallic “aroma” rather than metallic “taste.” However, it also appears after neurological injuries, such as a chorda tympani section, which suppresses taste perception mediated by the facial nerve.

METHODS: In 120 healthy volunteers, the perception of an iron sulfate solution was assessed at different lingual locations—corresponding to the facial and glossopharyngeal nerves—and with either open (n=60) or closed (n=60) noses to evaluate if it is a taste or an aroma.

RESULTS: Nasal occlusion significantly reduced the perception of iron sulfate. It prevented perception in 31.7% of participants, though it did not completely eliminate it in the remaining 68.3%. Additionally, in open-nosed subjects, the intensity of iron sulfate perception was significantly greater when applied to the base of the tongue (glossopharyngeal nerve) than to the tip (facial nerve). These differences did not persist with nasal occlusion.

CONCLUSION: Nasal occlusion diminished but did not abolish the perception of iron sulfate. With an open nose, a differential taste perception was significant, but not with a closed nose. Therefore, metallic perception involves both retro-olfactory and gustatory components, suggesting it is a metallic flavor.

KEYWORDS: metallic taste, flavor, taste, smell

INTRODUCTION

The prevalence of metallic taste (MT) affects approximately 29% of people undergoing cancer treatment (11). MT is known to vanish with nasal occlusion, leading some authors to suggest a retro-olfaction mechanism (12, 16, 80, 114, 125, 127). However, its perception also depends on neurological factors. Taste is mediated by two nerves: the facial nerve for the anterior tongue and the glossopharyngeal nerve for the posterior tongue. The release of facial nerve inhibition on the glossopharyngeal nerve (13-15, 65, 79, 123, 230) could be a potential cause of MT. This hypothesis has been proposed to explain the appearance of metallic taste after chorda tympani sectioning—a branch of the facial nerve—during middle ear surgery (13-15, 230).

Under normal circumstances, the facial nerve appears to exert an inhibitory effect on the glossopharyngeal nerve, thus limiting taste perception in the posterior part of the tongue. When the chorda tympani is severed, the abrupt cessation of sensory input to the anterior tongue causes this inhibition to be lifted, possibly explaining MT perception. By analogy, anticancer treatments, which can cause neurotoxicity (3), might also release facial nerve inhibition on the glossopharyngeal nerve, leading to MT in these circumstances.

While much data is available on the overall perception of iron sulfate (12, 80-82, 113-116, 125, 127, 231), insufficient data exists on tests at precise locations on the tongue to distinguish perceptions mediated by the facial or glossopharyngeal nerves. So, is metallic “taste” a taste or an aroma?

We questioned whether differences in the perception of induced metallic taste exist depending on the test’s location in the oral cavity and on retro-olfaction. To answer this, we assessed the difference in the perception of an iron sulfate solution at different lingual locations (facial and glossopharyngeal nerve territories) and whether the nose was open/occluded in healthy volunteers.

METHODS

Objective: The primary objective of this study was to evaluate the variability in the intensity of iron sulfate perception based on tongue location in healthy volunteers with and without nasal occlusion.

Recruitment: Patients in the waiting room of the ENT consultation department were offered the opportunity to participate in supraliminal tests of iron sulfate perception intensity. Voluntary adult patients were included, while non-inclusion criteria were a history of head and neck cancer, consultations for taste/smell disorders, refusal to participate, minors, adults under guardianship, or insufficient French language proficiency to answer the questions. No exclusion criteria were applied. Tests were conducted without nasal occlusion in the first 60 participants (50%) and with nasal occlusion in the last 60 (50%) to prevent retro-olfactory airflow. The study complies with the Declaration of Helsinki for Medical Research involving Human Subjects and was authorized by the CPP Est IV on 16/12/2021 (no. 2021-A02149-32) for the open nose and on 05/08/2022 (no. 2022-A01475-38) for the occluded nose. All participants provided informed written or verbal consent. The study has been registered on Clinicaltrials.gov (NCT05227157).

Data Collection: Data collected included gender, age, smoking status, open/occluded nose status, and quantification of supraliminal sensory intensity testing.

Testing Process: After confirming eligibility and consent, a cotton swab soaked in a 10 g/L iron sulfate solution diluted in Evian water was applied to each predefined lingual location (see Figure 25 and video). The glossopharyngeal nerve mediates taste at the posterior tongue (base), and the facial nerve mediates taste at the anterior tongue (tip). Participants rated the perceived intensity on a scale from 0 (no perception) to 5 (highest imaginable intensity) at each of the four predefined lingual locations. The mouth was rinsed with Evian water between tests.

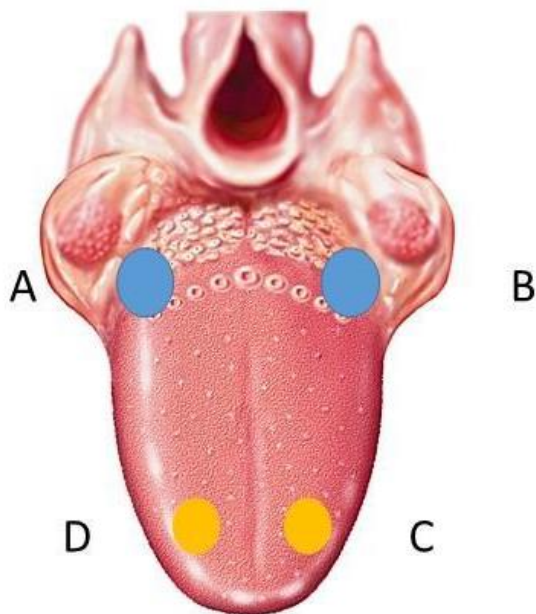


Figure 25: test location

Data Analysis:

- Group comparability was ensured by comparing age means using Student's t-test and qualitative data using the chi-square test.
- In the overall population, and sub-populations with open/occluded noses, arithmetic means were compared for quantitative data from supraliminal sensory tests: i. between each site (left front, right front, left back, right back), ii. Between the right and left sides, and iii. Between the anterior and posterior parts of the tongue.
- Means for each of the four sites were then compared according to open or occluded nose status.

RESULTS

A total of 120 participants were recruited from December 16, 2021, to August 8, 2023. In the open nose group, 29 were females (48.3%) and 31 were males (51.7%). In the occluded nose group, 36 were females (60%) and 24 were males (40%). There was no significant difference in gender distribution between the two groups ($p=0.27$). Figure 26 presents the age distribution according to nose occlusion status. Participants in the open nose group were significantly older than those in the occluded nose group (mean age 47.9 ± 18.8 years and 39.0 ± 16.0 years, respectively; $p=0.0056$). Tobacco consumption between the two groups was not significantly different ($p=0.06559$).

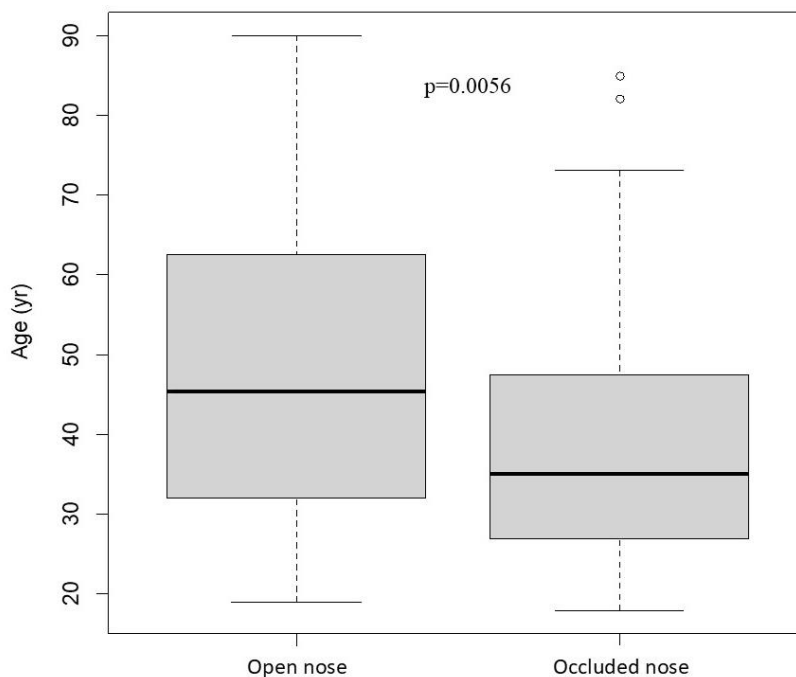


Figure 26: Age according to the nasal occlusion

Figure 27 presents the intensity test results of MT perception in the subpopulation without nasal occlusion ($n=60$) and with nasal occlusion ($n=60$), according to test location. Comparison by location according to open/occluded nose status showed that all perceptions at each location were significantly higher when the nose was open ($p < 10^{-5}$ for right back, left back, left front, and right front).

Résultats : qu'est-ce que la perception métallique ?

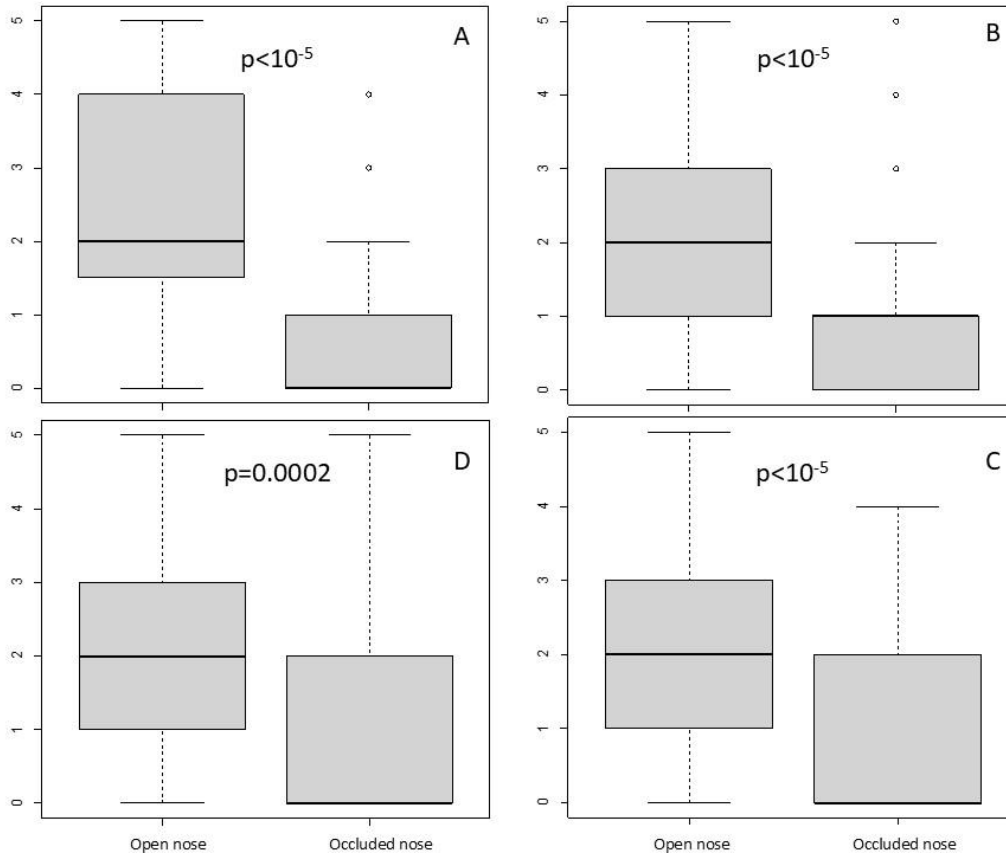


Figure 27: metallic intensity perception according to the test location

A= back and right, B= back and left, C= front and left, D= front and right (cf figure 30)

Figure 28 presents the intensity test results of MT perception in the subpopulation without nasal occlusion ($n=60$) and with nasal occlusion ($n=60$), according to the sum of test locations: left, right, front, and back. All perceptions at each location were significantly higher with an open nose ($p < 10^{-5}$).

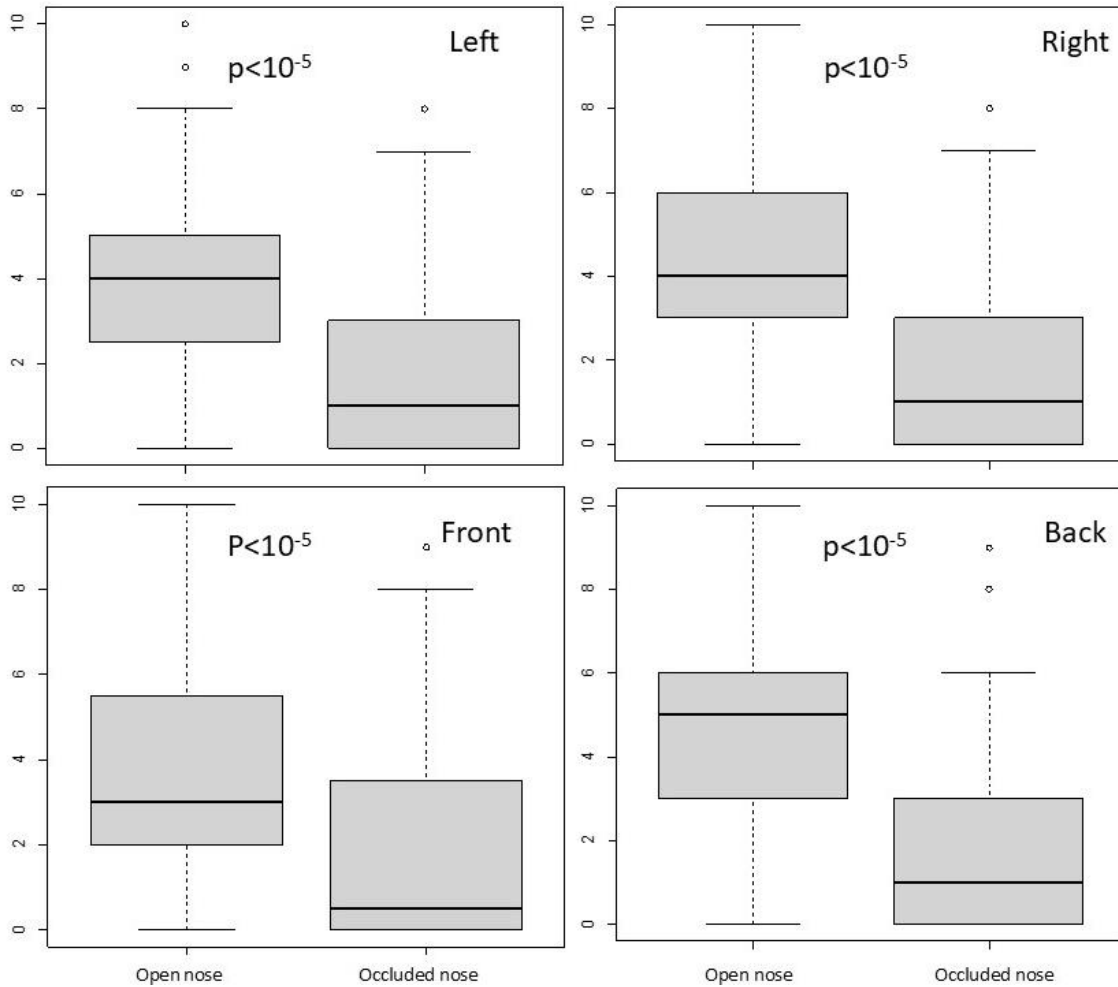


Figure 28: metallic intensity perception according to the test location

Back is the arithmetic sum of back left and back right. Front is the arithmetic sum of front left and front right. Right is the arithmetic sum of front right and back right. Left is the arithmetic sum of back left and front left.

Among the 60 participants without nasal occlusion, only one had no perception of the iron sulfate solution in any location (1.7%). Comparison of perceptions on the right and left sides showed a non-significant difference in means of $-0.4/5$ ($p=0.060$). Comparison between perceptions at the front and back of the tongue showed a significant difference in means of $-0.76/5$ ($p=0.020$): intensity was significantly higher at the base of the tongue than at the tip.

Among the 60 participants with nasal occlusion, 19 had no perception of the iron sulfate solution in any location (31.7%). Comparison of perceptions on the right and left sides showed a non-significant difference in means of $-0.18/5$ ($p=0.5704$). Comparison between perceptions at the front and back of the tongue showed a non-significant difference in means of $+0.4/5$ ($p=0.1914$).

DISCUSSION

We were able to show, in healthy volunteers: i. that nasal occlusion significantly reduced the perception of iron sulfate (it even prevented it in 24% of people, without causing it to disappear in the others); ii. That the intensity of perception of iron sulfate was significantly greater when the product was applied to the base of the tongue than to the tip, whereas there was no significant difference between the right and left sides in open-nose subjects; iii. That the latter differences did not persist in the case of nasal occlusion. These results are consistent with those published by other teams, who have also shown that nasal occlusion increases iron sulfate detection thresholds (12, 80, 81, 114, 115, 127) without making it disappear. Only Hettinger et al. (125), in 12 healthy subjects, found no perception of iron sulfate during nasal occlusion. We can deduce that the perception of iron sulfate is mainly linked to olfaction, since nasal occlusion drastically reduces its perception. In fact, there are differences in perception between groups, regardless of the territory, and between the anterior and posterior parts of the tongue. With the nose open, perception at the base of the tongue (closer to the back of the nose, i.e., perception by retro-olfaction) should counterbalance the intensity differential between the front and back of the tongue. However, this perception was not completely abolished in the majority of patients (76%) during nasal occlusion, despite the 0.5/10 mean difference in gustatory perception between the territory of the glossopharyngeal nerve (base of the tongue) and the territory of the facial nerve (tip of the tongue) no longer being significant ($p=0.080$).

There may be two explanations: the perception of iron sulfate may be a flavor, essentially combining retro-olfactory perception with other chemosensory components of the perception. Indeed, Omur-Ozbek et al. suggest a potential role for trigeminal stimulation. In their study, Fe^{2+} was perceived as astringent during nasal occlusion and then as merely metallic after removal of the nasal clips (81). Similarly, Stevens et al., with their concept of multidimensional taste scales applied to iron sulfate in 47 healthy volunteers, showed that metallic taste was not grouped with traditional tastes (the closest was bitter) but rather with oral chemical sensations such as aluminum (astringency, trigeminal sensitivity) and glutamate (umami taste), even after nasal occlusion (113).

Another explanation could be linked to the metal's related olfactory detection threshold, such as 1-octen-3-one and 1-nonen-3-one (22), trans-4,5-epoxydecenal, (Z)-1,5-octadien-3-one and 1-octen-3-one (232-235), and epoxydecenal (236), which could be acute, on the order of parts per billion. In this case, incomplete nasal occlusion would have occurred in the 74% of people with nasal occlusion who still perceive the olfactory component of metallic taste. But in that case, as the tongue base is closer to the retronasal pathway than the tip of the tongue, the intensity should be higher at the tongue base than at the tip of the tongue, which was not highlighted in our data (Figure 27 and Figure 28).

Finally, other research supports the notion that MT perception involves oral somesthesia. Studies by Stevens and colleagues using multidimensional taste scales have shown that metallic taste was associated with oral chemical sensations such as astringency and trigeminal sensitivity, rather than traditional gustatory qualities like sweet, sour, salty, and bitter (113). Additionally, Hettinger et al. demonstrated the role of olfaction and somatosensory pathways in the perception of non-traditional taste stimuli, further highlighting the complexity of metallic taste perception (125). These findings suggest that metallic taste may be a multidimensional sensory experience involving both olfactory and trigeminal contributions, i.e. mainly a 'flavor' involving, chemosensory informations namely smell, taste and chemisthesis.

CONCLUSION

This study demonstrated that nasal occlusion significantly reduced the perception of iron sulfate, confirming that this perception was primarily linked to olfaction. Perception varied depending on the tongue's position, with higher intensity at the base compared to the tip, a difference nullified by nasal occlusion. Despite occlusion, 76% of subjects still perceived the metallic taste, suggesting a possible involvement of a real metallic taste, and/or a trigeminal stimulation, or very low olfactory detection thresholds. Therefore, MT should be considered as a flavor.

4. Evaluating the Etiology of Metallic Taste During Head and Neck Cancer Treatments: A Study of Facial and Glossopharyngeal Nerve Interactions

Travail présenté au congrès de l'International Symposium on Olfaction and Taste, Reykjavik, juin 2024.

Article soumis à European Journal of Neurosciences le 27 juillet 2024, il **présente l'évaluation de l'hypothèse de levée d'inhibition du nerf facial sur le nerf glossopharyngien, objectif secondaire de TORCAD.**

Authors: Guillaume Buiret^{1,2,3} MD, BRIGNOT Hélène³, SEPTIER Chantal³, THOMAS-DANGUIN Thierry PhD³, FERON Gilles PhD³

Affiliations:

Service ORL et Chirurgie Cervicofaciale, Centre Hospitalier de Valence, 179 Boulevard du Maréchal Juin, 26953 Valence, France

Université de Bourgogne Franche Comté, Esplanade Erasme, 21000 Dijon France

Centre des Sciences du Goût et de l'Alimentation, 9^E Boulevard Jeanne d'Arc, France

ABSTRACT

INTRODUCTION: Metallic taste is frequently described during head and neck cancer treatments but very little is known about its etiologies. One hypothesis to explain the metallic taste is the removal of facial nerve inhibition on the glossopharyngeal nerve. Indeed, the decrease of taste afferents mediated by the facial nerve (anterior two-thirds of the tongue) due to cancer or its treatments, would reveal those mediated by the glossopharyngeal nerve (posterior one-third of the tongue) and thus lead to metallic taste perception. The aim of this study was to evaluate the validity of this hypothesis.

METHODS: Selective supraliminal taste tests on the tip and the base of the tongue were regularly performed on 44 patients with head and neck cancers before, during, and after their treatment. Sweet, salty, bitter, sour, and metallic tastes were tested. Patients were grouped based on whether they reported experiencing metallic taste or not.

RESULTS: 12 patients complained about metallic taste (27.2%), always during the treatment phase. Most of them (83.3%) were treated by surgery and radiotherapy or radiochemotherapy. Supraliminal tastes were altered in every patient, especially during the treatment phase. Test results showed that perceived intensity was significantly reduced in patients reporting metallic taste for salt, sweet and sour. This was observed more on the base of tongue than on the tip of the tongue. Metallic taste was significantly linked with mucositis ($p=0.027$) but with neither candidiasis ($p=0.38$) nor salivary flow ($p=0.63$).

CONCLUSION: The hypothesis of removal of facial nerve inhibition on the glossopharyngeal nerve cannot explain metallic taste in head and neck cancer.

INTRODUCTION

Head and Neck Cancers (HNC), encompassing a diverse range of malignant tumors affecting anatomical structures crucial for speech, swallowing, and breathing, present a substantial challenge in the field of oncology. First, even if the incidence is decreasing, they are the fifth most common cancers in men worldwide (237). HNC are characterized by their significant impact on patients' quality of life due to symptoms that impair essential oral and throat functions (238-240). Among the numerous manifestations associated with these cancers, Metallic Taste (MT) stands out as one of the most complex and poorly understood symptoms. MT is described by patients as a persistent sensation of metallic flavor in the mouth. It can manifest at various stages of the disease, whether during diagnosis, treatment, or remission. This disruption of the sense of taste has profound consequences on

appetite, nutrition, and quality of life of patients and can even influence their adherence to treatment, as it may contribute to weight loss and malnutrition (37, 44, 241). While MT is widely acknowledged by healthcare professionals as a common side effect of cancers in general and Head and Neck Cancers (HNC) particularly (11, 78), its understanding, management, and underlying mechanisms remain inadequately explored. This knowledge gap hinders our ability to offer appropriate treatment strategies to alleviate this symptomatic manifestation.

According to Logan et al. (122) or Reith et al. (79), the causes of MT are not understood. It may be linked with oral health issues (mucositis, candidiasis), a neuropathy, the reduction in taste receptors, a direct stimulation of a specific MT receptor close to bitter receptors (T2R) or receptors of the TRPV1 family, the toxicity to salivary glands, the intra-oral lipoperoxidation. Among the possible causes, the removal of facial nerve inhibition on the glossopharyngeal nerve may be hypothesized (123). Under normal circumstances, the facial nerve may exert an inhibitory effect on the glossopharyngeal nerve to regulate taste sensation. Because of the cancer and/or its treatments, the decrease of taste afferents mediated by the facial nerve (anterior two-thirds of the tongue) would reveal those mediated by the glossopharyngeal nerve (posterior one-third of the tongue), leading to abnormal taste sensations such as MT. This lack of inhibition effect is commonly evoked after middle ear surgery (13-15, 123, 230) but has never been proven in HNC.

In case of release of inhibition of the facial nerve on the glossopharyngeal nerve, the following results are expected: for one or more tastes, the taste on the tongue base should be significantly more intense than on the tip of the tongue in case of MT. In this study, we tested this hypothesis by performing supraliminal taste tests in both territories of the taste nerves on the tongue in patients with HNC, regardless of their treatments. The perceived intensity of sweet, salty, bitter, sour, metallic tastes was recorded following the application of specific taste-eliciting compounds on the anterior and posterior parts of the tongue, as well as on both sides (left and right). Since MT could occur at various stages of the treatment protocol, each patient was tested at multiple time points before, during, and after treatment.

METHODS

Study population:

The prospective study involved a cohort of 44 patients diagnosed with HNC. These patients were included at the onset of their cancer, before the occurrence of MT and were followed-up during one year, unless a recurrence or another cancer occurs.

Supraliminal recognition then intensity taste testing:

Selective supraliminal taste tests were conducted on each patient at regular intervals. Identical tests were performed before any treatment ((m) moment 1); after surgery if any (m2); in the middle (m3) and at the end (m4) of the radiotherapy if any; at 3 (m5)-6 (m6)-9 (m7) and 12 (m8) months.

Taste assessments were carried out on four distinct regions of the tongue: the tip (taste mediated by the facial nerve) and the base (taste mediated by the glossopharyngeal nerve) and each on the right and on the left. Figure S1 in the annex 7 presents the test locations on the tongue.

A cotton swab, soaked in a solution of tastant diluted in Evian water, was applied to each of the predefined lingual locations (tip/base, left/right) following a sequence that avoided anticipation through iterative testing. The evaluation encompassed the following tastes (tastants): sweet (sucrose, 850g/l), salty (sodium chloride, 75g/l), bitter (anhydrous caffeine, 20g/l), sour (citric acid, 50g/l), and metallic (iron sulfate, 10 g/l). All tastants were purchased at Merck (Saint-Quentin-Fallavier, France). Each patient was asked first to identify the taste and then to rate the perceived intensity on a numerical scale from 0 (no perception) to 5 (very intense) at each of the four predefined lingual locations. The mouth was rinsed with Evian water between each test.

Other tests:

The oral mucositis and candidiasis were determined by oral examination by an ENT (0 if no, 1 if yes). The non-stimulated salivary flow was determined by weighing the saliva collected over a 10-minute period as described previously (230).

Data Analysis:

At the end of the study, patients were divided into two groups based on whether they reported experiencing metallic taste (MT) or not.

Confidence intervals by moments and tongue locations were calculated for different time points and tongue locations to compare taste intensity between patients who reported experiencing MT and those who did not. According to Neyman and Pearson (222), if the confidence intervals don't cross on the graph, the test was considered as significant.

Links between oral mucositis and MT status and candidiasis status and MT status were determined by chi-squared tests.

The potential link between salivary flow reduction and MT was determined by comparing the mean flow according to MT status by a t-test.

A p-value of <0.05 was considered statistically significant for every test in this study.

Ethical Considerations:

This study was conducted in accordance with the French ethical guidelines and principles related to biomedical research. An informed consent was obtained from all participating patients. The whole study received approval from the ethic committee CPP Est I, Dijon, France (2017-A03641-52). The protocol was registered on Clinicaltrials.gov (NCT03558789).

RESULTS

44 patients were recruited and followed up for one year. The flowchart is reported in Figure 29. The characteristics of the population are presented in Tableau 12. As in every study on HNC, the patients were predominantly male, middle-aged, and with advanced Tumor and Node stages. The treatment was predominantly multimodal.

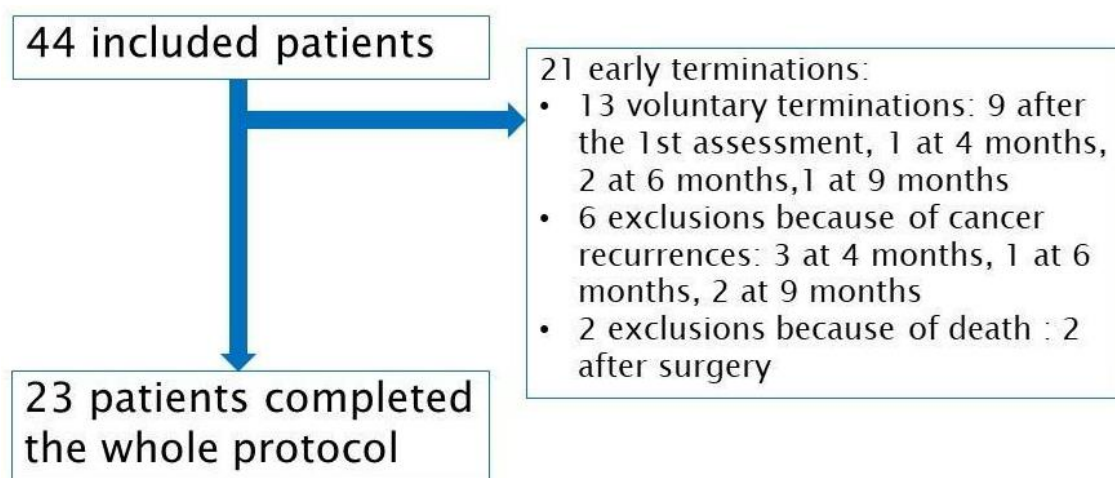


Figure 29 : study flowchart

Sex: males n(%) ; females n(%)	34 (77.3%), 10 (22.7%)
Mean age at diagnosis ± SD (year)	63.1 ± 9.8
Location n (%):	
Hypopharynx	3 (6.8%)
Larynx	8 (18.2%)
Oropharynx	14 (31.8%)
Oral Cavity	14 (31.8%)
Unknown primary	5 (11.4%)

Tumor stage n (%):	
1	9 (20.4%)
2	18 (40.9%)
3	8 (18.2%)
4	4 (9.1%)
x	5 (11.4%)
Node stage n (%):	
0	20 (45.5%)
1	10 (22.7%)
2a	4 (9.1%)
2b	5 (11.4%)
2c	2 (4.5%)
3a	3 (6.8%)
Smoking at inclusion n (%):	
Yes	20 (45.5%)
No	5 (11.4%)
Quit	19 (43.1%)
Treatment	
Withdrawal of consent before treatment	5 (11.4%)
Chemotherapy, surgery, radiotherapy	1 (2.2%)
Surgery	14 (31.8%)
Surgery, radiotherapy	12 (27.3%)
Surgery, radiochemotherapy	12 (27.3%)

Tableau 12: population characteristics

Among the 23 patients who completed the whole study, 12 complained of MT (27.2%) ; they all reported MT during the treatment phase (moments 2 to 5, one patient still at moment 6). Ten of these patients (83.3%) were treated by surgery and radiotherapy or radiochemotherapy, and two others (16.7%) by surgery only.

The Figure 30, Figure 31, Figure 32, and Figure 33 represent the evolution of mean taste intensity as a function of the time of treatment, and according to the reported MT status for the bitter, salty, sour, sweet respectively. The figure S2 in the annex 7 represent the same results for the metallic taste. The results of all chi-2 tests to evaluate differences between groups of patients for taste recognition and t-tests for taste intensity are presented in the annex 7 (table S1 and S2) respectively. No differences were observed for taste recognition. Most of the intensities were not significantly different in function of MT status and tongue location. Nevertheless, intensities were significantly reduced in patients with metallic taste for salty (Figure 31), sour (Figure 32) and sweet (Figure 33) tastes, during treatments (most of the time at the end of radio(chemo)therapy, i.e. moment 4). The differences were observed on the base of tongue locations as well as on the tip of the tongue.

There was only one patient who reported MT at moment 6 so no confidence interval was made possible and statistical significance was not retained.

For each taste, intensities recorded when applying stimuli on the tip of the tongue (i.e. facial nerve mediation) and on the tongue base (i.e. glossopharyngeal nerve mediation) were added up. The mean differences (posterior minus anterior) were then compared according to the MT status. The results are presented in Tableau 13. For salty and sour tastes, the mean intensity was significantly lower on the tongue base than on the tip of the tongue in the case of patients reporting MT ($p=0.031$ and 0.028 respectively). This was almost significant for sweet taste ($p=0.056$).

	patients reporting MT	patients reporting No MT	p-value
Bitter	0.2 ± 1.5	0.7 ± 1.9	0.177
Salty	-1.3 ± 2.1	-0.1 ± 1.7	0.031
Sour	-0.7 ± 2.0	0.4 ± 1.6	0.028
Sweet	-1.3 ± 2.3	-0.2 ± 1.4	0.056

Tableau 13: intensity differences (posterior minus anterior) of tastes according to metallic taste (MT) status

A negative value means that the intensity was higher on the tip of the tongue than on the tongue base. A positive value mean means that the intensity was higher on the tongue base than on the tip of the tongue.

To summarize, salty and sour tastes intensities on the tip of the tongue were lower than on the tongue base for patients reporting MT compared to patients who did not report MT. The difference was marginally significant for sweet taste but not significant for MT.

Besides, MT was significantly linked with mucositis (p=0.027) but not with neither candidiasis (p=0.38) nor with salivary flow (p=0.63).

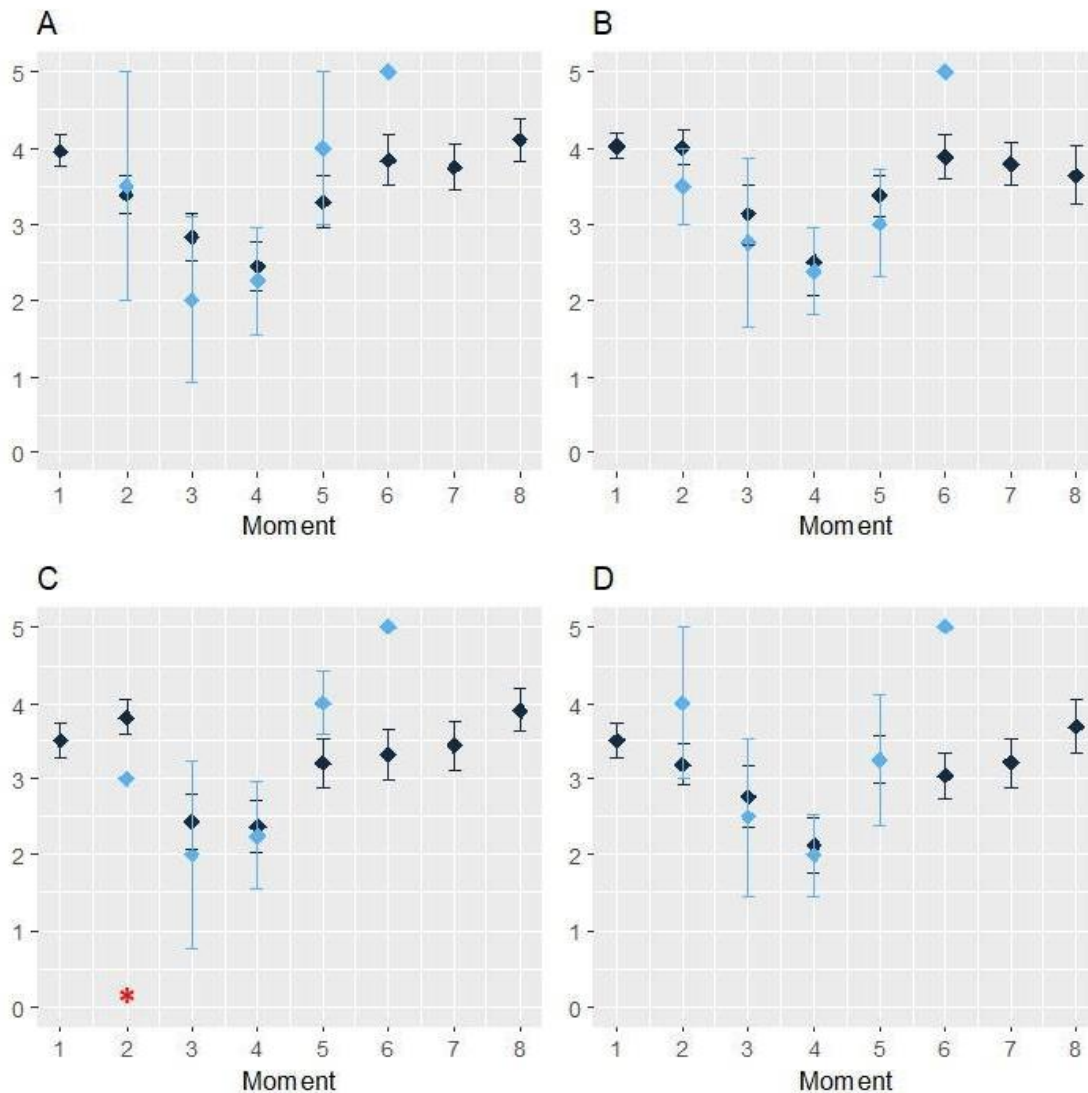


Figure 30: evolution of results for bitter taste according to the precise location on the tongue

Blue = metallic taste, black = no metallic taste. A = right tongue base, B = left tongue base, C = left tip of the tongue, D = right tip of the tongue

*: significance of t-test<0.05

Moments: (m) moment 1); after surgery if any (m2); in the middle (m3) and at the end (m4) of the radiotherapy if any; at 3 (m5)-6 (m6)-9 (m7) and 12 (m8) months.

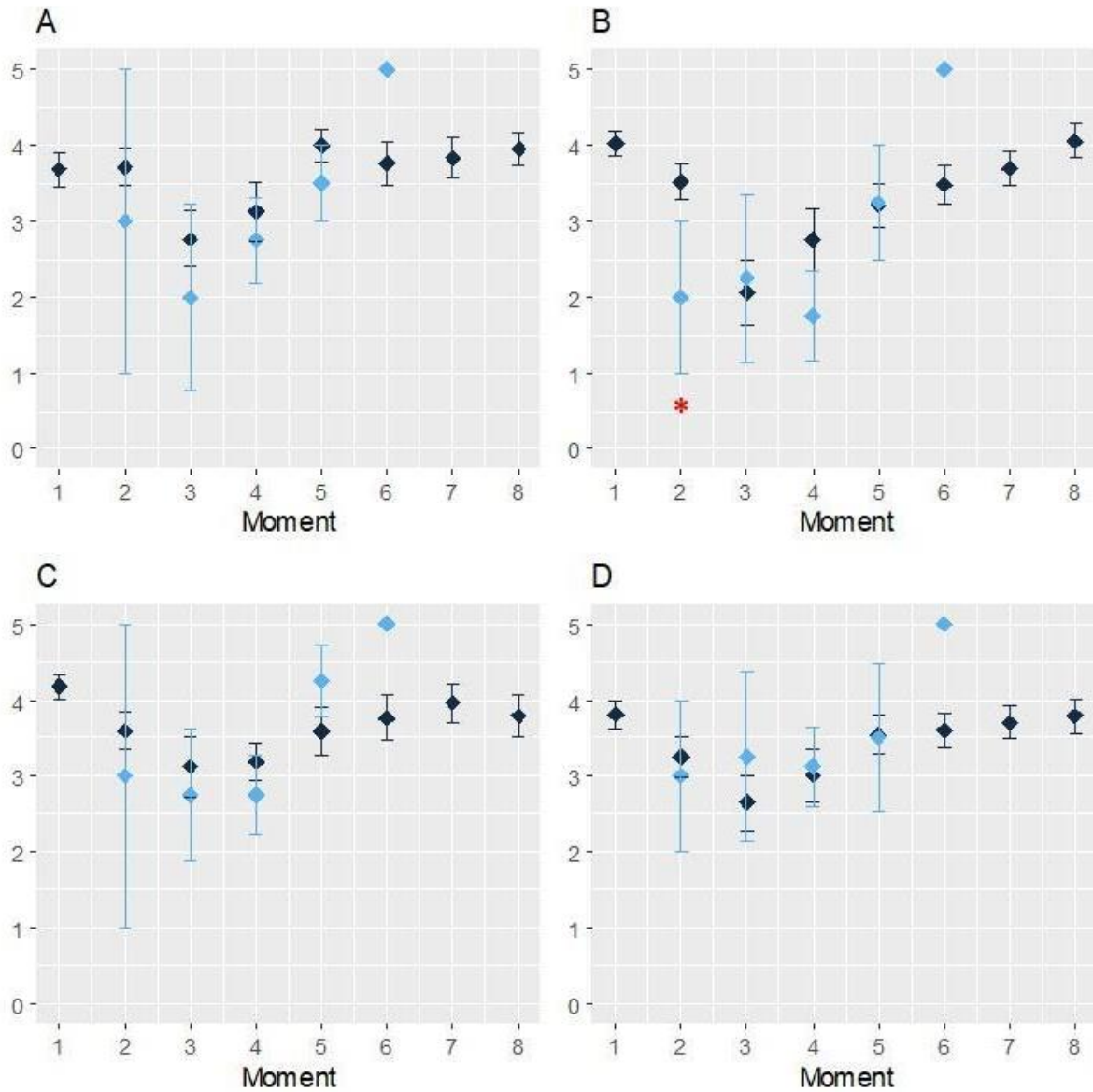


Figure 31: evolution of taste results for salty taste according to the precise location on the tongue

Blue = metallic taste, black = no metallic taste. A = right tongue base, B = left tongue base, C = left tip of the tongue, D = right tip of the tongue

*: significance of t-test<0.05

Moments: (m) moment 1); after surgery if any (m2); in the middle (m3) and at the end (m4) of the radiotherapy if any; at 3 (m5)-6 (m6)-9 (m7) and 12 (m8) months.

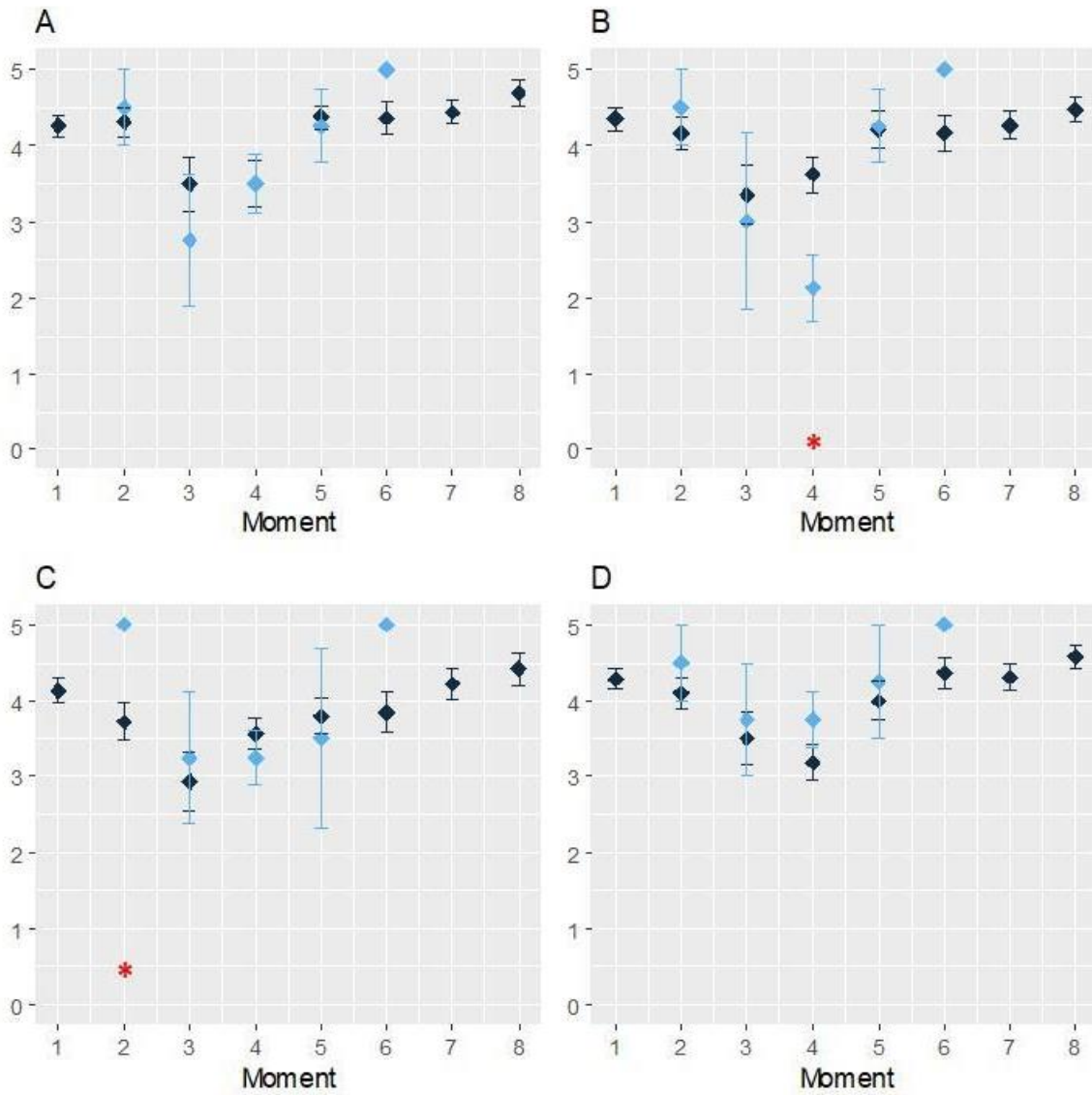


Figure 32: evolution of taste results for sour according to the precise location on the tongue

Blue = metallic taste, black = no metallic taste. A = right tongue base, B = left tongue base, C = left tip of the tongue, D = right tip of the tongue

*: significance of t-test < 0.05

Moments: (m) moment 1; after surgery if any (m2); in the middle (m3) and at the end (m4) of the radiotherapy if any; at 3 (m5)-6 (m6)-9 (m7) and 12 (m8) months.

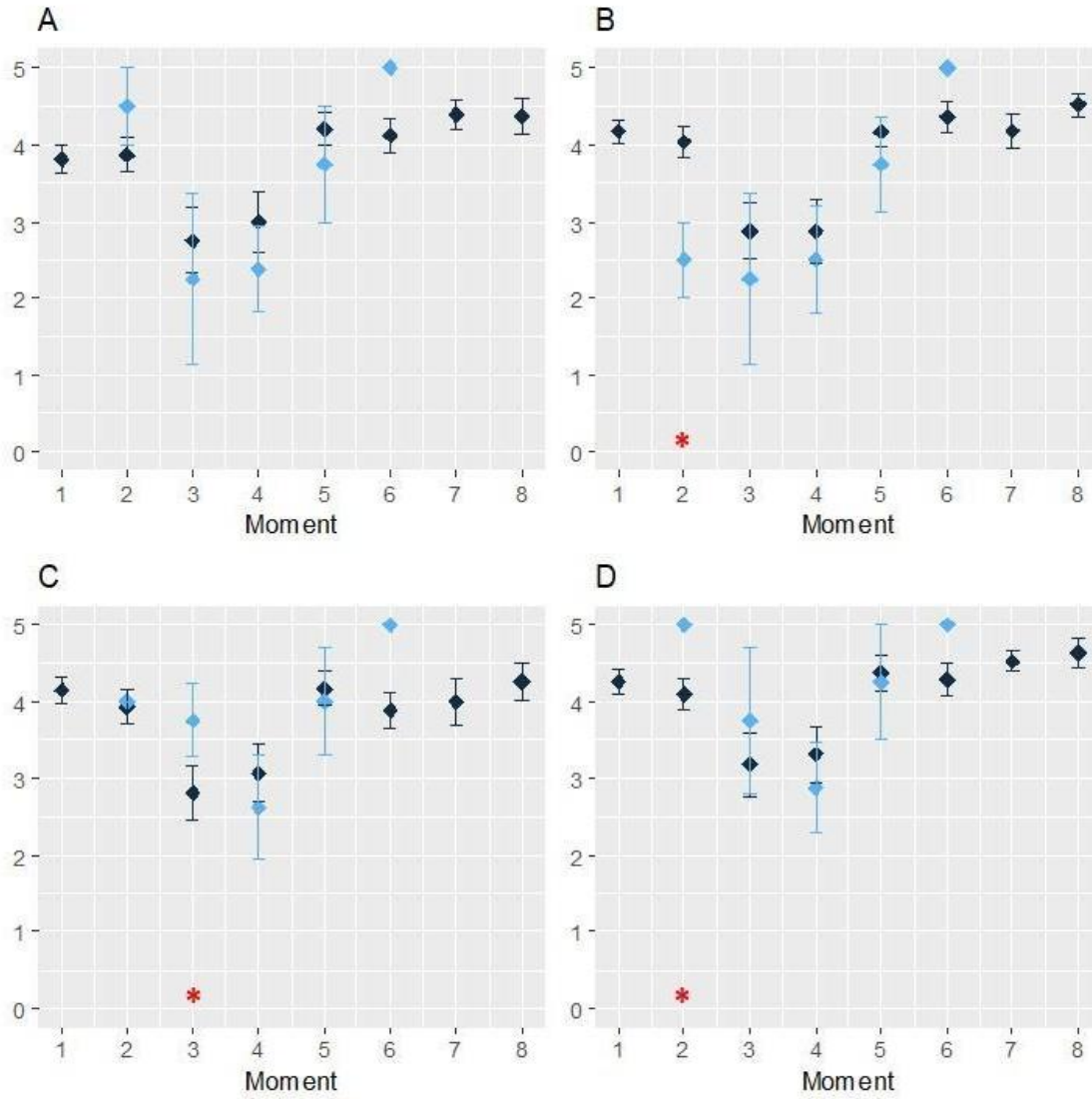


Figure 33: evolution of taste results for sweet according to the precise location on the tongue

Blue = metallic taste, black = no metallic taste. A = right tongue base, B = left tongue base, C = left tip of the tongue, D = right tip of the tongue

*: significance of t-test < 0.05

Moments: (m) moment 1; after surgery if any (m2); in the middle (m3) and at the end (m4) of the radiotherapy if any; at 3 (m5)-6 (m6)-9 (m7) and 12 (m8) months.

DISCUSSION

Our findings reveal that the intensities of all tastes decreased across various regions of the tongue during HNC treatments. Although the decline was more pronounced at the base of the tongue than at the tip, there were no significant differences for most of the tests. This disproves the hypothesis that the removal of facial nerve inhibition on the glossopharyngeal nerve is responsible for this phenomenon (123). Instead, this aligns with Deshpande et al. (242), who suggested that dysgeusia in HNC is more likely due to a reduced density of taste buds rather than nerve damage.

Changes in Taste Perception During HNC Treatments

The observed pattern of side-effects peaking during irradiation and then recovering, as seen in our study, is commonly reported in other series for taste intensity tests (36, 223-225) and salivary flows (53). Sapir et al. also did not find any correlation between dysgeusia and salivary flow, although they did find a correlation between dysgeusia and xerostomia questionnaire responses (53).

All taste intensities decreased during HNC treatments, with a subsequent recovery. There were some differences between the two groups of patients based on reported MT, regarding salty and sour tastes, possibly due to higher alteration of ion channel receptors. Interestingly, our findings showed an inverse relationship between the expected results for different tongue regions. The base of the tongue, typically less sensitive to these tastes, showed a greater decrease in taste intensity compared to the tip. This could suggest a more complex mechanism involving different receptor types and their distribution across the tongue. Finally, the recovery phase observed in taste intensities mirrors patterns found in other studies (36, 223-225).

Metallic Taste (MT) and Its Mechanisms

When specifically considering MT, its incidence in our study was 27.2%, which is consistent with other series (11). Previous studies on tympanic cord anesthesia, a branch of the facial nerve involved in taste, have shown a significant increase in bitter intensity at the base of the tongue and MT (13-15, 230). However, our results do not support a potential link between bitter receptors and MT (19), as bitter intensity did not significantly differ between MT and non-MT patients (Figure 30, Tables S1 and S2 in annex 7). This lack of significant differences highlights the need for further research into the specific mechanisms underlying these changes in taste perception during and after HNC treatments. Moreover, MT was significantly associated with mucositis but not with candidiasis or salivary flow. Given that the removal of facial nerve inhibition on the glossopharyngeal nerve seems to be disproved and MT is linked to mucositis but not candidiasis, the most probable explanation for MT is inflammation, specifically lipoperoxidation. Malondialdehyde, a byproduct of lipoperoxidation, has been shown to be elevated in HNC patients (185). Our study included salivary samplings to measure salivary levels of malondialdehyde.

In summary, our study challenges existing hypotheses regarding the cause of MT in HNC patients and suggests that inflammation, rather than nerve damage, may play a significant role. The dynamic changes in taste perception, along with the interplay between different types of taste receptors and regions of the tongue, warrant further investigation. Understanding these mechanisms can lead to more effective interventions for managing dysgeusia in these patients. Furthermore, the complex interplay between taste perception and the altered microenvironment of the oral cavity in HNC patients, including changes in salivary composition and flow, remains a hypothesis that requires testing.

CONCLUSION

The hypothesis of removal of facial nerve inhibition on the glossopharyngeal nerve cannot explain metallic taste in HNC. MT was significantly linked with mucositis, suggesting a possible role of inflammation, potentially lipoperoxidation.

5. Exploring the Role of Oxidative Stress in Metallic Taste During Head and Neck Cancer Treatment: A Study of Salivary Malondialdehyde and Therapeutic Interventions

A soumettre à Rhinology, ce travail présente l'évaluation de l'hypothèse de lipoperoxydation salivaire, objectif principal de TORCAD.

ABSTRACT

INTRODUCTION : Head and neck cancers (HNC) treatments often cause a metallic taste (MT), adversely affecting patients' quality of life. This study aims to investigate the lipoperoxydation hypothesis of MT by examining salivary malondialdehyde (MDA) levels, a marker of oxidative stress, in HNC patients undergoing treatment.

METHODS: This prospective cohort study included 44 newly diagnosed HNC patients. Saliva samples were collected before, during and up to one year after the HNC treatment. Analyses including MDA and other markers were performed. Additionally, a bovine lactoferrin mouthwash was evaluated for its efficacy in alleviating MT.

RESULTS: Out of the 44 patients, 12 (27.2%) reported MT, primarily during treatment phases. Salivary MDA levels significantly increased during radiotherapy, peaking mid-treatment, before declining post-treatment. Despite this fluctuation, no significant relationship was found between MDA levels and MT. Bovine lactoferrin mouthwash alleviated MT at least partially in 63.2% of the occurrences. Other salivary markers such as protein concentration, antioxidant properties, catalase activity, and superoxide dismutase activity showed no significant link to MT.

DISCUSSION: The increase in MDA levels during radiotherapy indicated heightened oxidative stress. However, the lack of a significant association between MDA and MT suggests other factors may contribute to MT development. The partial efficacy of lactoferrin mouthwash highlighted a potential benefits. Future research should explore other mechanisms, such as the role of oral microbiota, to better understand and manage MT in HNC patients.

INTRODUCTION

Head and neck cancers (HNC) include cancers of the oral cavity, pharynx, and larynx, with tobacco use, alcohol consumption, and human papillomavirus (HPV) being the primary risk factors. In France they are the fifth most common group of cancers (243). The treatments are multimodal, including surgery, radiotherapy, chemotherapy targeted therapies and, more recently, immunotherapy. Due to the cancer end/or its treatments, one of the lesser-studied but clinically significant symptom is the development of a metallic taste (MT). However, little or nothing is known about the origin of MT in HNC. There are two main hypotheses (79): the release of inhibition of the facial nerve on the glossopharyngeal nerve (14, 15) and the oral mucosa lipoperoxydation (82).

Malondialdehyde (MDA) is the final stable product of lipid peroxidation and is considered a reliable marker of oxidative stress. It has been documented in various inflammatory or precancer diseases (185, 244, 245), HNC (185) as well as in smokers (142). Given that saliva plays also a crucial role in oral health and taste perception (52, 246), it is also a relevant medium for assessing oxidative stress marker like MDA (140-142, 244).

Bovine lactoferrin was shown to alleviate MT induced by ferrous sulfate mouthwash in healthy volunteers (82). It has immunoregulatory mechanisms, especially in vitro and in animals supplemented per os (247). But no trial has yet been performed to evaluate its putative effect on MT.

By conducting serial saliva collections and analyses over a one-year period on patients treated for a HNC, we sought to determine if changes in salivary MDA concentrations or other salivary composition parameters were associated with the onset and severity of MT, thereby providing insights into potential markers and underlying mechanisms of MT in HNC patients. We also used bovine lactoferrin mouthwash to try to alleviate MT.

METHODS

Study Design

This prospective cohort study was conducted over a two-year period, involving 44 patients newly diagnosed with HNC. Participants were recruited from the ENT departments of a regional teaching hospital. They were followed up for one year after the end of their treatment, unless a recurrence or another cancer occurred. At the end of the study, they were categorized into two distinct groups based on the presence or absence of reported MT.

Participants

Inclusion criteria were: adults aged 18 years and older, newly diagnosed with HNC, and scheduled to undergo treatment (surgery, radiation, and/or chemotherapy). Exclusion criteria included previous treatment for HNC, other malignancies, having a total laryngectomy, or being pregnant.

Data Collection

The population's characteristics were assessed at the beginning of the study. Assessments were performed on each patient at regular intervals: before any treatment; after surgery if any; midway and upon completion of radio(chemo)therapy if any; and at 3-6-9 and 12 months.

Several questionnaires were used: EORTC QLQ30 (9) and HN35 (10) questionnaires to evaluate Quality of Life (QoL), a specific questionnaire to assess MT impact (11) in patients complaining about MT, measurement of weight and a Visual Analog Scale (VAS) to quantify food intake (12) ranging from 0 (total abstention) to 10 (normal eating).

An oral examination looking for mucositis and/or candidiasis was performed at each assessment.

Similarly, unstimulated whole saliva samples were also collected. Participants were instructed to refrain from eating, drinking, smoking, or performing oral hygiene activities for at least one hour before sample collection. Saliva was collected by having participants drool into sterile tubes over a ten-minute period. Saliva flow was calculated from the weight of saliva assuming that 1 g is equal to 1 mL. Samples were immediately placed on ice, transported to the laboratory, weighed and stored at -80°C until analysis. Aliquotes of saliva samples were previously defrosted and then centrifuged 15 min at 15 000 g. Analyses were performed on the resulting supernatants.

Salivary MDA analysis

To summarize, salivary MDA levels were measured using a modified thiobarbituric acid reactive substances (TBARS) assay. 2,6-di-tert-butyl-4-méthylphénol (BHT) was added to an aliquot of whole saliva samples until a concentration of 280µM and then the saliva samples were clarified by centrifugation 15 min at 15000 g at 4°C. 150µL of supernatant were mixed with thiobarbituric acid in duplicates and incubated at 60°C for 30 minutes. The fluorescence of the reaction mixture was measured (excitation: 532 nm; emission : 553 nm) using a microplate reader (Ensignt, PerkinElmer, Waltham, MA). MDA concentrations were calculated using the linear regression obtained from a standard range prepared with known concentrations of MDA. The salivary MDA concentration is the mean of the 2 replicates expressed in µM MDA equivalent.

Other biochemical analysis

If appropriate, all enzyme activities were expressed in milli International Enzyme Activity Units (mIU) per ml of saliva. One IU was defined as the amount of enzyme that catalyses the conversion of 1 µmol of substrate per minute. Protein concentration (mg/mL) was measured with a Quick Start Bradford protein assay (Bio-Rad, France) (excitation: 595 nm) using a microplate reader (Ensignt, PerkinElmer, Waltham, MA).

Total antioxidant status quantification

Total antioxidant status was determined using an ORAC Assay kit (Cell Biolabs Inc, Oxiselect™, San Diego, CA). This assay uses the free radicals initiator AAPH (2,2'-azobis-2-methyl-propanimidamide dihydrochloride). Peroxyl radicals then oxidize fluorescein with a consequent loss of fluorescence. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble vitamin E

analogue, served as a standard to inhibit fluorescein decay in a dose dependent manner. Kinetics of the intensity of fluorescence was recorded at 37°C (excitation: 485 nm; emission : 538 nm) using a microplate reader (Ensign, PerkinElmer, Waltham, MA). The antioxidant capacity of saliva was expressed as micromolar Trolox equivalents per mL.

Catalase

Catalase Activity was determined using a Catalase Fluorescent Activity Kit (Arbor Assay, Ann Arbor, MI, USA) by means of a fluorescent reaction coupled to HRP (horseradish peroxidase) which permit following the rate of oxidation of hydrogen peroxide added to samples. Increasing levels of catalase in the samples causes a decrease in hydrogen peroxide concentration and a reduction in fluorescent product (excitation at 570 nm, emission at 590nm) determined using a microplate reader (Ensign, PerkinElmer, Waltham, MA). A bovine catalase standard is used to generate a standard curve. Results were expressed in terms of units of catalase activity per mL of saliva.

SOD

SOD Activity was determined by SOD Assay Kit (Sigma-Aldrich, Saint-Louis, MI) using Dojindo's highly water-soluble tetrazolium salt, WST-1 (2-(4-Iodophenyl)- 3-(4-nitrophenyl)-5-(2,4-disulfophenyl)- 2Htetrazolium, monosodium salt), producing a water-soluble formazan dye upon reduction with a superoxide anion. The rate of the reduction with O₂ are linearly related to the xanthine oxidase (XO) activity, and is inhibited by SOD. The IC₅₀ (50% inhibition activity of SOD) is determined by absorbance reading at 440nm using a microplate reader (Ensign, PerkinElmer, Waltham, MA). Since the absorbance at 440 nm is proportional to the amount of superoxide anion, the SOD activity as an inhibition activity could be quantified by measuring the decrease in the color development. A bovine SOD standard (Sigma-Aldrich, Saint-Louis, MI) was used to generate a standard curve. Results were expressed in terms of units of SOD activity per mL of saliva.

GST

Glutathion S Transferase Pi (GSTp) was quantified using Enzyme-Linked Immunosorbent Assay kit from AbbeXa Ltd. (Cambridge, UK). The concentrations were obtained in ng/mL of saliva.

Bovine lactoferrin mouthwash

A mouthwash of 10ml with a lactoferrin-concentration of 50mg/l was proposed to patients who complained of a spontaneous MT. Lactoferrin powder, produced by Ingredia, France, was diluted with Evian water.

Statistical Analysis

Descriptive statistics were used to summarize the demographic and clinical characteristics of the study population. Quantitative data were compared using mean comparisons through ANOVA, while qualitative data were analyzed using the chi-square test. The primary outcome was the association between salivary MDA levels and the presence of MT. Mean comparison (with t-tests if the distribution was normal or Wilcoxon test if not) of salivary MDA according to the presence or not of MT was used to assess the relationship between MDA levels and MT.

Ethical Considerations:

This study was performed in line with the principles of the Declaration of Helsinki and adhered strictly to the French ethical guidelines and principles, with all participating patients providing informed consent prior to their involvement. Approval for the study was granted by the CPP Est I, Dijon, France (#2017-A03641-52). Additionally, the study is registered on ClinicalTrials.gov (NCT03558789).

RESULTS

Participant Characteristics

A total of 44 patients were enrolled in the study. The flowchart of the study is presented in Figure 34.

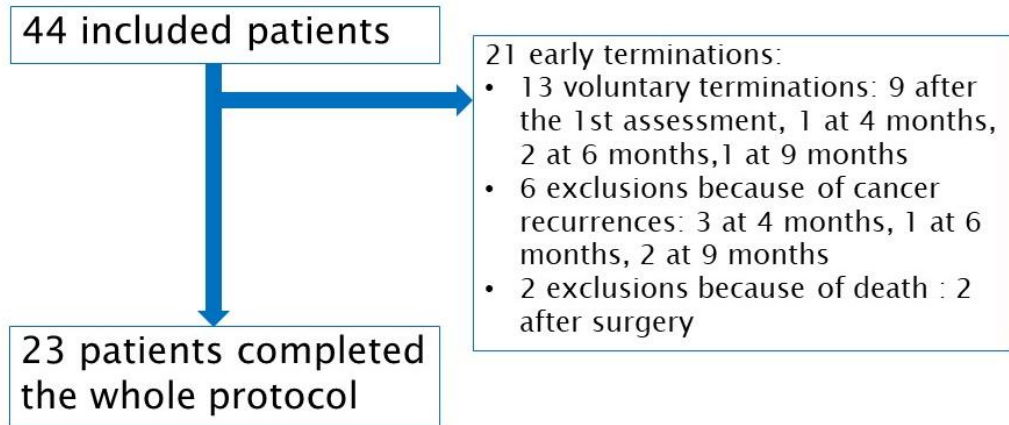


Figure 34: study flowchart

Tableau 14 displays the demographic characteristics of the study population at inclusion according to the MT status. Patients were predominantly male, of middle age, and with advanced Tumor and Node stage. The treatment modalities were predominantly multimodal.

	MT	No MT	p-value
Sex: males n(%) ; females n(%)	9 (75%), 3 (25%)	25 (78.1%), 7 (21.9%)	1
Mean age at diagnosis ± SD (year)	61.7 ± 10.2	63.6 ± 9.8	0.58
Location n (%): Hypopharynx Larynx Oropharynx Oral Cavity Unknown primary	0 (0%) 1 (8.3%) 4 (33.3%) 5 (41.7%) 2 (16.7%)	3 (9.4%) 7 (21.9%) 10 (31.2%) 9 (28.1%) 3 (9.4%)	0.57
T stage n (%): 1 2 3 4 x	3 (25%) 6 (50%) 1 (8.3%) 0 (0%) 2 (16.7%)	6 (18.7%) 12 (37.5%) 7 (21.9%) 4 (12.5%) 3 (8.8%)	0.69
N stage n (%): 0 1 2a 2b 2c 3a	4 (33.3%) 4 (33.3%) 1 (8.3%) 2 (16.7%) 0 (0%) 1 (8.3%)	16 (50%) 6 (18.7%) 3 (9.4%) 3 (9.4%) 2 (6.2%) 2 (6.2%)	0.77
Smoking at inclusion n (%): Yes No Quit	3 (25%) 3 (25%) 6 (50%)	17 (50%) 2 (6.2%) 13 (40.6%)	0.11

Treatment			0.54
Withdrawal of consent before treatment	0 (0%)	5 (15.6%)	
Chemotherapy, surgery, radiotherapy	0 (0%)	1 (3.1%)	
Surgery	2 (16.7%)	12 (37.5%)	
Surgery, radiotherapy	7 (58.3%)	5 (15.6%)	
Surgery, radiochemotherapy	3 (25%)	9 (28.1%)	

Tableau 14: studied population characteristics at inclusion according to metallic taste status.

MT: metallic taste

Twelve complained of MT (27.2% of the whole sample; 52.2% of the 23 patients who completed the whole study) at different moment (19 occurrences), mainly during the treatment phase (periods 2 to 4). The number and moments of MT are presented in Figure 35B). There were no significant demographic characteristics differences at inclusion, between MT and no MT status. At Moment 2 (after surgery, if any), two patients complained about MT; at Moment 3 (middle of radiotherapy or radiochemotherapy, if any), four patients; at Moment 4 (end of radiotherapy or radiochemotherapy, if any), eight patients; at Moment 5 (3 months after the end of treatments), four patients; at Moment 6 (six months after the end of treatments), one patient.

Figure S1 in annex 8 shows the evolution of the salivary flow according to the different moments and the MT status

Salivary Malondialdehyde Levels

Figure 35A represents the evolution of salivary MDA concentration according to the different moments. Baseline salivary MDA levels varied widely among participants, with a mean of 0.11 μM (standard deviation [SD] 0.06 μM). Over the one-year follow-up, MDA levels showed a significant increase, peaking at the middle of the radiotherapy (mean 0.26 μM , SD 0.27 μM , p-value of the comparison between Moments 1 and 3=0.034) before declining slightly (at 12 months: mean 0.15 μM , SD 0.1 μM , p=0.125 in comparison with Moment 1). Figure 35B represents the evolution of salivary MDA concentration and the MT status during the one-year-follow-up. Wilcoxon tests revealed no significant link between salivary MDA levels and metallic taste (p=0.44, 0.96, 1, and 0.92 for moments 2, 3, 4 and 5).

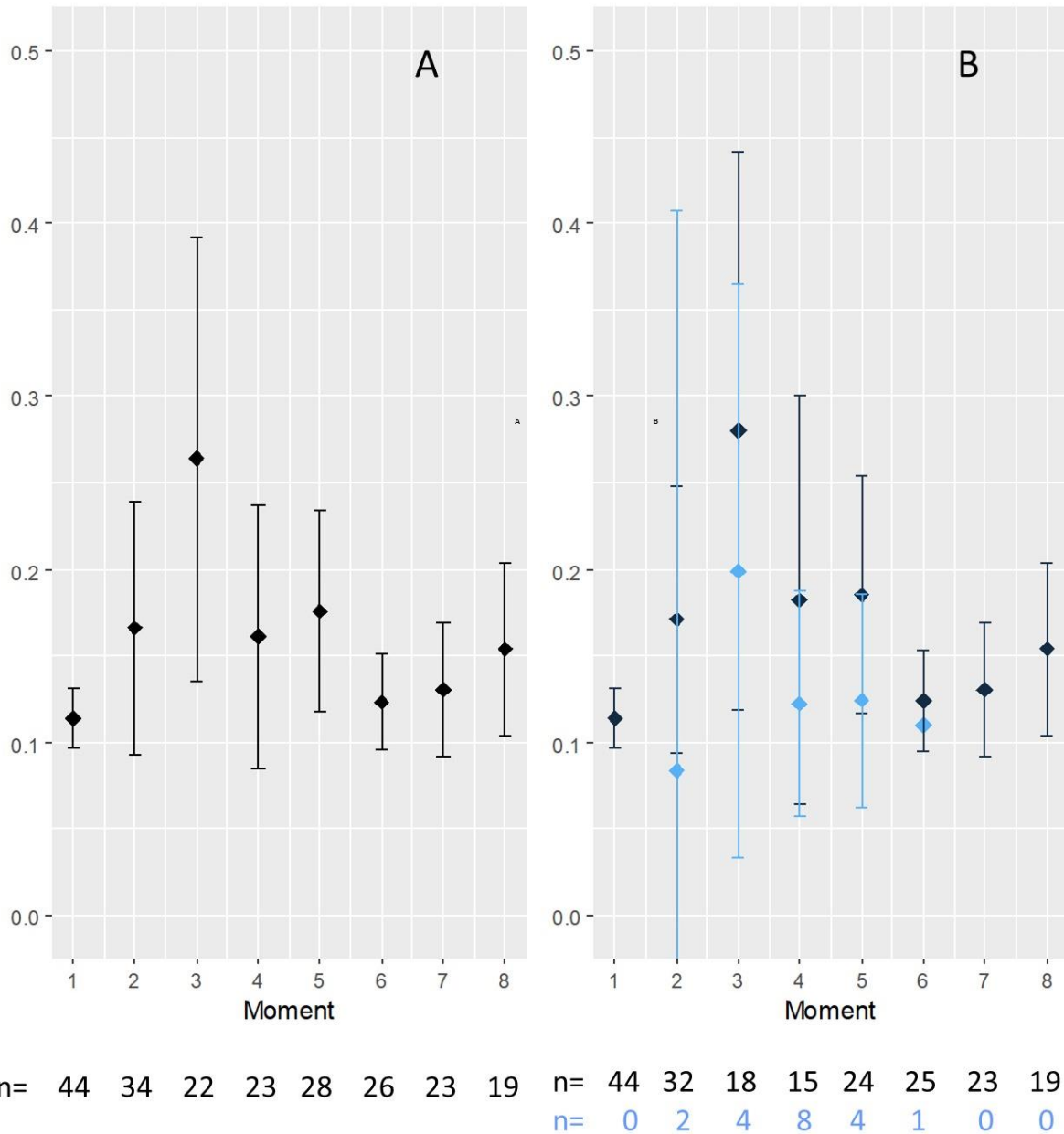


Figure 35: evolution of salivary MDA concentration ($\mu\text{mol}/10 \text{ min}$) during the follow-up.

A = global results on the whole population. B = according to the metallic taste status (Blue = metallic taste, black = no metallic taste)
 Moments: (m) moment 1); after surgery if any (m2); in the middle (m3) and at the end (m4) of the radiotherapy if any; at 3 (m5)-6 (m6)-9 (m7) and 12 (m8) months.

MT and lactoferrin mouthwash

Figure 36 shows the efficacy of bovine lactoferrin mouthwash in patients complaining about MT. Lactoferrin mouthwash helped to alleviate at least partially MT in 12 out of the 19 MT occurrence (efficacy rate: 63.2%).

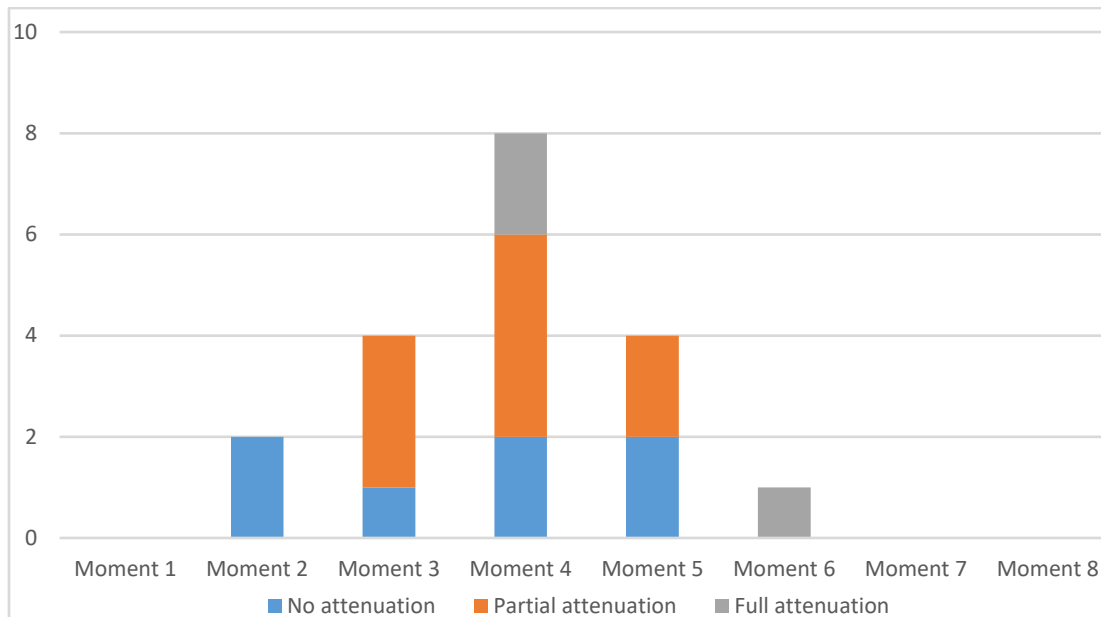


Figure 36: qualitative efficacy of lactoferrin mouthwash in patients with metallic taste according to the evaluation moment, based on patient-reported perceptions of attenuation across three modalities.

Other salivary analyzes (figures are presented in the annex 8)

Baseline salivary protein mean was 0.98 mg/ml (SD 0.73 mg/ml). Over the one-year follow-up, protein levels showed a stable concentration (Figure S2A in annex 8). Figure S2B in annex 8 represents the evolution of salivary protein concentration over time in two groups (MT vs no-MT). Wilcoxon tests revealed no significant link between salivary protein concentration and MT (all $p > 0.05$).

Baseline salivary antioxidant properties (ORAC) mean was 1663 $\mu\text{mol/l}$ (SD 892 $\mu\text{mol/l}$). Over the one-year follow-up, antioxidant properties remained stable (Figure S3A, annex 8). Figure S3B in annex 8 represents the evolution of salivary antioxidant properties over time in two groups (MT vs no-MT). Wilcoxon tests revealed no significant link between salivary antioxidant properties and MT (all $p > 0.05$).

Baseline catalase activity mean was 162 IU/ml (SD 148 IU/ml). Over the one-year follow-up, protein levels showed a stable concentration (Figure S4A, annex 8). Figure S4B in annex 8 represents the evolution of salivary catalase activity over time in two groups (MT vs no-MT). Wilcoxon tests revealed no significant link between salivary protein concentrations and MT (all $p > 0.05$).

Baseline SuperOxide Dismutase (SOD) activity mean was 6.05 IU/ml (SD 5.10 IU/ml). Over the one-year follow-up, SOD showed a stable concentration (Figure S5A, annex 8). Figure S5B in annex 8 represents the evolution of salivary SOD activity over time in two groups (MT vs no-MT). Wilcoxon tests revealed no significant link between salivary protein levels and MT (all $p > 0.05$).

Baseline salivary GSTp mean was 38.4 ng/ml (SD 41.8 ng/ml). Over the one-year follow-up, GSTp levels slightly decreased after surgery and/or radiotherapy (period 2-4) and returned to baseline later (Figure S6A, annex 8). Figure S6B in annex 8 represents the evolution of salivary GSTp concentration over time in two groups (MT vs no-MT). Wilcoxon tests revealed no significant link between salivary GSTp concentration and MT (all $p > 0.05$).

DISCUSSION

Our study aimed to explore the relationship between salivary markers, especially MDA levels, and MT in patients undergoing treatment for HNC.

MDA levels and MT

The current findings indicate a significant increase in salivary MDA levels during the radiotherapy or radiochemotherapy phase (moments 3 and 4 of Figure 2), which subsequently declined, while other

salivary markers such as protein concentration, antioxidant properties, catalase activity, and SOD activity remained relatively stable over the same one-year follow-up (Figure S2-S6). The observed increase in salivary MDA levels during radiotherapy suggests elevated oxidative stress in patients undergoing cancer treatment. MDA is a well-known marker of lipid peroxidation and oxidative stress, and its elevation during radiotherapy aligns with previous findings that highlight the oxidative damage induced by cancer therapies (140, 244). Agha-Hosseini et al. reported significantly higher salivary MDA levels in patients with oral lichen planus compared to healthy controls, emphasizing the role of oxidative stress in oral pathologies (140). Similarly, Ergun et al. found elevated MDA levels in patients with lichen planus, further supporting the relevance of MDA as a marker for oxidative stress in oral diseases (244). Rai et al. (245) and Saral et al. (83) found similar results. At last, smoking also increases salivary MDA (142, 143). Additionally, as we found, the study by Kaur et al. (185) investigating salivary markers in various oral conditions found significantly elevated MDA levels in patients with 30 oral squamous cell carcinoma and 30 precancerous conditions compared to 30 controls. The subsequent decline in MDA levels post-treatment in our study indicates a reduction in oxidative stress as patients recover. This highlights the role of oxidative stress in HNC and suggests a relationship between MDA levels and symptoms like MT. However, despite the fluctuations in MDA levels, no significant link with MT was observed, suggesting that factors other than this form of oxidative stress may play a concomitant or more prominent role in the development of MT. Furthermore, despite the 95% confidence intervals largely crossed, the MDA concentration mean was lower in the MT group. Other products of oral lipoperoxidation could lead to the formation of volatile compounds with very low detection thresholds, such as certain aldehydes or ketones (128, 129). Maybe those volatile markers of MT should be looked at instead of MDA, which is a general marker of lipoperoxidation. It should have been possible to evaluate the release of these volatiles by measuring nasal effluvia during oral consumption. However such a methodology should be adapted to be implemented to a population of cancer patients. Results on lactoferrin supports the oxidation hypothesis. Indeed, lactoferrin is an iron-binding milk protein, known for its antimicrobial, antiviral, immunostimulating and anti-inflammatory properties (247, 248). It has been proposed as a potential therapeutic agent for managing taste disturbances (82). In our study, the lactoferrin mouthwash helped to alleviate MT in 12 MT occurrences out of 19 (63.2%), which supports the oxidation hypothesis. Basically, the hypothesis of lipoperoxidation still holds but needs further explorations regarding identification and measurement of the molecules at the origin of MT.

Other salivary markers and MT

Previous research has shown that salivary protein composition can be altered in various conditions, including cancer therapy, impacting oral health and potentially taste perception (249, 250). Moreover, catalase and SOD are vital in managing oxidative stress, and their activity can be indicative of the overall oxidative status within the oral environment (251, 252). Indeed, our analysis revealed no significant link with MT. These findings suggest that the biochemical changes related to redox status in saliva during cancer treatment do not directly contribute to the development of MT specifically. Indeed those markers had been previously tested with taste disorders including MT (253), without identifying subgroups expressing specific taste disorders associated with specific marker modifications. Moreover, Walliczek-Dworschak et al. compared, among other parameters, 81 patients with taste disorder (the causes were not specified) including 11 patients with MT, to 40 healthy volunteers (254). They showed in MT patients similar mean salivary protein, catalase concentrations (8.8 (SD10.5) and 10.7 (SD 13.2), $p=0.40$) and total antioxidant status (476.4 (SD 388.4) and 992.3 (SD 596.5), $p=0.09$). However, for other taste disorders (ageusia, hypoguesia, bitter taste disorder, salt dysguesia), authors showed a significantly higher TAC compared to healthy subjects.

Limitations

A limitation of our study is the small sample size, which may have reduced the statistical power to detect significant associations. Additionally, the reliance on self-reported measures for MT, without incorporating objective taste tests, may limit the robustness of our findings. Future studies should aim to include larger cohorts and a combination of subjective and objective measures to provide a more comprehensive understanding of the relationship between salivary markers and MT.

CONCLUSION

Our study provides valuable insights into the changes in salivary markers during cancer in the global population, regardless the MT status. The significant increase in salivary MDA levels highlighted the oxidative stress induced by radiotherapy. However there was no significant difference in the mean salivary MDA levels between patients reporting MT and those who did not. As MDA is the final stable product of lipoperoxidation, it doesn't seem the lipoperoxidation could explain MT. However the palliation effect of lactoferrin mouthwash suggests potential benefits of antioxidants. Is there another marker that would stimulate more the retronasal path than taste which could trigger MT? Is the oral microbiota involved? Overall, our findings underscore the need for larger, more comprehensive studies focused on HNC patients treated by radiotherapy and or radiochemotherapy to fully elucidate the mechanisms underlying MT and to develop effective interventions for managing this distressing symptom in cancer patients.

6. Evolution des capacités de reconnaissance et d'intensité gustatives sur différentes régions de la langue chez des patients atteints de cancer des voies aérodigestives supérieures

Soumis aux Annales de la SFORL, ce travail présente l'évolution des capacités gustatives (reconnaissance et intensité) à différentes localisations de la langue pour l'ensemble des patients de TORCAD.

La traduction anglaise est fournie en [annexe 9](#).

RESUME

CONTEXTE : cette étude examinait l'évolution des altérations gustatives subjectives et objectives chez les patients souffrant de Cancer des Voies AéroDigestives Supérieures (CVADS), une condition souvent négligée mais qui impacte significativement la qualité de vie et l'alimentation des patients. La recherche se concentre sur l'analyse temporelle et à différents endroits de la langue des changements avant, pendant et après le traitement du cancer.

METHODE : un suivi prospectif et longitudinal des altérations gustatives subjectives et objectives a été réalisé chez 44 patients diagnostiqués avec un CVADS. Elle a consisté en des tests sensoriels supraliminaires de reconnaissance et d'intensité réalisés à différentes localisations sur la langue et à plusieurs moments clés avant, pendant et jusqu'à un an après le traitement. Des questionnaires ont été utilisés pour évaluer la qualité de vie. Les résultats ont été analysés en fonction des différentes étapes du traitement et des caractéristiques démographiques des patients.

RESULTATS : les altérations gustatives subjectives et objectives étaient déjà présentes avant le traitement chez certains patients, se détérioraient significativement dans le temps et puis s'amélioraient tout en persistant jusqu'à un an après le traitement. L'atteinte était significativement plus prononcée à la base de la langue qu'à la pointe. De plus, une tendance entre l'atteinte gustative subjective et le type de traitement du CVADS (plus fréquente en cas de radiothérapie ou radiochimiothérapie) était mise en évidence ($p=0.0360$).

CONCLUSION : l'étude souligne la fréquence et l'importance des altérations gustatives chez les patients atteints de CVADS. Il est donc important de délivrer une information préalable et de réaliser une évaluation précoce, un suivi et une prise en charge appropriée pour améliorer leur qualité de vie et leur tolérance au traitement.

INTRODUCTION

Les cancers des voies aérodigestives supérieures (CVADS) restent fréquents, bien que leur incidence diminue (243). Outre une mortalité qui reste élevée, ils impactent également la qualité de vie des patients de manière significative (43, 239, 255), à la fois par le cancer en lui-même et les traitements, en général multimodaux. Parmi les nombreuses séquelles physiques et psychologiques causées par ces cancers et leurs traitements, les altérations gustatives occupent une place importante mais souvent négligée comme les soins de support en général, au profit de la survie globale. En effet, les patients atteints de CVADS sont souvent confrontés à des modifications gustatives significatives en raison des effets des traitements, notamment la chimiothérapie et/ou la radiothérapie (3, 220, 242).

Les altérations gustatives peuvent impacter les apports nutritionnels en réduisant les ingesta chez des personnes déjà dénutries (11, 37, 44, 241). Irune et al. (43) et Buiret et al. (19) décrivaient différents mécanismes, possiblement concomitants : atteinte muqueuse, atteinte directe par la radiothérapie externe (diminution du nombre des cellules gustatives, neurotoxicité), chimiotoxicité (diminution du nombre des cellules gustatives et de leurs récepteurs, passage intra salivaire), xérostomie, chirurgie (diminution du nombre des cellules gustatives, atteinte neurologique). Des études, reprises dans la méta-analyse de Hovan (3) montraient des prévalences élevées de dysgueusie chez les patients sous chimiothérapie (56.3%), radiothérapie (66.5%) et radiochimiothérapie combinée (76%). Abendstein (255) et Asif (223) montraient des impacts significatifs sur la qualité de vie et le goût global durant et

après les traitements, bien que des interventions comme l'utilisation de l'amifostine, un agent cytoprotecteur vis-à-vis de la chimiothérapie et de la radiothérapie, actif sur les cellules saines mais pas sur les cellules cancéreuses par des effets sur les facteurs de transcription, l'expression des gènes, la stabilité de la chromatine et l'activité enzymatique (256), pouvaient réduire ces symptômes (257). Nous avons suivi de façon prospective et longitudinale l'évolution des altérations gustatives chez les patients atteints de CVADS, en analysant les changements observés pour quatre saveurs (sucré, salé, acide et amer) avant, pendant et jusqu'à un an après le traitement. Ces données pourront contribuer à la mise en place de stratégies de prise en charge plus adaptées, permettant de minimiser l'impact des traitements sur les perceptions gustatives et, par conséquent, sur la qualité de vie des patients.

METHODES

Tous les patients diagnostiqués pour un CVADS entre avril 2021 et mars 2023 s'étaient vus proposer d'intégrer l'étude TORCAD sur le goût métallique avant, pendant et après le CVADS. Peu de patients avaient accepté car le recueil des données était long et un peu fastidieux.

Les critères d'inclusion étaient d'être majeur et de présenter un CVADS avec une espérance de vie de plus d'une année. Les critères de non-inclusion étaient de ne pas se voir proposer une laryngectomie totale (l'odorat par voie orthonasale devant être conservé), ne pas avoir déjà eu un cancer, CVADS ou non (le traitement précédent pourrait avoir modifié les capacités gustatives des individus), ne pas être enceinte (la grossesse perturbe le goût). Les critères d'exclusion étaient la récurrence du cancer durant l'année de suivi post-thérapeutique. Dans ce cas, les données acquises jusqu'à la récurrence étaient gardées. De même, en cas de retrait du consentement, les données acquises jusqu'au retrait du consentement étaient gardées.

Le traitement proposé faisait l'objet d'une réunion de concertation pluridisciplinaire. En cas de radiothérapie ou de radiochimiothérapie, une gastrostomie radiologique prophylactique était systématiquement réalisée.

Les patients avaient plusieurs évaluations : T1 avant tout traitement ; T2 après chirurgie avant radiothérapie le cas échéant ; T3 au milieu de la radiothérapie le cas échéant ; T4 : à la fin de la radiothérapie le cas échéant ; T5 : trois mois après la fin du traitement ; T6 : six mois après la fin du traitement ; T7 : neuf mois après la fin du traitement ; T8 : 12 mois après la fin du traitement.

A chaque moment d'évaluation, des tests sensoriels de reconnaissance et d'intensité étaient réalisés et des questionnaires portant sur la qualité de vie étaient remplis. Les tests sensoriels de reconnaissance des saveurs et d'évaluations de l'intensité supraliminaire étaient réalisés avec des cotons-tiges, imbibés de solution gustative et appliqués sur différentes régions de la langue : la base de langue droite (A), la base de langue gauche (B), la pointe de langue gauche (C) et la pointe de langue droite (D). La technique du test est présentée dans la vidéo. Pour les solutions gustatives, les composés suivants étaient utilisés : solution de sucre blanc (Sigma-Aldrich ref 16104) à 850 g/l pour le sucré, solution de sel de table à 75 g/l pour le salé, solution de caféine anhydre (Sigma-Aldrich ref W222402) à 20 g/l pour l'amertume et solution d'acide citrique (Sigma-Aldrich ref W230618) à 50 g/l pour l'acidité. L'eau d'Évian, du fait de ses propriétés organoleptiques neutres, était utilisée pour diluer les composés sapides et pour le rinçage de la bouche entre chaque test. A chaque position sur la langue, les patients devaient reconnaître la saveur, parmi quatre possibilités (sucré, salé, amer, acide) puis évaluer sur une échelle de Likert de 0 à 5 l'intensité de leur perception. L'ordre des saveurs et la localisation de l'application du coton-tige étaient fixés à l'avance dans le protocole et identiques pour tous les individus. Toutefois la multiplicité des tests (quatre saveurs appliquées sur les quatre localisations de la langue) et plusieurs semaines entre chaque test ne permettaient pas aux personnes d'anticiper les réponses des tests. Les questionnaires utilisés étaient l'EORTC QLQ30 (187) et HN35 (76) pour la qualité de vie. D'autres paramètres étaient mesurés : le poids, une échelle visuelle analogique pour les ingesta (221), le flux salivaire évalué via la quantité de salive crachée en 10 min sans stimulation (258), la présence d'une mucite et/ou d'une candidose, le tabagisme (actif ou non).

Éthique et statistiques

L'étude était validée par le CPP Est I sous le numéro 2017-A03641-52. Elle est référencée sur clinicaltrials sous le numéro NCT03558789.

Les moyennes et écart-types étaient calculés pour les données quantitatives. Des fréquences étaient calculées pour les données qualitatives.

Des ANOVA sur données répétées étaient calculées pour les données quantitatives. Les interactions entre critères qualitatifs étaient testées par tests du chi-2.

Les réponses aux tests gustatifs étaient traitées par un modèle linéaire généralisé (binomial pour la reconnaissance des saveurs, gaussien pour l'intensité perçue), sur données répétées. La représentation graphique des résultats montrait une évolution en U. Un polynôme de degré 2 était donc introduit pour prendre en considération cette évolution temporelle.

Le logiciel R (version 4.3.1) était utilisé.

RESULTATS

Quarante-quatre patients étaient recrutés. La Figure 37 rapporte le diagramme de flux.

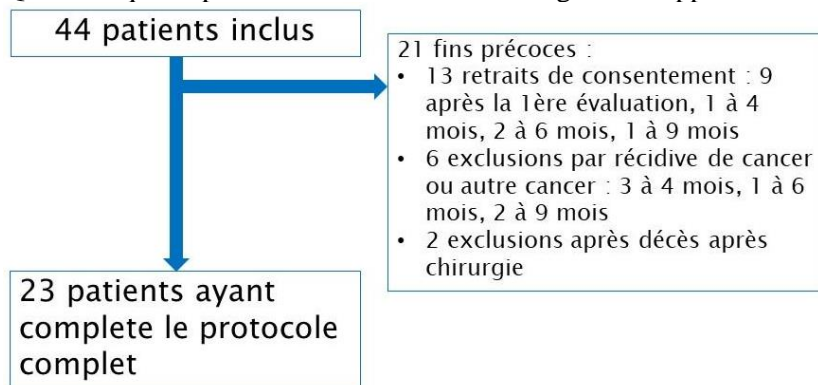


Figure 37: diagramme de flux de l'étude

Le Tableau 15 présente les caractéristiques démographiques et les traitements des patients.

Sexe : hommes n(%) ; femmes n(%)	34 (77.2%) ; 10 (22.8%)
Age moyen au diagnostic \pm écart-type (ans)	63.0 \pm 9.8
Localisation du cancer n (%) :	
Hypopharynx	3 (6.8%)
Larynx	8 (18.2%)
Oropharynx	14 (31.8%)
Cavité orale	14 (31.8%)
Adénopathies en apparence primitive	5 (11.4%)
Stade tumoral T n (%) :	
1	9 (20.5%)
2	18 (40.9%)
3	8 (18.2%)
4	4 (9.1%)
x	5 (11.4%)
Stade ganglionnaire N n (%) :	
0	20 (45.5%)
1	10 (22.7%)
2a	4 (9.1%)
2b	5 (11.4%)
2c	2 (4.5%)
3a	3 (6.8%)
Traitement	
Retrait du consentement avant traitement	5 (11.4%)
Chimiothérapie, chirurgie (curage), radiothérapie	1 (2.3%)
Chirurgie	14 (31.8%)

Chirurgie, radiothérapie	12 (27.3%)
Chirurgie, radiochimiothérapie	12 (27.3%)

Tableau 15: caractéristiques démographiques de la population et traitements réalisés

La Figure 38 représente l'évolution subjective de la dysgueusie rapportée à la question 14 du questionnaire de l'EORTC HN35. Les atteintes gustatives subjectives variaient significativement avec le moment d'évaluation ($p < 10^{-5}$). Pour rappel, les moments 3 et 4 étaient obtenus uniquement en cas de radiothérapie et de radiochimiothérapie. Seize personnes n'avaient eu aucune dysgueusie rapportée à la question 14 du questionnaire de l'EORTC HN35 (36.4%). Après le retrait des neuf personnes n'ayant eu qu'une évaluation initiale et ayant retiré leur consentement après la première évaluation, sept personnes n'avaient rapporté aucune dysgueusie (15.9%), six avaient été traitées par chirurgie exclusive et une par chirurgie et radiothérapie d'un cancer du larynx. Il existait une tendance de relation entre le type de traitement et l'évaluation subjective de la dysgueusie, rapportée à la question n°14 de l'EORTC HN35 ($p = 0.0360$).

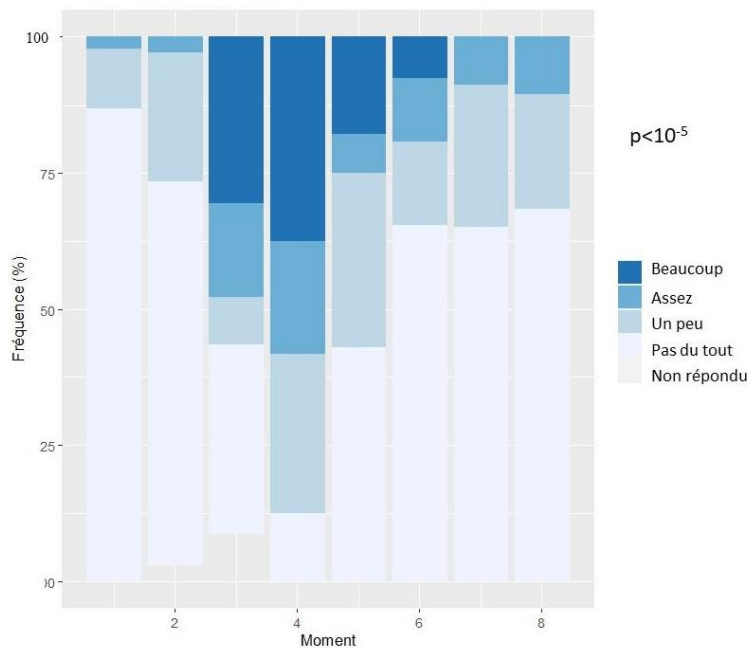


Figure 38 : évolution de la réponse à la question « Durant la semaine passée, avez-vous eu des problèmes de goût ? » (EORTC HN35 n°14)

Les évolutions du débit salivaire, du poids, de l'échelle visuelle analogique des ingesta et le tabagisme au moment de l'évaluation sont présentées dans les figures S1 à 4 dans l'annexe 10. Le débit salivaire diminuait significativement dans le temps ($- 0.31\text{g}/10\text{min} / \text{moment}$, $p < 10^{-5}$), de même que le poids ($- 0.85\text{kg}/\text{moment}$, $p = 0.0012$).

La Figure 39 représente l'évolution de la reconnaissance des saveurs en fonction du moment et selon la localisation sur la langue et la Figure 40 de l'intensité des saveurs en fonction du moment de l'évaluation et selon la localisation sur la langue.

Pour la grande majorité des tests, il existait une répartition significativement différente ou une tendance des résultats des tests gustatifs de reconnaissance et d'intensité selon le moment du test, avec des diminutions des deux paramètres essentiellement aux moments 3 et 4, correspondant respectivement au milieu et à la fin de la radio(chimio)thérapie puis un retour progressif à l'état de base.

Les résultats des tests gustatifs de reconnaissance étaient ensuite comparés selon qu'ils étaient réalisés sur la base de langue (points A et B) ou sur la pointe (points C et D). Pour toutes les saveurs, l'atteinte était significativement plus importante en base de langue qu'en pointe de langue ($p < 10^{-5}$ pour le salé, l'amer et le sucré, $p = 0.004$ pour l'acide). Pour les tests gustatifs d'intensité, l'atteinte était aussi significativement plus importante en base de langue qu'en pointe de langue ($p < 10^{-5}$) pour chaque saveur.

L'effectif de l'étude n'avait pas la puissance suffisante pour étudier, pour chaque saveur et chaque localisation, l'impact sur l'identification et l'intensité selon le type de traitement du CVADS.

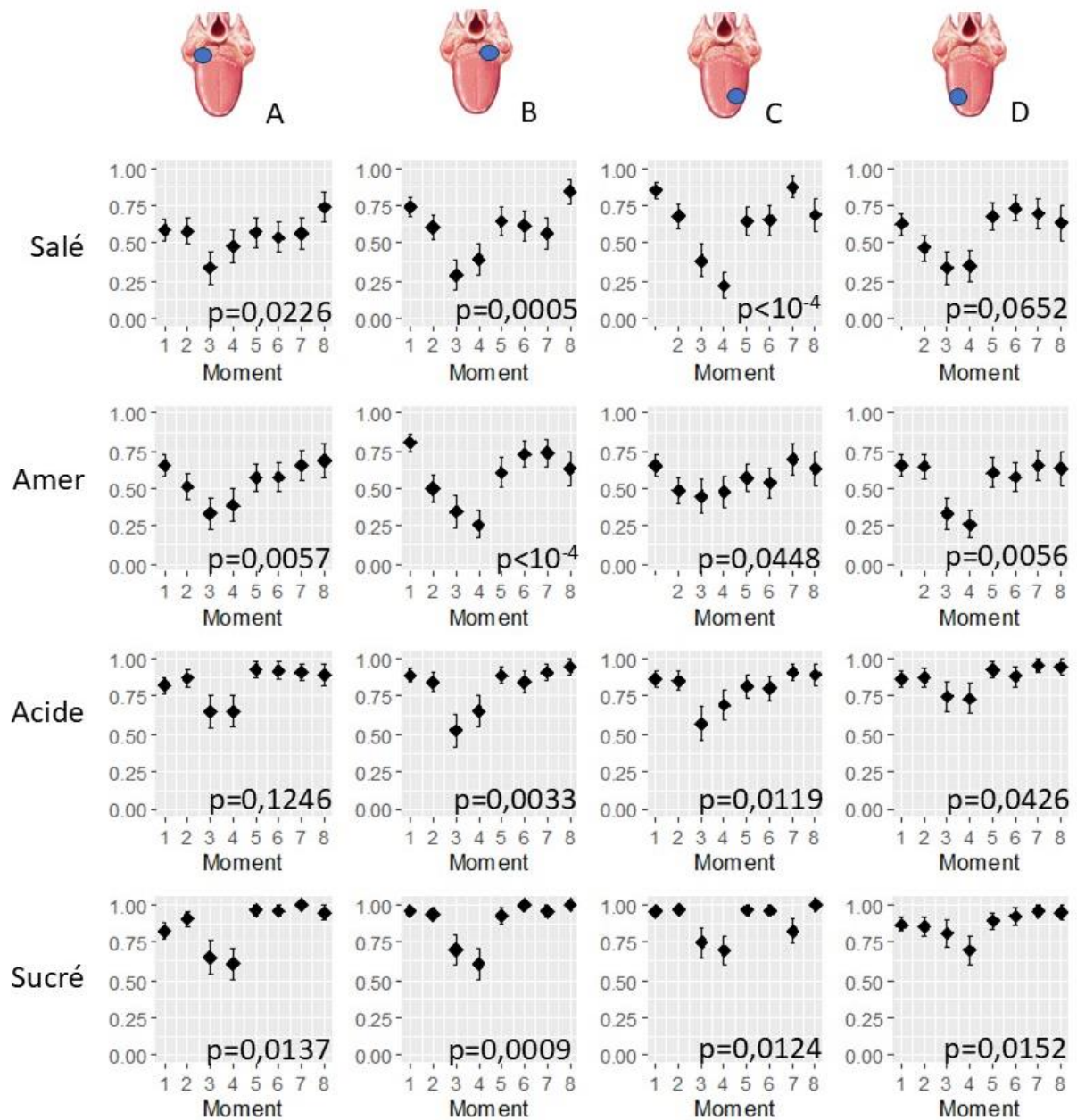


Figure 39: évolution de la reconnaissance des saveurs en fonction du moment de l'évaluation et selon la localisation sur la langue

La valeur de p, sur chaque graphique est le résultat de la modélisation de la reconnaissance de la saveur (0 : reconnaissance incorrecte de la saveur, 1 : reconnaissance correcte de la saveur) pour les différents moments de l'évaluation.

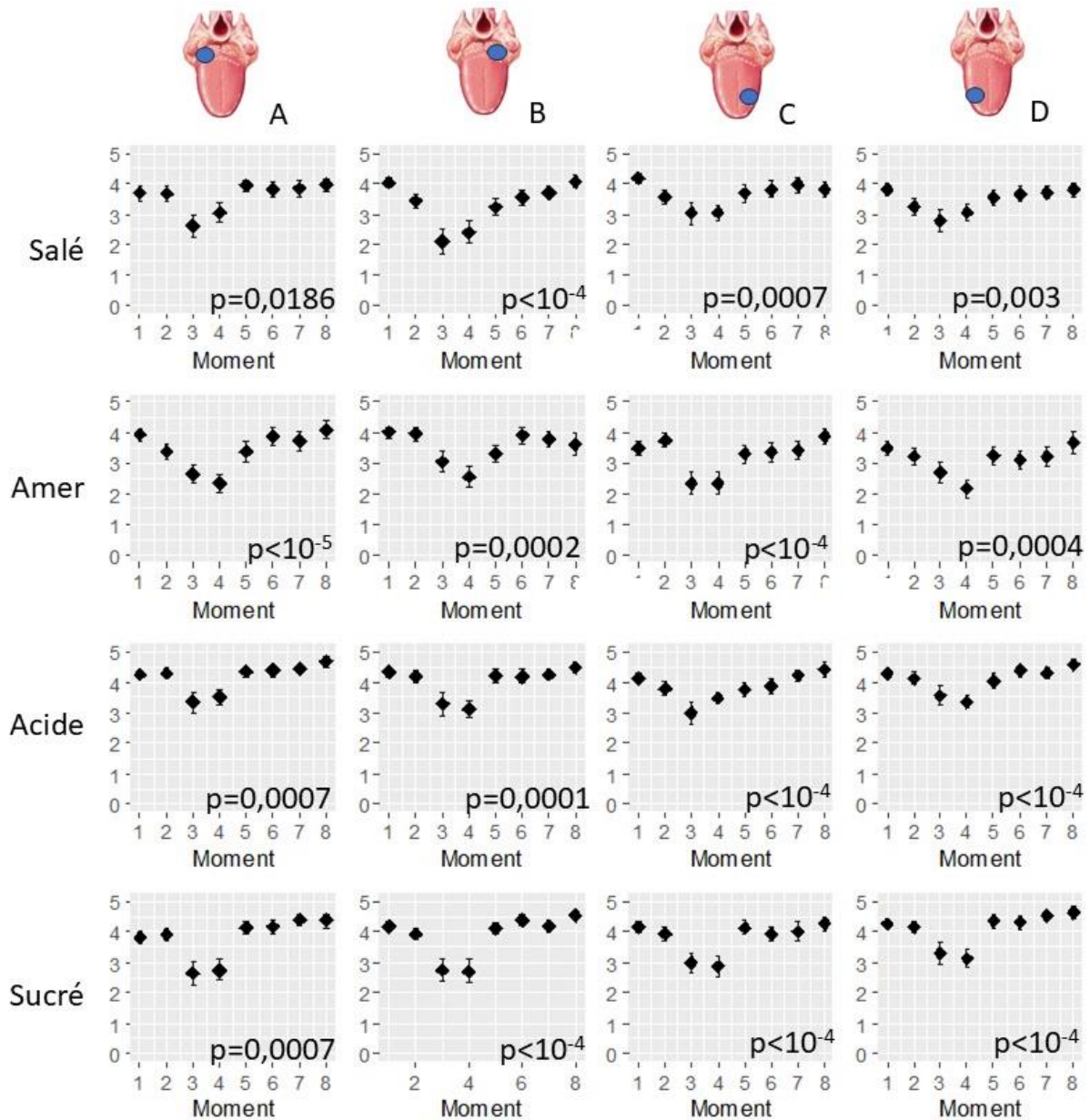


Figure 40: évolution de l'intensité des saveurs en fonction du moment de l'évaluation et selon la localisation sur la langue

La valeur de p, sur chaque graphique est le résultat de la modélisation de l'intensité de la saveur évaluée sur une échelle de Likert de 0 à 5 pour les différents moments de l'évaluation.

DISCUSSION

Les résultats de cette étude mettaient en lumière la fréquence et l'intensité des altérations gustatives chez les patients atteints de CVADS. Nous avons montré qu'une atteinte gustative, évaluée de manière subjective (questionnaire) et objective (identification et intensité des saveurs) existait déjà chez certains patients avant le traitement du cancer, ce qui est retrouvé chez d'autres auteurs (43, 259). Les deux modalités (subjective et objective) étaient surtout atteintes lors de la radiothérapie ou de la radiochimiothérapie (moments 3 et 4), là aussi concordant avec la littérature (53, 54, 75, 223, 241, 260-263) puis un retour au niveau de base au-delà de 6 mois après la fin du traitement. Enfin l'atteinte était significativement plus importante en base de langue qu'en pointe de langue. Cette dernière donnée s'explique probablement par deux raisons. D'une part, l'oropharynx étant la localisation de cancer la plus fréquente (à égalité avec la cavité orale), une dose d'irradiation plus importante était délivrée sur l'oropharynx. D'autre part, une épargne de la pointe de langue et du bloc incisivocanin était réalisée en modulation d'intensité par les radiothérapeutes dans les cancers en dehors de ceux de

la cavité orale, donc une moindre dose en pointe de langue était délivrée. Chen et al. (264) avaient ainsi montré qu'une irradiation de la langue à plus de 50Gy était un facteur de risque de dysgueusie. De plus, l'atteinte prolongée (plus d'une année) du goût (de manière subjective et objective) qui est constatée dans notre étude l'était aussi pour d'autres auteurs (30, 224). Enfin, bien que le manque de puissance lié à notre effectif n'ait pas permis de vérifier si le type de traitement avait un impact significatif sur les altérations par saveur et par localisation, la tendance observée entre le type de traitement et les réponses à la question 14 de l'EORTC HN35 ($p=0,0360$) suggère une possible relation. De plus, le fait que la dysgueusie objective et subjective était principalement présente aux moments 3 et 4 (milieu et fin de l'irradiation) renforce cette hypothèse d'imputabilité du traitement. Kamprad et al. (260) et Ogama et al. (265) avaient ainsi montré une altération des seuils de reconnaissance des saveurs débutant à 20Gy et maximale de 40 à 60Gy. Chez Kamprad et al. (260), les seuils de perception des saveurs étaient bien plus altérés si toute la langue était irradiée et la récupération variait selon le volume lingual irradié (8 semaines en moyenne si les deux tiers de la langue était irradiée et 6 mois si toute langue était irradiée).

Globalement, la perception de toutes les saveurs était touchée dans notre étude. D'autres auteurs avaient obtenu des résultats identiques. Certains avaient même montré une atteinte différentielle des saveurs. Ainsi, Baharvand et al. (266), chez 22 patients avec un CVADS irradié, avaient montré une différence de temporalité des atteintes sensorielles (d'abord les saveurs salée et amère puis acide puis sucrée) sur des tests de seuil de perception des saveurs. Chez Conger et al. (267), l'amertume et l'acidité étaient les premières saveurs touchées (dès 2.4 et 4 Gy respectivement). Chez Mossman et al. (225) et Fernando et al. (268), c'était surtout l'amertume et le salé qui étaient touchés. Pour Kamprad et al. (260), toutes les saveurs étaient impactées dès 20Gy d'irradiation. Maes et al. (224) avaient montré une atteinte des saveurs amère, salée, sucrée avant tout traitement (mais pas acide) puis de toutes les saveurs durant l'irradiation (par ordre décroissant d'atteinte : amer, salé, sucré, acide). Mirza et al. (63) n'avaient pas montré de différences significative d'atteinte gustative dans le temps mais leur effectif était réduit (8 patients avec un CVADS) et les seuils de reconnaissance des saveurs à la limite de la significativité. Ruo-Reda et al. (34) avaient réalisé une revue de littérature sur l'impact de l'irradiation sur le goût. Si les résultats étaient parfois discordants d'une étude à l'autre, une atteinte plus fréquente de l'amertume, l'acidité et du salé et moindre du sucré était constatée.

La concordance observée dans notre étude entre l'évaluation gustative subjective (question EORTC HN35 14) et objective (reconnaissance et intensité), principalement dans les moments 3 et 4, ce qui avait également été par Maes (224) et Baharvand et al. (266). Plusieurs auteurs avaient toutefois noté des discordances entre réponses subjectives et objectives (17, 31, 32, 34, 269) dans un sens (atteinte subjective majeure rapportée avec absence d'anomalie dans les tests objectifs) comme dans l'autre (modifications majeures des seuils de reconnaissance ou d'intensité dans les tests objectifs avec retentissement mineur subjectif rapporté).

Il est important de reconnaître que les altérations gustatives n'étaient pas seulement physiologiques, mais qu'elles pouvaient également avoir des répercussions psychologiques et sociales importantes. Les patients pouvaient ressentir de la frustration, de la tristesse ou de la colère face à la perte de plaisir liée à l'alimentation, ou des difficultés dans les relations sociales (ex. perte de perception des odeurs corporelles) ou liées à l'environnement (ex. perte de perception des marqueurs olfactifs de danger), ce qui pouvait affecter leur qualité de vie globale. Il est donc essentiel de fournir un soutien psychologique et émotionnel aux patients pour les aider à faire face à ces défis. S'il est essentiel de prévenir les patients de l'impact des traitements sur le goût, en particulier la radiothérapie et la radiochimiothérapie, peu d'études avaient été réalisées sur les moyens de prévenir et/ou traiter la dysgueusie. Néanmoins Buntzel et al. avaient montré dans une étude randomisée vs placebo que l'amifostine réduisait significativement la dysgueusie (38.1% vs 46.4%, $p=0.04$) (257). Najafzade et al. (270) dans une étude prospective randomisée comparant la supplémentation préventive en sulfate de zinc (20 patients) vs placebo (15 patients) avaient montré une moindre atteinte de la perception des saveurs dans le groupe supplémenté, de même que Buntzel et al. avec une supplémentation par sélénium (271). Jham et al. (272) avaient montré dans une étude randomisée que le bethanechol vs placebo ne prévenait pas mieux la dysgueusie ($p=0.26$).

Enfin, le fait que quatre des cinq interactions significatives entre modifications de la composition salivaire et résultats des tests sensoriels d'intensité et de reconnaissance concernaient le MDA est intrigant. Aucune donnée de la littérature n'existe à ce sujet.

Il est important de reconnaître les limites de notre étude, qui découlaient principalement de la petite taille de l'échantillon de population. En effet, il avait été difficile de motiver les patients pour participer à de longues séances de tests répétés, ce qui avait entraîné un taux d'attrition élevé. Enfin, les tests statistiques menés pour évaluer le lien entre la reconnaissance et le moment (Figure 39) ou le moment et l'intensité de la saveur (Figure 40), partaient du principe d'une indépendance entre chaque valeur car les intervalles de temps sont suffisamment longs (trois mois) pour que les évaluations successives soient relativement indépendantes (273, 274).

CONCLUSION

Les altérations gustatives subjectives et objectives observées chez les patients de notre étude atteints de CVADS étaient des complications fréquentes et prolongées. La reconnaissance et l'évaluation d'intensité de toutes les saveurs étaient soit significativement abaissées ($p \leq 0.005$), soit montraient une tendance à l'abaissement ($0.05 \leq p < 0.005$, plus fréquemment en cas de radiothérapie ou de radiochimiothérapie).

Une évaluation précoce et une prise en charge adéquate de ces altérations sont essentielles pour améliorer la qualité de vie des patients et favoriser une meilleure tolérance au traitement. Des recherches supplémentaires sont nécessaires pour mieux comprendre les mécanismes sous-jacents aux altérations gustatives chez les patients atteints de CVADS et pour développer des stratégies de gestion de ces complications plus efficaces.

DISCUSSION - SYNTHESE

This part of the manuscript provides a synthesis of the discussions from the articles that were written, analyzing the incidence of metallic taste (MT) in the literature, exploring the causes of malnutrition in head and neck cancer (HNC), determining the frequency and impact of MT, testing hypotheses on potential causes of MT, and evaluating the effectiveness of a putative antidote.

1. Which concept is adapted to talk about Metallic Taste?

MT is a common and distressing symptom reported by patients undergoing treatment for HNC. MT is often described as a persistent, unpleasant sensation that can lead to reduced appetite and poor nutritional status (275, 276). Our research work supports the idea that MT includes gustatory, olfactory, and overall flavor components, significantly impacting patients' quality of life. Several studies, including Lawless et al. (16) and Omur-Ozbek et al. (2011), have identified metallic salts such as iron, calcium, and magnesium as primary contributors to MT. The study we led in 120 healthy volunteers with open (n=60) and closed (n=60) nose showed that MT was primarily a smell but that the perception of ferrous sulfate in the oral cavity remained in 68.3% of the patients, however with a significantly lower intensity, when the nose was closed. Therefore, the metallic perception is primarily a combination of retro-olfactory and gustatory sensations. The involvement of oral chemesthetic or somesthetic perception remains uncertain, as we were unable to differentiate it from the gustatory component in our protocol. Overall, this thesis work concludes that MT is a flavor perception involving at least olfactory and gustatory components.

2. Longitudinal global impact of treatments on nutritional status, saliva and taste

Our manuscript on the “evolution of taste recognition and intensity in different regions of the tongue in patients with head and neck cancer” (chapter 7 on the Results section) showed that cancer treatments, such as radiochemotherapy and radiotherapy, had profound and significant impacts on taste perception confirming what is often described in the literature (53, 54, 75, 223, 241, 260-263), including the development of MT. These treatments are known to damage taste buds and salivary glands (decrease of salivary flow), leading to altered taste sensations and decreased taste sensitivity (34, 65, 276), as it was shown in our own data (chapter 6/7 in the Results section of this manuscript). We also showed that there was a significant decrease of oral intakes and weight, especially during and just after the treatment phase, despite some of the patients (every patient with radiotherapy and radiochemotherapy) had an enteral nutrition support. As there is no evidence of prophylactic or therapeutic treatment of taste impairment, an enteral nutritional support therefore seems necessary until the taste natural recovery.

The decline in taste sensitivity and the increase in MT prevalence have significant nutritional consequences. Patients often experience reduced appetite, leading to decreased food intake and subsequent weight loss, which can further complicate their overall health (63, 275, 276). The long-term effect on salivary glands may explain a part of long-term taste alterations, as supported by Ruo Redda et al. (34) and Hong et al. (65), as the last of the taste symptoms and the salivary lack were concomitant in our series. Even if a nutritional support was provided to all of our patients, more nutritional interventions are probably crucial to mitigate these effects and improve patients' quality

of life (26, 63, 275, 276), such as a closer follow-up of oral intakes with a better adaptation of enteral nutrition and/or an increase of physical activity to increase the lean body mass. Finally, there was a slow recovery of taste abilities, oral intakes, and weight, during the 6 last months of the one-year-follow-up.

3. Metallic Taste

27.2% of our patients complained about MT, especially during the radiotherapy or the radiochemotherapy, which was concordant with our SLR (29%). A significant decline in quality of life related to taste because of MT was also demonstrated.

Understanding the underlying causes of MT is crucial for developing effective treatments and interventions. Our research in the literature identified two main hypotheses that could trigger MT in HNC patients. On one hand, oral lipoperoxidation (lipid peroxidation produces specific compounds responsible for the metallic taste sensation (81, 129)) and on the other hand, release of inhibition of the facial nerve on the glossopharyngeal nerve (123)

Other hypotheses (proteomic, with a specific MT receptor, or galvanism) were not tested as there wasn't enough proves of concept.

In our different studies and reports, we showed that an oral lipoperoxidation occurred during and after the HNC treatment but we failed to prove that it could explain MT. Indeed, not only there was no significant differences of salivary MDA concentrations according to the declared presence or absence of MT but the means of salivary MDA even were lower in the MT group. Other salivary markers such as protein concentration, antioxidant properties, catalase activity, and superoxide dismutase activity showed no significant link to MT. These results suggest that other factors may contribute to MT development.

Concerning the second hypothesis, we also showed that there was no release of inhibition of the facial nerve on the glossopharyngeal nerve. Indeed, even if the gustatory function was altered for all tastes during the treatment phase, taste recognition capabilities and perceived intensity were lower in the territory of the glossopharyngeal nerve (tongue base) than in the one of the facial nerve (tip of the tongue).

We also showed that MT was significantly linked with the mucositis ($p=0.027$) but not with neither candidiasis ($p=0.38$) nor with salivary flow ($p=0.63$). The link with mucositis was previously exposed in the circumstances of MT (chapter 4.1 of the "Etat de l'Art" section. But mucositis is also a symptom of inflammation, it is not by itself a potential cause of MT.

Lactoferrin complexes iron, reducing its concentration in saliva and thus limiting undesirable oral oxidation reactions (137). A lactoferrin mouthwash helped to alleviate at least partially MT in 12 out of the 19 MT occurrence (efficacy rate: 63.2%). This is one piece of evidence that oxidation is involved in the MT onset.

4. Perspectives

Other directions to explore the origine of MT may be:

- pH. On one hand, an acidification of the mouth occurs after the treatment of HNC (277). On the other hand, Epke et al. showed that electricity in the mouth caused an hydrolysis with

the production of H⁺ that could lead to a MT perception (12). We didn't assess the pH in our patients

- the oral microbiota which changes during HNC (as a cause or a consequence?) (278). Radiotherapy significantly reduced the quality and quantity of saliva in head and neck cancer patients. These reductions were associated with increased *C. albicans* counts (279) and decreased diversity of microbiome (280), especially during oral mucositis (281). Could these changes lead to MT ?
- Other products of oral lipoperoxidation could lead to the formation of volatile compounds with very low detection thresholds (128, 129), such as certain aldehydes or ketones (see **Erreur ! Source du renvoi introuvable.**). Maybe those volatile markers of MT should be looked at instead of MDA, which is a general marker of lipoperoxidation. It should have been possible to evaluate the release of these volatile compounds by measuring nasal effluvia during oral consumption. However such a methodology should be adapted to be implemented to a population of cancer patients. Basically, the hypothesis of lipoperoxidation still holds but we may not have considered the right target compounds. MDA showed off a lipoperoxidation during the treatment phase but was not appropriate to discriminate on MT.
- MT could also be a symptom of oral allodynia induced by radiotherapy and chemotherapy, as it can occur in the burning mouth syndrome.
- Finally we found a significant link between oral mucositis and MT. Mucositis is a wound of the oral mucosa through which a small quantity of hemoglobin could flow and/or on which metalloproteins could act to heal the wound.

CONCLUSION

Ce travail de recherche a permis de répondre à plusieurs questions :

Tout d'abord, la revue de la littérature a permis de déterminer que 29% des patients traités pour un cancer rapportaient un goût métallique. Ce sujet est largement sous-traité dans la littérature car seulement 22 articles en mentionnant l'incidence ont pu être identifiés dans notre méta-analyse. Ensuite, dans l'étude GOUTMETALSAIN, nous avons montré que le goût métallique est une mauvaise appellation. En effet, nous devrions utiliser le terme « flaveur » métallique dans la mesure où l'odorat a un rôle majeur, mais non exclusif, dans sa perception. Néanmoins, comme l'usage du terme « goût métallique » est consacré, nous l'avons utilisé tout au long de ce travail.

Enfin, dans l'étude TORCAD, nous avons montré :

- Que le « goût » métallique touchait 27.2% de nos patients atteints d'un cancer des voies aérodigestives supérieures, confirmant l'incidence estimée sur la base des données de la littérature en oncologie générale.
- Que l'intensité était modérée à sévère chez 42.9% des personnes en souffrant
- Que le goût métallique apparaissait surtout aux périodes 3 et 4, correspondant au milieu et à la fin de la radiothérapie ou de la radiochimiothérapie, avec un lien significatif avec la présence ou non d'une mucite orale.
- Que le goût métallique avait un impact significatif sur la qualité de vie liée au goût mais pas sur l'échelle visuelle analogique des ingestas ni sur le poids. Toutefois chez les patients traités par radiothérapie ou radiochimiothérapie, une gastrostomie prophylactique était systématique, ce qui constitue une variable confondante majeure.
- Que l'hypothèse de la levée d'inhibition du nerf facial sur le nerf glossopharyngien n'était pas valide
- Que l'hypothèse de la lipopéroxydation avec un taux supérieur de MDA en cas de MT n'était pas valide
- Que le goût métallique pouvait être atténué par le bain de bouche de lactoferrine dans 63% des cas (de manière complète dans 16% et de manière partielle dans 47%).

L'étude a révélé que 27,2% des patients souffrent de « goût » métallique, principalement durant les phases critiques de radiothérapie et de radiochimiothérapie, ce qui entraîne une diminution de l'appétit et une perte de poids. Bien que l'hypothèse de la lipopéroxydation et de la levée d'inhibition du nerf facial n'ait pas été confirmée, une découverte importante a été l'efficacité partielle du bain de bouche de lactoferrine pour atténuer le « goût » métallique. Ces résultats soulignent l'importance de mieux comprendre et de gérer le « goût » métallique, symptôme qui a touché plus d'un quart de nos patients, pour améliorer leur qualité de vie. Les interventions nutritionnelles ou culinaires adaptées sont essentielles pour atténuer les effets délétères des traitements anticancéreux sur la perception du goût et l'état nutritionnel global.

Pour aller plus loin dans la recherche des causes du goût métallique dans les cancers des voies aérodigestives supérieures, il existe plusieurs perspectives que nous n'avons pas entièrement explorées dans cette étude : l'acidification de la cavité buccale après le traitement, les modifications du microbiote buccal, des arômes issus de la lipopéroxydation, la mucite (hémoglobine, métalloprotéines), l'allodynie orale induite par la radiothérapie et la chimiothérapie.

Au total, auront été produits : un chapitre de livre, un article publié dans une revue internationale avec comité de lecture, un article publié dans une revue nationale avec comité de lecture, 4 articles soumis à une revue internationale à comité de lecture, un articles à soumettre à une revue

internationale à comité de lecture , un article à soumettre à une revue nationale à comité de lecture, 2 posters présentés en congrès de société savante nationale, 2 posters présentés en congrès de société savante internationale (avec soutenance orale des posters).

BIBLIOGRAPHIE

1. Thomas-Danguin T. Flavor. In: M. D. Binder NH, & U. Windhorst editor. *Encyclopedia of Neuroscience*: Springer-Verlag Berlin and Heidelberg GmbH & Co. K; 2009. p. 1580-2.
2. De Conno F, Ripamonti C, Sbanotto A, Ventafridda V. Oral complications in patients with advanced cancer. *Journal of palliative care*. 1989;5(1):7-15.
3. Hovan AJ, Williams PM, Stevenson-Moore P, Wahlin YB, Ohrn KE, Elting LS, et al. A systematic review of dysgeusia induced by cancer therapies. *Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer*. 2010;18(8):1081-7.
4. Heckel M, Stiel S, Ostgathe C. Smell and taste in palliative care: a systematic analysis of literature. *Eur Arch Otorhinolaryngol*. 2015;272(2):279-88.
5. Spotten LE, Corish CA, Lorton CM, Ui Dhuibhir PM, O'Donoghue NC, O'Connor B, et al. Subjective and objective taste and smell changes in cancer. *Ann Oncol*. 2017;28(5):969-84.
6. van Oort S, Kramer E, de Groot JW, Visser O. Taste alterations and cancer treatment. *Current opinion in supportive and palliative care*. 2018;12(2):162-7.
7. Boltong A, Keast R. The influence of chemotherapy on taste perception and food hedonics: a systematic review. *Cancer Treat Rev*. 2012;38(2):152-63.
8. Coa KI, Epstein JB, Ettinger D, Jatoi A, McManus K, Platek ME, et al. The impact of cancer treatment on the diets and food preferences of patients receiving outpatient treatment. *Nutrition and cancer*. 2015;67(2):339-53.
9. Belqaid K, Orrevall Y, McGreevy J, Mansson-Brahme E, Wismer W, Tishelman C, et al. Self-reported taste and smell alterations in patients under investigation for lung cancer. *Acta Oncol*. 2014;53(10):1405-12.
10. Brisbois TD, de Kock IH, Watanabe SM, Baracos VE, Wismer WV. Characterization of chemosensory alterations in advanced cancer reveals specific chemosensory phenotypes impacting dietary intake and quality of life. *Journal of pain and symptom management*. 2011;41(4):673-83.
11. Buiret G, Thomas-Danguin T, Feron G. Metallic taste prevalence in patients treated for cancer: a systematic literature review and meta-analysis. *Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer*. 2022;30(7):5691-702.
12. Epke EM, McClure ST, Lawless HT. Effects of Nasal Occlusion and Oral Contact on Perception of Metallic Taste from Metal Salts. *Food quality and preference*. 2009;20(2):133-7.
13. Kveton JF, Bartoshuk LM. The effect of unilateral chorda tympani damage on taste. *Laryngoscope*. 1994;104(1 Pt 1):25-9.
14. Lehman CD, Bartoshuk LM, Catalanotto FC, Kveton JF, Lowlicht RA. Effect of anesthesia of the chorda tympani nerve on taste perception in humans. *Physiology & behavior*. 1995;57(5):943-51.
15. Yanagisawa K, Bartoshuk LM, Catalanotto FA, Karrer TA, Kveton JF. Anesthesia of the chorda tympani nerve and taste phantoms. *Physiology & behavior*. 1998;63(3):329-35.
16. Lawless HT, Stevens DA, Chapman KW, Kurtz A. Metallic taste from electrical and chemical stimulation. *Chemical senses*. 2005;30(3):185-94.
17. I I, Renken RJ, Gietema JA, Slart R, Mensink MGJ, Lefrandt JD, et al. Changes in taste and smell function, dietary intake, food preference, and body composition in testicular cancer patients treated with cisplatin-based chemotherapy. *Clin Nutr*. 2017;36(6):1642-8.
18. Hirai R, Takao K, Onoda K, Kokubun S, Ikeda M. Patients with phantogeusia show increased expression of T2R taste receptor genes in their tongues. *Ann Otol Rhinol Laryngol*. 2012;121(2):113-8.
19. Buiret G, Riffard G. Causes et traitements de la dénutrition et des altérations sensorielles chez les patients atteints d'un cancer des voies aérodigestives supérieures. *Cahiers de Nutrition et de Diététique*. 2021;56(4):249-59.
20. Leone N, Voirin N, Roche L, Binder-Foucard F, Woronoff AS, Delafosse P, et al. Projection de l'incidence et de la mortalité par cancer en France métropolitaine en 2015 - Rapport technique 2015 [Available from: <http://www.e-cancer.fr/Expertises-et-publications/Catalogue-des-publications/Projection-de-l-incidence-et-de-la-mortalite-par-cancer-en-France-metropolitaine-en-2015-Rapport-technique>].

21. Hebuterne X, Lemarie E, Michallet M, de Montreuil CB, Schneider SM, Goldwasser F. Prevalence of malnutrition and current use of nutrition support in patients with cancer. *JPEN J Parenter Enteral Nutr.* 2014;38(2):196-204.
22. Langius JA, van Dijk AM, Doornaert P, Kruizenga HM, Langendijk JA, Leemans CR, et al. More than 10% weight loss in head and neck cancer patients during radiotherapy is independently associated with deterioration in quality of life. *Nutrition and cancer.* 2013;65(1):76-83.
23. Grossberg AJ, Chamchod S, Fuller CD, Mohamed AS, Heukelom J, Eichelberger H, et al. Association of Body Composition With Survival and Locoregional Control of Radiotherapy-Treated Head and Neck Squamous Cell Carcinoma. *JAMA oncology.* 2016;2(6):782-9.
24. Wang C, Vainshtein JM, Veksler M, Rabban PE, Sullivan JA, Wang SC, et al. Investigating the clinical significance of body composition changes in patients undergoing chemoradiation for oropharyngeal cancer using analytic morphomics. *SpringerPlus.* 2016;5:429.
25. Valentini V, Marazzi F, Bossola M, Micciche F, Nardone L, Balducci M, et al. Nutritional counselling and oral nutritional supplements in head and neck cancer patients undergoing chemoradiotherapy. *Journal of human nutrition and dietetics : the official journal of the British Dietetic Association.* 2012;25(3):201-8.
26. Ravasco P, Monteiro-Grillo I, Marques Vidal P, Camilo ME. Impact of nutrition on outcome: a prospective randomized controlled trial in patients with head and neck cancer undergoing radiotherapy. *Head Neck.* 2005;27(8):659-68.
27. Silander E, Nyman J, Bove M, Johansson L, Larsson S, Hammerlid E. Impact of prophylactic percutaneous endoscopic gastrostomy on malnutrition and quality of life in patients with head and neck cancer: a randomized study. *Head Neck.* 2012;34(1):1-9.
28. Tourvieille L, Buiet G, Finot M, Guillermet A, Combe C. Efficacité et tolérance des gastrostomies percutanées radiologiques en cancérologie des voies aérodigestives supérieures. *Nut Clin Metab.* 2019;33(4):278-82.
29. Couch ME, Dittus K, Toth MJ, Willis MS, Guttridge DC, George JR, et al. Cancer cachexia update in head and neck cancer: Definitions and diagnostic features. *Head Neck.* 2015;37(4):594-604.
30. Molassiotis A, Rogers M. Symptom experience and regaining normality in the first year following a diagnosis of head and neck cancer: a qualitative longitudinal study. *Palliative & supportive care.* 2012;10(3):197-204.
31. McLaughlin L. Taste dysfunction in head and neck cancer survivors. *Oncology nursing forum.* 2013;40(1):E4-13.
32. Bolze MS, Fosmire GJ, Stryker JA, Chung CK, Flipse BG. Taste acuity, plasma zinc levels, and weight loss during radiotherapy: a study of relationships. *Radiology.* 1982;144(1):163-9.
33. Boltong A, Keast R, Aranda S. Experiences and consequences of altered taste, flavour and food hedonics during chemotherapy treatment. *Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer.* 2012;20(11):2765-74.
34. Ruo Redda MG, Allis S. Radiotherapy-induced taste impairment. *Cancer Treat Rev.* 2006;32(7):541-7.
35. I I, Timmermans ER, Renken RJ, Ter Horst GJ, Reyners AK. Metallic Taste in Cancer Patients Treated with Systemic Therapy: A Questionnaire-based Study. *Nutrition and cancer.* 2017;69(1):140-5.
36. McLaughlin L. Taste dysfunction and eating behaviors in survivors of head and neck cancer treatment. *Medsurg nursing : official journal of the Academy of Medical-Surgical Nurses.* 2014;23(3):165-70, 84.
37. Ravasco P. Aspects of taste and compliance in patients with cancer. *Eur J Oncol Nurs.* 2005;9 Suppl 2:S84-91.
38. Aviv JE, Hecht C, Weinberg H, Dalton JF, Urken ML. Surface sensibility of the floor of the mouth and tongue in healthy controls and in radiated patients. *Otolaryngol Head Neck Surg.* 1992;107(3):418-23.
39. Bodin I, Jaghagen EL, Isberg A. Intraoral sensation before and after radiotherapy and surgery for oral and pharyngeal cancer. *Head Neck.* 2004;26(11):923-9.

40. Jaghagen EL, Bodin I, Isberg A. Pharyngeal swallowing dysfunction following treatment for oral and pharyngeal cancer--association with diminished intraoral sensation and discrimination ability. *Head Neck*. 2008;30(10):1344-51.
41. Peterson DE, Boers-Doets CB, Bensadoun RJ, Herrstedt J. Management of oral and gastrointestinal mucosal injury: ESMO Clinical Practice Guidelines for diagnosis, treatment, and follow-up. *Ann Oncol*. 2015;26 Suppl 5:v139-51.
42. Wang JJ, Liang KL, Twu CW, Lin JC, Jiang RS. Olfactory change after intensity-modulated radiotherapy for nasopharyngeal carcinoma. *International forum of allergy & rhinology*. 2015;5(11):1059-62.
43. Irune E, Dwivedi RC, Nutting CM, Harrington KJ. Treatment-related dysgeusia in head and neck cancer patients. *Cancer Treat Rev*. 2014;40(9):1106-17.
44. Sanchez-Lara K, Sosa-Sanchez R, Green-Renner D, Rodriguez C, Laviano A, Motola-Kuba D, et al. Influence of taste disorders on dietary behaviors in cancer patients under chemotherapy. *Nutr J*. 2010;9:15.
45. Volkow ND, Wang GJ, Baler RD. Reward, dopamine and the control of food intake: implications for obesity. *Trends in cognitive sciences*. 2011;15(1):37-46.
46. Chasen MR, Bhargava R. A descriptive review of the factors contributing to nutritional compromise in patients with head and neck cancer. *Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer*. 2009;17(11):1345-51.
47. Gellrich NC, Handschel J, Holtmann H, Kruskemper G. Oral cancer malnutrition impacts weight and quality of life. *Nutrients*. 2015;7(4):2145-60.
48. Agarwal J, Dutta D, Palwe V, Gupta T, Laskar SG, Budrukkar A, et al. Prospective subjective evaluation of swallowing function and dietary pattern in head and neck cancers treated with concomitant chemo-radiation. *Journal of cancer research and therapeutics*. 2010;6(1):15-21.
49. Silver HJ, Dietrich MS, Murphy BA. Changes in body mass, energy balance, physical function, and inflammatory state in patients with locally advanced head and neck cancer treated with concurrent chemoradiation after low-dose induction chemotherapy. *Head Neck*. 2007;29(10):893-900.
50. Murtaza B, Hichami A, Khan AS, Ghiringhelli F, Khan NA. Alteration in Taste Perception in Cancer: Causes and Strategies of Treatment. *Frontiers in physiology*. 2017;8:134.
51. Hong JH, Duncan SE, Dietrich AM, O'Keefe SF, Eigel WN, Mallikarjunan K. Interaction of copper and human salivary proteins. *Journal of agricultural and food chemistry*. 2009;57(15):6967-75.
52. Munoz-Gonzalez C, Feron G, Canon F. Main effects of human saliva on flavour perception and the potential contribution to food consumption. *Proc Nutr Soc*. 2018;77(4):423-31.
53. Sapir E, Tao Y, Feng F, Samuels S, El Naqa I, Murdoch-Kinch CA, et al. Predictors of Dysgeusia in Patients With Oropharyngeal Cancer Treated With Chemotherapy and Intensity Modulated Radiation Therapy. *Int J Radiat Oncol Biol Phys*. 2016;96(2):354-61.
54. Tomita Y, Osaki T. Gustatory impairment and salivary gland pathophysiology in relation to oral cancer treatment. *Int J Oral Maxillofac Surg*. 1990;19(5):299-304.
55. Buglione M, Cavagnini R, Di Rosario F, Maddalo M, Vassalli L, Grisanti S, et al. Oral toxicity management in head and neck cancer patients treated with chemotherapy and radiation: Xerostomia and trismus (Part 2). Literature review and consensus statement. *Critical reviews in oncology/hematology*. 2016;102:47-54.
56. Cho MA, Ko JY, Kim YK, Kho HS. Salivary flow rate and clinical characteristics of patients with xerostomia according to its aetiology. *Journal of oral rehabilitation*. 2010;37(3):185-93.
57. Kuten A, Ben-Aryeh H, Berdicevsky I, Ore L, Szargel R, Gutman D, et al. Oral side effects of head and neck irradiation: correlation between clinical manifestations and laboratory data. *Int J Radiat Oncol Biol Phys*. 1986;12(3):401-5.
58. Baltacioglu E, Akalin FA, Alver A, Deger O, Karabulut E. Protein carbonyl levels in serum and gingival crevicular fluid in patients with chronic periodontitis. *Archives of oral biology*. 2008;53(8):716-22.

59. Crowder SL, Douglas KG, Yanina Pepino M, Sarma KP, Arthur AE. Nutrition impact symptoms and associated outcomes in post-chemoradiotherapy head and neck cancer survivors: a systematic review. *Journal of cancer survivorship : research and practice*. 2018;12(4):479-94.
60. Doty RL, Bromley SM. Effects of drugs on olfaction and taste. *Otolaryngol Clin North Am*. 2004;37(6):1229-54.
61. Ackerman BH, Kasbekar N. Disturbances of taste and smell induced by drugs. *Pharmacotherapy*. 1997;17(3):482-96.
62. Rademacher WMH, Aziz Y, Hielema A, Cheung KC, de Lange J, Vissink A, et al. Oral adverse effects of drugs: taste disorders. *Oral Dis*. 2019.
63. Mirza N, Machtay M, Devine PA, Troxel A, Abboud SK, Doty RL. Gustatory impairment in patients undergoing head and neck irradiation. *Laryngoscope*. 2008;118(1):24-31.
64. Conger AD, Wells MA. Radiation and aging effect on taste structure and function. *Radiation research*. 1969;37(1):31-49.
65. Hong JH, Omur-Ozbek P, Stanek BT, Dietrich AM, Duncan SE, Lee YW, et al. Taste and odor abnormalities in cancer patients. *The journal of supportive oncology*. 2009;7(2):58-65.
66. Nguyen HM, Reyland ME, Barlow LA. Mechanisms of taste bud cell loss after head and neck irradiation. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2012;32(10):3474-84.
67. Yamazaki M, Fujii S, Ochiai A. Reduction of type II taste cells correlates with taste dysfunction after X-ray irradiation in mice. *Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology*. 2010;39(3):212-8.
68. Porter SR, Fedele S, Habbab KM. Taste dysfunction in head and neck malignancy. *Oral Oncol*. 2010;46(6):457-9.
69. Epstein JB, Thariat J, Bensadoun RJ, Barasch A, Murphy BA, Kolnick L, et al. Oral complications of cancer and cancer therapy: from cancer treatment to survivorship. *CA: a cancer journal for clinicians*. 2012;62(6):400-22.
70. Brisbois TD, Hutton JL, Baracos VE, Wismer WV. Taste and smell abnormalities as an independent cause of failure of food intake in patients with advanced cancer--an argument for the application of sensory science. *Journal of palliative care*. 2006;22(2):111-4.
71. Cowart BJ. Taste dysfunction: a practical guide for oral medicine. *Oral Dis*. 2011;17(1):2-6.
72. Shi HB, Masuda M, Umezaki T, Kuratomi Y, Kumamoto Y, Yamamoto T, et al. Irradiation impairment of umami taste in patients with head and neck cancer. *Auris Nasus Larynx*. 2004;31(4):401-6.
73. Cohen J, Wakefield CE, Laing DG. Smell and Taste Disorders Resulting from Cancer and Chemotherapy. *Current pharmaceutical design*. 2016;22(15):2253-63.
74. Bressan V, Stevanin S, Bianchi M, Aleo G, Bagnasco A, Sasso L. The effects of swallowing disorders, dysgeusia, oral mucositis and xerostomia on nutritional status, oral intake and weight loss in head and neck cancer patients: A systematic review. *Cancer Treat Rev*. 2016;45:105-19.
75. Lilja M, Markkanen-Leppanen M, Viitasalo S, Saarilahti K, Lindford A, Lassus P, et al. Olfactory and gustatory functions after free flap reconstruction and radiotherapy for oral and pharyngeal cancer: a prospective follow-up study. *Eur Arch Otorhinolaryngol*. 2018;275(4):959-66.
76. Bjordal K, Hammerlid E, Ahlner-Elmqvist M, de Graeff A, Boysen M, Evensen JF, et al. Quality of life in head and neck cancer patients: validation of the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-H&N35. *J Clin Oncol*. 1999;17(3):1008-19.
77. Wickham RS, Rehwaldt M, Kefer C, Shott S, Abbas K, Glynn-Tucker E, et al. Taste changes experienced by patients receiving chemotherapy. *Oncology nursing forum*. 1999;26(4):697-706.
78. I I, Renken RJ, Ter Horst GJ, Reyners AK. Metallic taste in cancer patients treated with chemotherapy. *Cancer Treat Rev*. 2015;41(2):179-86.
79. Reith AJM, Spence C. The Mystery of "Metal Mouth" in Chemotherapy. *Chemical senses*. 2020;45(2):73-84.

80. Lawless HT, Schlake S, Smythe J, Lim J, Yang H, Chapman K, et al. Metallic taste and retronasal smell. *Chemical senses*. 2004;29(1):25-33.
81. Omur-Ozbek P, Dietrich AM. Retronasal perception and flavour thresholds of iron and copper in drinking water. *Journal of water and health*. 2011;9(1):1-9.
82. Omur-Ozbek P, Dietrich AM, Duncan SE, Lee Y. Role of lipid oxidation, chelating agents, and antioxidants in metallic flavor development in the oral cavity. *Journal of agricultural and food chemistry*. 2012;60(9):2274-80.
83. Saral Y, Coskun BK, Ozturk P, Karatas F, Ayar A. Assessment of salivary and serum antioxidant vitamins and lipid peroxidation in patients with recurrent aphthous ulceration. *Tohoku J Exp Med*. 2005;206(4):305-12.
84. Guillemaud A, Pouillart P, Labrosse-Canat H, Gautheron L, Buiret G. Proposition d'un référentiel national de prise en charge des modifications des capacités olfactive, gustative et/ou de la déglutition dans les cancers des voies aérodigestives supérieures. *Nut Clin Metab*. 2018;32(1):49-56.
85. van der Molen L, van Rossum MA, Burkhead LM, Smeele LE, Rasch CR, Hilgers FJ. A randomized preventive rehabilitation trial in advanced head and neck cancer patients treated with chemoradiotherapy: feasibility, compliance, and short-term effects. *Dysphagia*. 2011;26(2):155-70.
86. Hutcheson KA, Bhayani MK, Beadle BM, Gold KA, Shinn EH, Lai SY, et al. Eat and exercise during radiotherapy or chemoradiotherapy for pharyngeal cancers: use it or lose it. *JAMA otolaryngology-- head & neck surgery*. 2013;139(11):1127-34.
87. Ahlberg A, Engstrom T, Nikolaidis P, Gunnarsson K, Johansson H, Sharp L, et al. Early self-care rehabilitation of head and neck cancer patients. *Acta Otolaryngol*. 2011;131(5):552-61.
88. Support AFdSOd. Dénutrition / réalimentation et cancer (VADS)2018. Available from: https://www.afsos.org/wp-content/uploads/2017/12/D%C3%A9nutrition_r%C3%A9alimentation-et-cancer_AFSOS.pdf.
89. Thibault R, Goujon N, Le Gallic E, Clairand R, Sebillé V, Vibert J, et al. Use of 10-point analogue scales to estimate dietary intake: a prospective study in patients nutritionally at-risk. *Clin Nutr*. 2009;28(2):134-40.
90. Hébuterne X, Senesse P. Nutrition chez le patient adulte atteint de cancer : Recommandations professionnelles de la Société Francophone Nutrition Clinique et Métabolisme (SFNEP). *Nut Clin Metab*. 2012;26(4):149-302.
91. Lonbro S, Dalgas U, Primdahl H, Johansen J, Nielsen JL, Overgaard J, et al. Lean body mass and muscle function in head and neck cancer patients and healthy individuals--results from the DAHANCA 25 study. *Acta Oncol*. 2013;52(7):1543-51.
92. Lonbro S, Dalgas U, Primdahl H, Johansen J, Nielsen JL, Aagaard P, et al. Progressive resistance training rebuilds lean body mass in head and neck cancer patients after radiotherapy--results from the randomized DAHANCA 25B trial. *Radiother Oncol*. 2013;108(2):314-9.
93. Rogers LQ, Courneya KS, Robbins KT, Malone J, Seiz A, Koch L, et al. Physical activity and quality of life in head and neck cancer survivors. *Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer*. 2006;14(10):1012-9.
94. Samuel SR, Maiya GA, Babu AS, Vidyasagar MS. Effect of exercise training on functional capacity & quality of life in head & neck cancer patients receiving chemoradiotherapy. *The Indian journal of medical research*. 2013;137(3):515-20.
95. Blanchard CM, Baker F, Denniston MM, Courneya KS, Hann DM, Gesme DH, et al. Is absolute amount or change in exercise more associated with quality of life in adult cancer survivors? *Preventive medicine*. 2003;37(5):389-95.
96. Capozzi LC, Nishimura KC, McNeely ML, Lau H, Culos-Reed SN. The impact of physical activity on health-related fitness and quality of life for patients with head and neck cancer: a systematic review. *British journal of sports medicine*. 2016;50(6):325-38.
97. Lonkvist CK, Lonbro S, Vinther A, Zerahn B, Rosenbom E, Primdahl H, et al. Progressive resistance training in head and neck cancer patients during concomitant chemoradiotherapy -- design of the DAHANCA 31 randomized trial. *BMC Cancer*. 2017;17(1):400.

98. McNeely ML, Parliament MB, Seikaly H, Jha N, Magee DJ, Haykowsky MJ, et al. Predictors of adherence to an exercise program for shoulder pain and dysfunction in head and neck cancer survivors. *Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer*. 2012;20(3):515-22.
99. McNeely ML, Parliament M, Courneya KS, Seikaly H, Jha N, Scrimger R, et al. A pilot study of a randomized controlled trial to evaluate the effects of progressive resistance exercise training on shoulder dysfunction caused by spinal accessory neurapraxia/neurectomy in head and neck cancer survivors. *Head Neck*. 2004;26(6):518-30.
100. McNeely ML, Parliament MB, Seikaly H, Jha N, Magee DJ, Haykowsky MJ, et al. Effect of exercise on upper extremity pain and dysfunction in head and neck cancer survivors: a randomized controlled trial. *Cancer*. 2008;113(1):214-22.
101. Bauml J, Kim J, Zhang X, Aggarwal C, Cohen RB, Schmitz K. Unsupervised exercise in survivors of human papillomavirus related head and neck cancer: how many can go it alone? *Journal of cancer survivorship : research and practice*. 2017;11(4):462-8.
102. Gerhard GS. Heme as a Taste Molecule. *Current Nutrition Reports*. 2020;9(3):290-5.
103. Gad A, Jayaram SH. Processing of Carbonated Beer by Pulsed Electric Fields. *Ieee Transactions on Industry Applications*. 2015;51(6):4759-65.
104. Povey MJW. Applications of ultrasonics in food science - novel control of fat crystallization and structuring. *Current Opinion in Colloid & Interface Science*. 2017;28:1-6.
105. Pena-Gonzalez E, Alarcon-Rojo AD, Garcia-Galicia I, Carrillo-Lopez L, Huerta-Jimenez M. Ultrasound as a potential process to tenderize beef: Sensory and technological parameters. *Ultrasonics Sonochemistry*. 2019;53:134-41.
106. Quiroz-Moreno AL, Fontes-Gagiola R, Rouzaud-Sandez O, Vidal-Quintanar RL. Evaluation of sensory rancidity of corn chips from nixtamalized dry corn masa produced at commercial level in Mexico. *Cyta-Journal of Food*. 2013;11:15-21.
107. Riera CE, Vogel H, Simon SA, le Coutre J. Artificial sweeteners and salts producing a metallic taste sensation activate TRPV1 receptors. *American journal of physiology Regulatory, integrative and comparative physiology*. 2007;293(2):R626-34.
108. Pereira CTM, Pereira DM, Bolini HMA. Influence of a prebiotic and natural sweeteners on the sensory profile of skyr yogurt with mango pulp. *Journal of food science*. 2021;86:2626-39.
109. Tan VWK, Wee MSM, Tomic O, Forde CG. Temporal sweetness and side tastes profiles of 16 sweeteners using temporal check-all-that-apply (TCATA). *Food Res Int*. 2019;121:39-47.
110. Tavares ER, Esmerino EA, Santos VD, da Silva ACL, Bolini HMA. Dynamic aspects of salt reduction in tomato sauce by use of flavor enhancers and a bitter blocker. *Food Science and Technology International*. 2020;26(6):549-59.
111. Ueno HM, Shiota M, Ueda N, Isogai T, Kobayashi T. Iron-Lactoferrin Complex Reduces Iron-Catalyzed Off-flavor Formation in Powdered Milk with Added Fish Oil. *Journal of Food Science*. 2012;77(8):C853-C8.
112. Setyawati R, Dwiyantri H, Aini N. The Effect of Fe-Fortification on Chemical and Sensory Properties of Cassava Biscuits Supplemented with Fish and Tempeh Flours. *Agritech*. 2018;38(4):396-403.
113. Stevens DA, Smith RF, Lawless HT. Multidimensional scaling of ferrous sulfate and basic tastes. *Physiol Behav*. 2006;87(2):272-9.
114. Hong JH, Kim KO. Operationally defined solubilization of copper and iron in human saliva and implications for metallic flavor perception. *Eur Food Res Technol*. 2011;233(6):973-83.
115. Lim J, Lawless HT. Oral sensations from iron and copper sulfate. *Physiology & behavior*. 2005;85(3):308-13.
116. Mirlohi S, Dietrich AM, Duncan SE. Age-associated variation in sensory perception of iron in drinking water and the potential for overexposure in the human population. *Environmental science & technology*. 2011;45(15):6575-83.
117. Doty RL, Shah M, Bromley SM. Drug-induced taste disorders. *Drug safety*. 2008;31(3):199-215.

118. Stevens DA, Baker D, Cutroni E, Frey A, Pugh D, Lawless HT. A direct comparison of the taste of electrical and chemical stimuli. *Chemical senses*. 2008;33(5):405-13.
119. Ayres S, Jr. Sore mouth caused by electrogalvanic current. *Journal of the Royal Society of Medicine*. 1984;77(8):708-9.
120. Sutow EJ, Maillet WA, Taylor JC, Hall GC. In vivo galvanic currents of intermittently contacting dental amalgam and other metallic restorations. *Dental materials : official publication of the Academy of Dental Materials*. 2004;20(9):823-31.
121. Bergman M, Ginstrup O, Nilner K. Potential and polarization measurements in vivo of oral galvanism. *Scandinavian journal of dental research*. 1978;86(2):135-45.
122. Logan HL, Bartoshuk LM, Fillingim RB, Tomar SL, Mendenhall WM. Metallic taste phantom predicts oral pain among 5-year survivors of head and neck cancer. *Pain*. 2008;140(2):323-31.
123. Bartoshuk LM, Catalanotto F, Hoffman H, Logan H, Snyder DJ. Taste damage (otitis media, tonsillectomy and head and neck cancer), oral sensations and BMI. *Physiology & behavior*. 2012;107(4):516-26.
124. Bartoshuk LM. History of taste research. *Handbook of Perception Volume 6A*. 2012;1.
125. Hettinger TP, Myers WE, Frank ME. Role of olfaction in perception of non-traditional 'taste' stimuli. *Chem senses*. 1990;15(6):755-60.
126. Skinner M, Lim M, Tarrega A, Ford R, Linforth R, Thomas A, et al. Investigating the oronasal contributions to metallic perception. *International Journal of Food Science and Technology*. 2017;52(6):1299-306.
127. Epke EM, Lawless HT. Retronasal smell and detection thresholds of iron and copper salts. *Physiology & behavior*. 2007;92(3):487-91.
128. Leffingwell JC, Leffingwell D. GRAS flavor chemicals-detection thresholds. *Perfumer Flavorist*. 1991;16(1):1-19.
129. Glindemann D, Dietrich A, Staerk HJ, Kusch P. The two odors of iron when touched or pickled: (skin) carbonyl compounds and organophosphines. *Angew Chem Int Ed Engl*. 2006;45(42):7006-9.
130. Lubran MB, Lawless HT, Lavin E, Acree TE. Identification of metallic-smelling 1-octen-3-one and 1-nonen-3-one from solutions of ferrous sulfate. *Journal of agricultural and food chemistry*. 2005;53(21):8325-7.
131. Piqueras-Fiszman B, Laughlin Z, Miodownik M, Spence C. Tasting spoons: Assessing how the material of a spoon affects the taste of the food. *Food Quality and Preference*. 2012;24(1):24-9.
132. Li I, Renken RJ, Ter Horst GJ, Reyners AK. The palatability of oral nutritional supplements: before, during, and after chemotherapy. *Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer*. 2016;24(10):4301-8.
133. Speck RM, DeMichele A, Farrar JT, Hennessy S, Mao JJ, Stineman MG, et al. Taste alteration in breast cancer patients treated with taxane chemotherapy: experience, effect, and coping strategies. *Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer*. 2013;21(2):549-55.
134. Lindsey AM, Piper BF. Anorexia, serum zinc, and immunologic response in small cell lung cancer patients receiving chemotherapy and prophylactic cranial radiotherapy. *Nutrition and cancer*. 1986;8(4):231-8.
135. Soares HP, Cusnir M, Schwartz MA, Pizzolato JF, Lutzky J, Campbell RJ, et al. Treatment of taste alterations in chemotherapy patients using the "miracle fruit": Preliminary analysis of a pilot study. *J Clin Oncol*. 2010;28(15):e19523.
136. Halyard MY, Jatoi A, Sloan JA, Bearden JD, 3rd, Vora SA, Atherton PJ, et al. Does zinc sulfate prevent therapy-induced taste alterations in head and neck cancer patients? Results of phase III double-blind, placebo-controlled trial from the North Central Cancer Treatment Group (N01C4). *Int J Radiat Oncol Biol Phys*. 2007;67(5):1318-22.
137. Wang A, Duncan SE, Lesser GJ, Ray WK, Dietrich AM. Effect of lactoferrin on taste and smell abnormalities induced by chemotherapy: a proteome analysis. *Food & function*. 2018;9(9):4948-58.

138. Johansson B, Stenman E, Bergman M. Clinical study of patients referred for investigation regarding so-called oral galvanism. *Scandinavian journal of dental research*. 1984;92(5):469-75.
139. Manifar S, Abbassi F, Mirzaii Dizgah I, Khatami R, Esmseil M, Almasi A. Alteration of Lipid Peroxidation and Total Antioxidant Capacity in Patients With Head and Neck Cancers Following Radiotherapy. *J Arch Mil Med*. 2015;3(4):e30431.
140. Agha-Hosseini F, Mirzaii-Dizgah I, Mikaili S, Abdollahi M. Increased salivary lipid peroxidation in human subjects with oral lichen planus. *Int J Dent Hyg*. 2009;7(4):246-50.
141. Kaur J, Politis C, Jacobs R. Salivary 8-hydroxy-2-deoxyguanosine, malondialdehyde, vitamin C, and vitamin E in oral pre-cancer and cancer: diagnostic value and free radical mechanism of action. *Clin Oral Investig*. 2016;20(2):315-9.
142. Kurtul N, Gokpinar E. Salivary lipid peroxidation and total sialic acid levels in smokers and smokeless tobacco users as Maras powder. *Mediators of inflammation*. 2012;2012:619293.
143. Nielsen F, Mikkelsen BB, Nielsen JB, Andersen HR, Grandjean P. Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors. *Clinical chemistry*. 1997;43(7):1209-14.
144. Smriti K, Pai KM, Ravindranath V, Pentapati KC. Role of salivary malondialdehyde in assessment of oxidative stress among diabetics. *J Oral Biol Craniofac Res*. 2016;6(1):41-4.
145. Marnett LJ. Lipid peroxidation-DNA damage by malondialdehyde. *Mutat Res*. 1999;424(1-2):83-95.
146. Awada M. L'oxydation modifie les effets métaboliques d'acides gras polyinsaturés de la série n-3 incorporés par différents vecteurs dans des régimes hyperlipidiques : contribution de l'absorption intestinale et de la réactivité cellulaire du 4 hydroxy-hexénaol Villeurbanne: Université Claude Bernard Lyon 1; 2012.
147. Lovrić J, Mesić M, Macan M, Koprivanac M, Kalava M, Bradamante V. Measurement of malondialdehyde (MDA) level in rat plasma after simvastatin treatment using two different analytical methods. *Periodicum biologorum*. 2008;110:63-7.
148. Petruska JM, Wong SH, Sunderman FW, Jr., Mossman BT. Detection of lipid peroxidation in lung and in bronchoalveolar lavage cells and fluid. *Free Radic Biol Med*. 1990;9(1):51-8.
149. Del Rio D, Stewart AJ, Pellegrini N. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. *Nutr Metab Cardiovasc Dis*. 2005;15(4):316-28.
150. Chellouai Z, Moussaoui R, Benaissa S, Nachi M, editors. Le dosage du malondialdéhyde (MDA) par chromatographie liquide: Aspects pré- analytiques et analytiques. *Journées de l'Innovation en Biologie*; 2019.
151. Shin HS, Jung DG. Sensitive Analysis of Malondialdehyde in Human Urine by Derivatization with Pentafluorophenylhydrazine then Headspace GC-MS. *Chroma* 2009;70:899-903
152. Tsikas D, Rothmann S, Schneider JY, Suchy M-T, Tretin A. Development, validation and biomedical applications of stable-isotope dilution GC-MS and GC-MS/MS techniques for circulating malondialdehyde (MDA) after pentafluorobenzyl bromide derivatization: MDA as a biomarker of oxidative stress and its relation to 15(S)-8-iso-prostaglandin F2 α and nitric oxide (NO). *J Chromatogr B Analyt Technol Biomed Life Sci*. 2016;1019:95-111.
153. Malaei R, Ramezani AM, Absalan G. Analysis of malondialdehyde in human plasma samples through derivatization with 2,4-dinitrophenylhydrazine by ultrasound-assisted dispersive liquid-liquid microextraction-GC-FID approach. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2018;1089:60-9.
154. Syslovà C, Kacer P, Kuzma M, Najmanová V, Fenclová Z. Rapid and easy method for monitoring oxidative stress markers in body fluids of patients with asbestos or silica-induced lung diseases. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2009;877:2477-86.
155. Mendonca R, Gning O, Di Cesare C, Lachat L, Bennett NC, Helfenstein F, et al. Sensitive and selective quantification of free and total malondialdehyde in plasma using UHPLC-HRMS. *J Lipid Res*. 2017;58(9):1924-31.
156. Hannan P, Khan J, Iqbal Z, Ullah I. Simultaneous Determination of Endogenous Antioxidants and MDA by RPHPLC Coupled with Electrochemical Detector in Serum Samples. *J Liq Chromatogr Relat Technol*. 2015;38:1052-60.

157. Rezaei Z, Jamshidzadeh A, Sanati E. A rapid and sensitive method for the determination of malondialdehyde as its hydralazine derivative in human plasma using high performance liquid chromatography. *Anal Methods*. 2013;5:2995-9.
158. Kim B, Jung W, Kho Y. Quantification of Malondialdehyde in Human Urine by HPLC-DAD and Derivatization with 2,4 Dinitrophenylhydrazine. *Kor Chem Soc*. 2017;38:642–5.
159. Hoyland D, Taylor A. A review of the methodology of the 2-thiobarbituric acid test. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*. 1991;40:271-*91.
160. Richard M, Guiraud P, Meo J, Favier A. High-performance liquid chromatographic separation of malondialdehyde—thiobarbituric acid adduct in biological materials (plasma and human cells) using a commercially available reagent. *J Chromatogr*. 1992;577:9-18.
161. Domijan AM, Ralic J, Radic Brkanac S, Rumora L, Zanic-Grubisic T. Quantification of malondialdehyde by HPLC-FL - application to various biological samples. *Biomed Chromatogr*. 2015;29(1):41-6.
162. Bouzid MA. Exercice physique, marqueurs antioxydants et peroxydation lipidique : effets de l'âge et du niveau d'aptitude physique. : Université du Droit et de la Santé - Lille II; 2014.
163. Kand'ár R, Mužáková V, Čegan A. Highly Specific, Simple and Rapid Method for the Determination of Malondialdehyde in Blood Using High-Performance Liquid Chromatography. *Clinical chemistry and laboratory medicine*. 2002;40:1032-5.
164. Young I, Trimble E. Measurement of malondialdehyde in plasma by high performance liquid chromatography with fluorimetric detection. *Annals of Clinical Biochemistry*. 1991;28:504-8.
165. Carbonneau MA, Peuchant E, Sess D, Canioni P, Clerc M. Free and bound malondialdehyde measured as thiobarbituric acid adduct by HPLC in serum and plasma. *Clinical chemistry*. 1991;37(8):1423-9.
166. Wong SH, Knight JA, Hopfer SM, Zaharia O, Leach CN, Jr., Sunderman FW, Jr. Lipoperoxides in plasma as measured by liquid-chromatographic separation of malondialdehyde-thiobarbituric acid adduct. *Clinical chemistry*. 1987;33(2 Pt 1):214-20.
167. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*. 1979;95(2):351-8.
168. Richard MJ, Portal B, Meo J, Coudray C, Hadjian A, Favier A. Malondialdehyde kit evaluated for determining plasma and lipoprotein fractions that react with thiobarbituric acid. *Clinical chemistry*. 1992;38(5):704-9.
169. Jo C, Ahn DU. Fluorometric analysis of 2-thiobarbituric acid reactive substances in turkey. *Poult Sci*. 1998;77(3):475-80.
170. Armstrong D. Free radical and antioxidant protocols. Introduction. *Methods Mol Biol*. 1081998. p. v-viii.
171. Carre G. Compréhension des mécanismes lors de la photocatalyse appliquée à la dégradation des microorganismes : application au traitement de l'air et aux textiles auto-décontaminants: Université de Strasbourg; 2013.
172. Wang J, Schipper HM, Velly AM, Mohit S, Gornitsky M. Salivary biomarkers of oxidative stress: A critical review. *Free Radic Biol Med*. 2015;85:95-104.
173. Noury P. Dosage en microplaque des substances réagissant à l'acide Thiobarbiturique (Tbars). Fiche technique. 2016 [Available from: <https://hal.science/hal-02602439/>].
174. Yagi K. Simple Assay for the Level of Total Lipid Peroxides in Serum or Plasma. *Free Radical and Antioxidant Protocols Methods in Molecular Biology*. 108. Totawa, NJ, USA: Humana Press; 1998. p. 101-6.
175. Hedrei Helmer S, Kerbaol A, Aras P, Jumarie C, Boily M. Effects of realistic doses of atrazine, metolachlor, and glyphosate on lipid peroxidation and diet-derived antioxidants in caged honey bees (*Apis mellifera*). *Environmental Science and Pollution Research*. 2014;22:8010-21.
176. Landry C. Évaluation de l'état du système redox et des rétinoïdes comme biomarqueurs chez la perchaude en lien avec le déclin de la population du lac Saint-Pierre. . Montreal: Université du Québec; 2017.

177. Buege JA, Aust SD. Microsomal lipid peroxidation. *Methods Enzymol.* 1978;52:302-10.
178. Armstrong D, Browne R. The analysis of free radicals, lipid peroxides, antioxidant enzymes and compounds related to oxidative stress as applied to the clinical chemistry laboratory. *Adv Exp Med Biol.* 1994;366:43-58.
179. Maboudou P, Mathieu D, Bachelet H, Wiart JF, Lhermitte M. Detection of oxidative stress. Interest of GC-MS for malondialdehyde and formaldehyde monitoring. *Biomed Chromatogr.* 2002;16(3):199-202.
180. Singh Z, Karthigesu P, Singh P, Kaur R. Use of Malondialdehyde as a Biomarker for Assessing Oxidative Stress in Different Disease Pathologies: a Review. *Iran J Public Health.* 2015;46 (supple 3):7-16.
181. Khoubnasabjafari M, Ansarin K, Jouyban A. Salivary malondialdehyde as an oxidative stress biomarker in oral and systemic diseases. *J Dent Res Dent Clin Dent Prospects.* 2016;10(2):71-4.
182. Tothova L, Kamodyova N, Cervenka T, Celec P. Salivary markers of oxidative stress in oral diseases. *Front Cell Infect Microbiol.* 2015;5:73.
183. Camejo G, Wallin B, Enojärvi M. Analysis of Oxidation and Antioxidants Using Microtiter Plates. In: Armstrong D, editor. *Free Radical and Antioxidant Protocols Methods in Molecular Biology™.* 108: Humana Press; 1998.
184. Lepage G, Munoz G, Champagne J, Roy CC. Preparative steps necessary for the accurate measurement of malondialdehyde by high-performance liquid chromatography. *Anal Biochem.* 1991;197(2):277-83.
185. Kaur R, Singh Z, Karthigesu IP, Singh P. Use of Malondialdehyde as a Biomarker for Assessing Oxidative Stress in Different Disease Pathologies: a Review. *Iran J Publ Health.* 2015;43:7–16.
186. Feinberg M. Interpretation of the Accuracy Profile. *Le Cahier des techniques de l'INRA.* 2010;Special Volume:45-60.
187. Aaronson NK, Ahmedzai S, Bergman B, Bullinger M, Cull A, Duez NJ, et al. The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology. *J Natl Cancer Inst.* 1993;85(5):365-76.
188. Spotten L, Corish C, Lorton C, Dhuibhir PU, O'Donoghue N, O'Connor B, et al. Subjective taste and smell changes in treatment-naive people with solid tumours. *Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer.* 2016;24(7):3201-8.
189. Newell S, Sanson-Fisher RW, Girgis A, Bonaventura A. How well do medical oncologists' perceptions reflect their patients' reported physical and psychosocial problems? Data from a survey of five oncologists. *Cancer.* 1998;83(8):1640-51.
190. Jensen SB, Mouridsen HT, Bergmann OJ, Reibel J, Brunner N, Nauntofte B. Oral mucosal lesions, microbial changes, and taste disturbances induced by adjuvant chemotherapy in breast cancer patients. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2008;106(2):217-26.
191. Drareni K, Dougkas A, Giboreau A, Laville M, Souquet PJ, Bensafi M. Relationship between food behavior and taste and smell alterations in cancer patients undergoing chemotherapy: A structured review. *Semin Oncol.* 2019;46(2):160-72.
192. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. Updating guidance for reporting systematic reviews: development of the PRISMA 2020 statement. *Journal of clinical epidemiology.* 2021;134:103-12.
193. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan-a web and mobile app for systematic reviews. *Systematic reviews.* 2016;5(1):210.
194. Sterne JA, Hernan MA, Reeves BC, Savovic J, Berkman ND, Viswanathan M, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. *BMJ.* 2016;355:i4919.
195. Schunemann HJ, Cuello C, Akl EA, Mustafa RA, Meerpohl JJ, Thayer K, et al. GRADE guidelines: 18. How ROBINS-I and other tools to assess risk of bias in nonrandomized studies should be used to rate the certainty of a body of evidence. *Journal of clinical epidemiology.* 2019;111:105-14.
196. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials.* 1986;7:177-88.
197. Agresti A, Coull BA. Order-restricted tests for stratified comparisons of binomial proportions. *Biometrics.* 1996;52(3):1103-11.

198. Newcombe RG. Interval estimation for the difference between independent proportions: comparison of eleven methods. *Stat Med*. 1998;17(8):873-90.
199. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ*. 2003;327(7414):557-60.
200. Sterne JA, Sutton AJ, Ioannidis JP, Terrin N, Jones DR, Lau J, et al. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. *BMJ*. 2011;343:d4002.
201. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ*. 1997;315 629-34.
202. Al-Taie A, Köseoğlu A. Determination of radiotherapy-related acute side effects; a starting point for the possible implementation of a clinical pharmacy services in the radiological unit in Turkey. *Journal of Young Pharmacists*. 2019;11(4):434-8.
203. Alvarez-Camacho M, Martinez-Michel L, Gonella S, Scrimger RA, Chu KP, Wismer WV. Physical symptom burden of post-treatment head and neck cancer patients influences their characterization of food: Findings of a repertory grid study. *Eur J Oncol Nurs*. 2016;22:54-62.
204. Amezaga J, Alfaro B, Rios Y, Larraioz A, Ugartemendia G, Urruticoechea A, et al. Assessing taste and smell alterations in cancer patients undergoing chemotherapy according to treatment. *Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer*. 2018;26(12):4077-86.
205. Bernhardson BM, Tishelman C, Rutqvist LE. Self-reported taste and smell changes during cancer chemotherapy. *Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer*. 2008;16(3):275-83.
206. Cusnir M, Soares H, Schwartz M, Pizzolato J, Ludsky J, Campbell R, et al. Treatment of taste alterations in chemotherapy patients using "Miracle fruit". *Psycho-oncology* 2012. p. 58.
207. Derksen JWG, Koopman M, Ten Bokkel Huinink D, Sommeijer DW, Dorresteijn B, Jourdan M, et al. Taste and smell alterations (TSAs) in patients (pts) with stage II-III colon cancer (CC): A pilot within the PROTECT study. *Annals of Oncology* 2019;30(0):v735.
208. Genvresse I, Lange C, Schanz J, Schweigert M, Harder H, Possinger K, et al. Tolerability of the cytoprotective agent amifostine in elderly patients receiving chemotherapy: a comparative study. *Anticancer Drugs*. 2001;12(4):345-9.
209. Halyard MY. Taste and smell alterations in cancer patients--real problems with few solutions. *The journal of supportive oncology*. 2009;7(2):68-9.
210. McDaniel RW, Rhodes VA. Development of a preparatory sensory information videotape for women receiving chemotherapy for breast cancer. *Cancer nursing*. 1998;21:143-8.
211. McGreevy J, Orrevall Y, Belqaid K, Wismer W, Tishelman C, Bernhardson BM. Characteristics of taste and smell alterations reported by patients after starting treatment for lung cancer. *Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer*. 2014;22(10):2635-44.
212. Ponticelli E, Clari M, Frigerio S, De Clemente A, Bergese I, Scavino E, et al. Dysgeusia and health-related quality of life of cancer patients receiving chemotherapy: A cross-sectional study. *European journal of cancer care*. 2017;26(2).
213. Rehwaldt M, Wickham R, Purl S, Tariman J, Blendowski C, Shott S, et al. Self-care strategies to cope with taste changes after chemotherapy. *Oncology nursing forum*. 2009;36(2):E47-56.
214. Ui-rehwDhuibhir P, Barrett M, O'Donoghue N, Gillham C, El Beltagi N, Walsh D. Self-reported and objective taste and smell evaluation in treatment-naive solid tumour patients. *Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer*. 2020;28(5):2389-96.
215. Wilken MK, Satiroff BA. Pilot study of "miracle fruit" to improve food palatability for patients receiving chemotherapy. *Clinical journal of oncology nursing*. 2012;16(5):E173-7.
216. Baker WL, White CM, Cappelleri JC, Kluger J, Coleman CI. Understanding heterogeneity in meta-analysis: the role of meta-regression. *Int J Clin Pract*. 2009;63(10):1426-34.

217. Sakashita S, Takayama K, Nishioka K, Katoh T. Taste Disorders in Healthy "Carriers" and "Non-Carriers" of *Candida Albicans* and in Patients with Candidosis of the Tongue. *J Dermatol* 2004 31 890-7.
218. Thomas-Danguin T. Flavor. In: M. D. Binder NH, & U. Windhorst, editor. *Encyclopedia of Neuroscience*. Berlin: Springer-Verlag and Heidelberg GmbH; 2009. p. 1580-2.
219. Defossez G, Le Guyader-Peyrou S, Uhry Z, Grosclaude P, Colonna M, Dantony E, et al. Estimations nationales de l'incidence et de la mortalité par cancer en France métropolitaine entre 1990 et 2018. Étude à partir des registres des cancers du réseau Francim 2019 [Available from: https://www.e-cancer.fr/content/download/266450/3759432/file/Synthese_Estimations%20nationales%20incidence%20et%20mortalite%20par%20cancer_juillet_2019.pdf].
220. Shi Q, Mendoza TR, Gunn GB, Wang XS, Rosenthal DI, Cleeland CS. Using group-based trajectory modeling to examine heterogeneity of symptom burden in patients with head and neck cancer undergoing aggressive non-surgical therapy. *Quality of life research : an international journal of quality of life aspects of treatment, care and rehabilitation*. 2013;22(9):2331-9.
221. Guerdoux-Ninot E, Flori N, Janiszewski C, Vaille A, de Forges H, Raynard B, et al. Assessing dietary intake in accordance with guidelines: Useful correlations with an ingesta-Verbal/Visual Analogue Scale in medical oncology patients. *Clin Nutr*. 2019;38(4):1927-35.
222. Neyman J, Pearson ES. On the problems of the most efficient tests of statistical hypotheses. *Philosophical Transactions of the Royal Society of London*. 1933;231A:289-338.
223. Asif M, Moore A, Yarom N, Popovtzer A. The effect of radiotherapy on taste sensation in head and neck cancer patients - a prospective study. *Radiat Oncol*. 2020;15(1):144.
224. Maes A, Huygh I, Weltens C, Vandeveld G, Delaere P, Evers G, et al. De Gustibus: time scale of loss and recovery of tastes caused by radiotherapy. *Radiother Oncol*. 2002;63(2):195-201.
225. Mossman KL, Henkin RI. Radiation-induced changes in taste acuity in cancer patients. *Int J Radiat Oncol Biol Phys*. 1978;4(7-8):663-70.
226. O'Neill M, Heron DE, Flickinger JC, Smith R, Ferris RL, Gibson M. Posttreatment quality-of-life assessment in patients with head and neck cancer treated with intensity-modulated radiation therapy. *Am J Clin Oncol*. 2011;34(5):478-82.
227. Ganzer H, Rothpletz-Puglia P, Byham-Gray L, Murphy BA, Touger-Decker R. The eating experience in long-term survivors of head and neck cancer: a mixed-methods study. *Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer*. 2015;23(11):3257-68.
228. Gill SS, Frew J, Fry A, Adam J, Paleri V, Dobrowsky W, et al. Priorities for the head and neck cancer patient, their companion and members of the multidisciplinary team and decision regret. *Clin Oncol (R Coll Radiol)*. 2011;23(8):518-24.
229. List MA, Rutherford JL, Stracks J, Pauloski BR, Logemann JA, Lundy D, et al. Prioritizing treatment outcomes: head and neck cancer patients versus nonpatients. *Head Neck*. 2004;26(2):163-70.
230. Sone M, Sakagami M, Tsuji K, Mishiro Y. Younger patients have a higher rate of recovery of taste function after middle ear surgery. *Arch Otolaryngol Head Neck Surg*. 2001;127(8):967-9.
231. Lim J, Lawless HT. Detection thresholds and taste qualities of iron salts. *Food Qual Pref*. 2006;17(6):513-21.
232. Guth H, Grosch W. Comparison of stored soya-bean and rapeseed oils by aroma extract dilution analysis. *Lebens Wiss u-Technol*. 1990;23:59-65.
233. Hinterholzer A, Lemos T, Schieberle P. Identification of key odorants in raw french beans and changes during cooking. *Z Lebens Unters Forsch A*. 1998;207:219-22.
234. Hinterholzer A, Schieberle P. Identification of the most odour active volatiles in fresh, hand-extracted juice of valencia late oranges by odour dilution techniques. *Flav Frag J*. 1998;13:49-55.
235. Buettner A, Schieberle P. Characterization of the most odor-active volatiles in fresh, hand-squeezed juice of grapefruit (*Citrus paradisi* Macfayden). *Journal of agricultural and food chemistry*. 1999;47(12):5189-93.

236. Buettner A, Schieberle P. Evaluation of key aroma compounds in hand-squeezed grapefruit juice (*Citrus paradisi* Macfayden) by quantitation and flavor reconstitution experiments. *Journal of agricultural and food chemistry*. 2001;49(3):1358-63.
237. Wild CP, Weiderpass E, Stewart EW. *World Cancer Report: Cancer Research for Cancer Prevention*. Lyon, France: International Agency for Research on Cancer; 2020.
238. Birkhaug EJ, Aarstad HJ, Aarstad AK, Olofsson J. Relation between mood, social support and the quality of life in patients with laryngectomies. *Eur Arch Otorhinolaryngol*. 2002;259(4):197-204.
239. de Graeff A, de Leeuw JR, Ros WJ, Hordijk GJ, Blijham GH, Winnubst JA. Long-term quality of life of patients with head and neck cancer. *Laryngoscope*. 2000;110(1):98-106.
240. Wan Leung S, Lee TF, Chien CY, Chao PJ, Tsai WL, Fang FM. Health-related quality of life in 640 head and neck cancer survivors after radiotherapy using EORTC QLQ-C30 and QLQ-H&N35 questionnaires. *BMC Cancer*. 2011;11:128.
241. Tribius S, Raguse M, Voigt C, Munscher A, Grobe A, Petersen C, et al. Residual deficits in quality of life one year after intensity-modulated radiotherapy for patients with locally advanced head and neck cancer: Results of a prospective study. *Strahlenther Onkol*. 2015;191(6):501-10.
242. Deshpande TS, Blanchard P, Wang L, Foote RL, Zhang X, Frank SJ. Radiation-Related Alterations of Taste Function in Patients With Head and Neck Cancer: a Systematic Review. *Curr Treat Options Oncol*. 2018;19(12):72.
243. Lapôtre-Ledoux B, Remontet L, Uhry Z, Dantony E, Grosclaude P, Molinié F, et al. Incidence des principaux cancers en France Métropolitaine en 2023 et tendances depuis 1990 2023 [Available from: <https://www.santepubliquefrance.fr/maladies-et-traumatismes/cancers/cancer-du-sein/documents/article/incidence-des-principaux-cancers-en-france-metropolitaine-en-2023-et-tendances-depuis-1990bui>].
244. Ergun S, Trosala SC, Warnakulasuriya S, Ozel S, Onal AE, Ofluoglu D, et al. Evaluation of oxidative stress and antioxidant profile in patients with oral lichen planus. *Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology*. 2011;40(4):286-93.
245. Rai B, Kaur J, Jacobs R, Singh J. Possible action mechanism for curcumin in pre-cancerous lesions based on serum and salivary markers of oxidative stress. *Journal of oral science*. 2010;52(2):251-6.
246. Epstein JB, Smutzer G, Doty RL. Understanding the impact of taste changes in oncology care. *Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer*. 2016;24(4):1917-31.
247. Caccavo D, Pellegrino NM, Altamura M, Rigon A, Amati L, Amoroso A, et al. Antimicrobial and immunoregulatory functions of lactoferrin and its potential therapeutic application. *Journal of endotoxin research*. 2002;8(6):403-17.
248. Kowalczyk P, Kaczynska K, Kleczkowska P, Bukowska-Osko I, Kramkowski K, Sulejczak D. The Lactoferrin Phenomenon-A Miracle Molecule. *Molecules*. 2022;27(9).
249. Humphrey SP, Williamson RT. A review of saliva: normal composition, flow, and function. *The Journal of prosthetic dentistry*. 2001;85(2):162-9.
250. Vissink A, Jansma J, Spijkervet FK, Burlage FR, Coppes RP. Oral sequelae of head and neck radiotherapy. *Critical reviews in oral biology and medicine : an official publication of the American Association of Oral Biologists*. 2003;14(3):199-212.
251. Shpitzer T, Hamzany Y, Bahar G, Feinmesser R, Savulescu D, Borovoi I, et al. Salivary analysis of oral cancer biomarkers. *Br J Cancer*. 2009;101(7):1194-8.
252. Battino M, Bullon P, Wilson M, Newman H. Oxidative injury and inflammatory periodontal diseases: the challenge of anti-oxidants to free radicals and reactive oxygen species. *Critical reviews in oral biology and medicine : an official publication of the American Association of Oral Biologists*. 1999;10(4):458-76.

253. Zhu Y, Feron G, Von Koskull D, Neiers F, Brignot H, Hummel T. The association between changes of gustatory function and changes of salivary parameters: A pilot study. *Clin Otolaryngol*. 2021;46(3):538-45.
254. Walliczek-Dworschak U, Schöps F, Feron G, Brignot H, Hähner A, Hummel T. Differences in the Density of Fungiform Papillae and Composition of Saliva in Patients With Taste Disorders Compared to Healthy Controls. *Chemical senses*. 2017;42:699-708.
255. Abendstein H, Nordgren M, Boysen M, Jannert M, Silander E, Ahlner-Elmqvist M, et al. Quality of life and head and neck cancer: a 5 year prospective study. *Laryngoscope*. 2005;115(12):2183-92.
256. Grdina DJ, Kataoka Y, Murley JS. Amifostine: mechanisms of action underlying cytoprotection and chemoprevention. *Drug Metabol Drug Interact*. 2000;16(4):237-79.
257. Buntzel J, Glatzel M, Mucke R, Micke O, Bruns F. Influence of amifostine on late radiation-toxicity in head and neck cancer--a follow-up study. *Anticancer research*. 2007;27(4A):1953-6.
258. Vandenberghe-Descamps M, Labouré H, Prot A, Septier C, Tournier C, Feron G, et al. Salivary flow decreases in healthy elderly people independently of dental status and drug intake. *J Text Stud*. 2016;47:353-60.
259. Mossman K, Shatzman A, Chencharick J. Long-term effects of radiotherapy on taste and salivary function in man. *Int J Radiat Oncol Biol Phys*. 1982;8(6):991-7.
260. Kamprad F, Ranft D, Weber A, Hildebrandt G. Functional changes of the gustatory organ caused by local radiation exposure during radiotherapy of the head-and-neck region. *Strahlenther Onkol*. 2008;184(3):157-62.
261. Kirca K, Kutluturkan S. Symptoms of patients with head and neck cancers undergoing radiotherapy. *European journal of cancer care*. 2017;26(6).
262. Martini S, Iorio GC, Arcadipane F, Olivero F, Silveti P, Rampino M, et al. Prospective assessment of taste impairment and nausea during radiotherapy for head and neck cancer. *Med Oncol*. 2019;36(5):44.
263. Sandow PL, Hejrat-Yazdi M, Heft MW. Taste loss and recovery following radiation therapy. *Journal of dental research*. 2006;85(7):608-11.
264. Chen WC, Tsai MS, Tsai YT, Lai CH, Lee CP, Chen MF. Long-Term Taste Impairment after Intensity-Modulated Radiotherapy to Treat Head-and-Neck Cancer: Correlations with Glossectomy and the Mean Radiation Dose to the Oral Cavity. *Chemical senses*. 2019.
265. Ogama N, Suzuki S, Umeshita K, Kobayashi T, Kaneko S, Kato S, et al. Appetite and adverse effects associated with radiation therapy in patients with head and neck cancer. *Eur J Oncol Nurs*. 2010;14(1):3-10.
266. Baharvand M, ShoalehSaadi N, Barakian R, Moghaddam EJ. Taste alteration and impact on quality of life after head and neck radiotherapy. *Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology*. 2013;42(1):106-12.
267. Conger AD. Loss and recovery of taste acuity in patients irradiated to the oral cavity. *Radiation research*. 1973;53(2):338-47.
268. Fernando IN, Patel T, Billingham L, Hammond C, Hallmark S, Glaholm J, et al. The effect of head and neck irradiation on taste dysfunction: a prospective study. *Clin Oncol (R Coll Radiol)*. 1995;7(3):173-8.
269. Mahmoud FA, Aktas A, Walsh D, Hullihen B. A pilot study of taste changes among hospice inpatients with advanced cancer. *The American journal of hospice & palliative care*. 2011;28(7):487-92.
270. Najafzade N, Hemati S, Gookizade A, Berjis N, Hashemi M, Vejdani S, et al. Preventive effects of zinc sulfate on taste alterations in patients under irradiation for head and neck cancers: A randomized placebo-controlled trial. *Journal of research in medical sciences : the official journal of Isfahan University of Medical Sciences*. 2013;18(2):123-6.
271. Buntzel J, Micke O, Glatzel M, Schäfer U, Riesenbeck D, Kisters K, et al. Selenium substitution during radiotherapy in head and neck cancer. *Trace Elements and Electrolytes*. 2010;27(4):235-9.

272. Jham BC, Chen H, Carvalho AL, Freire AR. A randomized phase III prospective trial of bethanechol to prevent mucositis, candidiasis, and taste loss in patients with head and neck cancer undergoing radiotherapy: a secondary analysis. *Journal of oral science*. 2009;51(4):565-72.
273. Gustavson K, von Soest T, Karevold E, Roysamb E. Attrition and generalizability in longitudinal studies: findings from a 15-year population-based study and a Monte Carlo simulation study. *BMC Public Health*. 2012;12:918.
274. Walters SJ, Bonacho Dos Anjos Henriques-Cadby I, Bortolami O, Flight L, Hind D, Jacques RM, et al. Recruitment and retention of participants in randomised controlled trials: a review of trials funded and published by the United Kingdom Health Technology Assessment Programme. *BMJ open*. 2017;7(3):e015276.
275. Epstein JB, Barasch A. Taste disorders in cancer patients: pathogenesis, and approach to assessment and management. *Oral Oncol*. 2010;46(2):77-81.
276. Zabernigg A, Gamper EM, Giesinger JM, Rumpold G, Kemmler G, Gattringer K, et al. Taste alterations in cancer patients receiving chemotherapy: a neglected side effect? *Oncologist*. 2010;15(8):913-20.
277. Viswanath L, Bhanumathi G, Reddy BK, Prabhakaran PS. Oral pH changes due to salivary gland irradiation during radiation therapy of oral and head and neck cancer. *Indian journal of dental research : official publication of Indian Society for Dental Research*. 2004;15(4):137-8.
278. Mascitti M, Togni L, Troiano G, Caponio VCA, Gissi DB, Montebugnoli L, et al. Beyond Head and Neck Cancer: The Relationship Between Oral Microbiota and Tumour Development in Distant Organs. *Front Cell Infect Microbiol*. 2019;9:232.
279. Arrifin A, Heidari E, Burke M, Fenlon MR, Banerjee A. The Effect of Radiotherapy for Treatment of Head and Neck Cancer on Oral Flora and Saliva. *Oral Health Prev Dent*. 2018;16(5):425-9.
280. Makinen AI, Pappalardo VY, Buijs MJ, Brandt BW, Makitie AA, Meurman JH, et al. Salivary microbiome profiles of oral cancer patients analyzed before and after treatment. *Microbiome*. 2023;11(1):171.
281. Vesty A, Gear K, Biswas K, Mackenzie BW, Taylor MW, Douglas RG. Oral microbial influences on oral mucositis during radiotherapy treatment of head and neck cancer. *Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer*. 2020;28(6):2683-91.
282. Roman K, Vladimira M, Alexander C. Highly specific, simple and rapid method for the determination of malondialdehyde in blood using high-performance liquid chromatography. *Clinical chemistry and laboratory medicine*. 2002;40(10):1032-5.
283. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. 2021;372:n71.

ANNEXES

ANNEX 1: Traduction anglaise de l'article : causes and treatments for undernutrition and sensory alterations in patients with head and neck cancer

ABSTRACT

Undernutrition is common and early in patients with head and neck cancer. Its origin are multifactorial: insufficient intakes (by far the most frequent and important), increased energy expenditure, metabolic changes related to cancer. One of the causes of the lack of intake is the impairment of the sensory perceptions of taste and olfactory that it is difficult to specify because all variations are possible (increase/decrease in perception thresholds, perversion of perceived information). Among them, the perception of a metallic taste is largely understudied while it is frequently reported by patients in routine clinical practice. It deserves a more careful study (incidence, cause, treatment).

The management of undernutrition must be early (ideally from diagnosis and before any treatment), multimodal (oral, enteral, and very rarely parenteral) and multiprofessional (ENT, dietician possibly assisted by a nutritionist, speech therapist, teacher of adapted physical activity ...).

INTRODUCTION

Head and Neck Cancers (HNCs) form a nosological entity. They share anatomical (oral cavity, oropharynx, hypopharynx, and larynx) and pathophysiological characteristics (identical risk factors, almost exclusively squamous cell carcinoma histopathologically, frequent synchronous cancers). Their incidence was estimated at 14,706 new cases in 2015 (20), making them one of the most common cancers in France. Their treatment is multimodal: surgery, radiotherapy, chemotherapy, and since 2018, immunotherapy. Diagnosis is often late, resulting in a poor prognosis.

Undernutrition is common in these patients. For instance, 49% of HNC patients were diagnosed as malnourished in the cross-sectional study of hospitalized patients by Hébuterne et al. (21).

Malnutrition is early (50% of patients had lost >5% of their weight at diagnosis in Langius et al.'s study (22)). It also seems to be an independent factor for overall survival (22, 23) and general health deterioration (21, 24). The causes of this undernutrition are multiple and often combined. Despite dietary advice, enrichment (25, 26), and/or prophylactic gastrostomy (27, 28), this malnutrition usually worsens during and long after the treatment of HNC(29, 30).

Sensory perception disorders (related to taste, smell, and oral somesthesia) are a possible cause of malnutrition. Gustatory and olfactory capacities are quantitatively and/or qualitatively altered, especially due to the proposed treatments. The diagnosis of these sensory disorders can be approached through questionnaires or psychophysical sensory tests. However, there are discrepancies in the results of these two approaches (17, 31-33): a sensory impairment may often be reported by patients with normal tests and vice versa (34). Results from objective psychophysical tests also vary from one study to another due to the lack of test standardization, making it difficult to use these tests routinely to refine the functional signs of patients.

Among dysgeusias, metallic taste (MT) is a common but underestimated complaint (35). Neither its frequency nor its cause(s) is precisely known.

In this article, we aim to provide an etiological update on these different symptoms, starting with the general causes of malnutrition in HNC patients, followed by those of taste and smell disorders, and ending with a focus on the causes of MT. Finally, we will recall the main treatment guidelines for malnutrition and taste and smell disorders.

1. CAUSES OF MALNUTRITION IN HNC PATIENTS

Three main mechanisms can contribute to malnutrition in HNC (Figure 1)

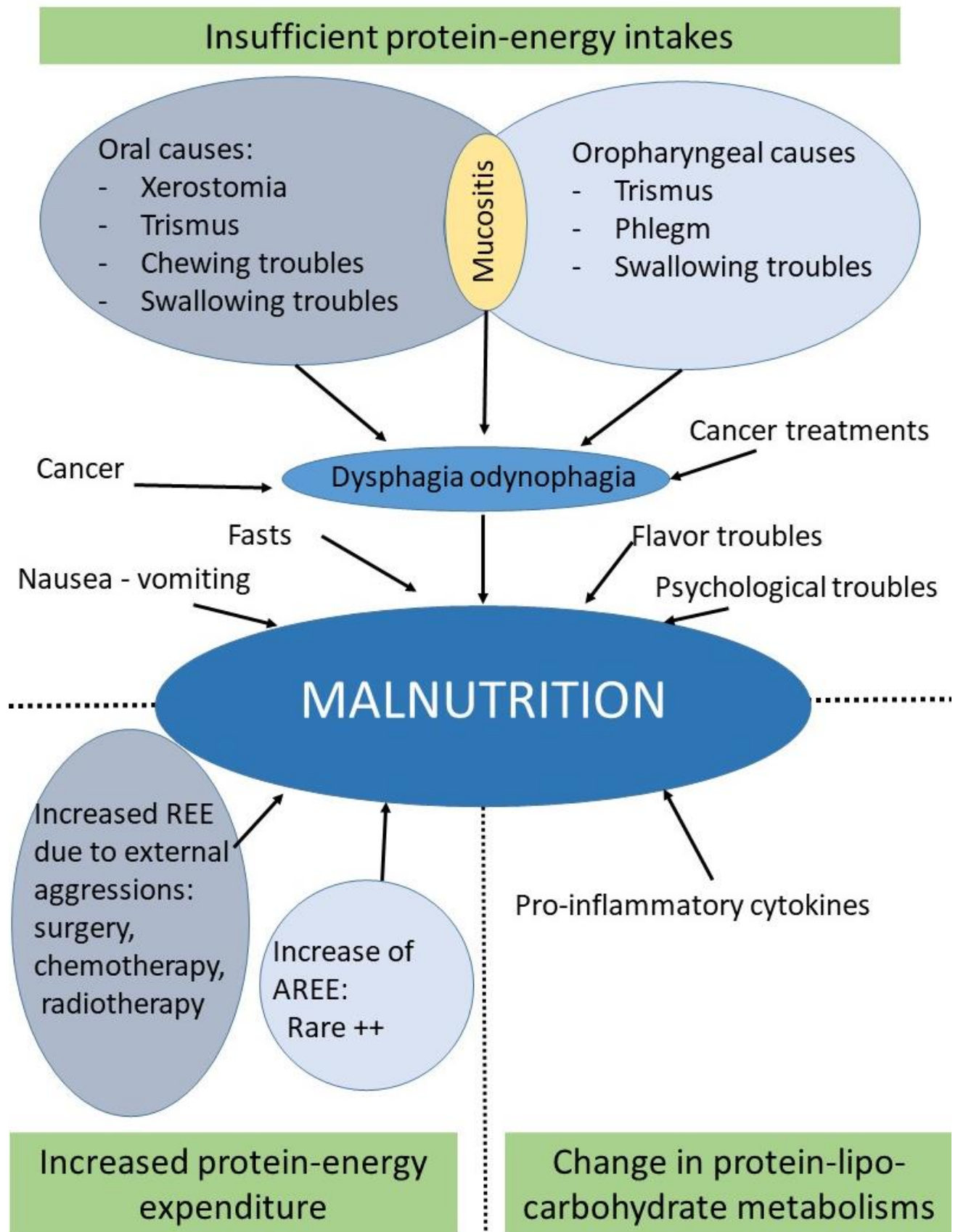


Figure 1: Diagramming of the causes of malnutrition in a patient with head and neck cancer

REE: Energy Expenditure for Rest, AREE: Activity-Related Energy Expenditure

3.1. Insufficient Energy Intake:

This is by far the most frequent mechanism. Given the cancer location in the aerodigestive tract, nutritional intake is frequently and rapidly affected. Various causes, sometimes interrelated or potentiating, include:

- Dysphagia (swallowing disorders, blockage, etc.) and odynophagia are common revealing signs of HNC. They can be caused by:
 - The cancer itself (tumor mass effect, motor, sensory, and/or painful nerve involvement) and/or by treatment and can lead to food aversions (36, 37).
 - Oral involvement: xerostomia, limited mouth opening, mastication disorders, poor dental condition, the need to extract imperfect teeth to avoid osteoradionecrosis in case of irradiation, an inappropriate denture also contribute to reducing calorie intake as patients modify their food texture and thus the quantity of ingested food. Oral somesthesia impairment can also limit swallowing capacity (38, 39).
 - Oropharyngeal involvement: the presence of post-radiation oropharyngeal mucus also affects swallowing. Similarly, limited mouth opening affects food intake. This can be due to the tumor, inflammation it generates on masticatory muscles, treatments (excision, reconstruction surgery, oropharyngeal radiotherapy or chemoradiotherapy). Just like oral causes, pharyngeal somesthesia impairment can limit swallowing capacity (40).
 - Mucositis: oral and/or oropharyngeal mucositis deserves special attention. Sometimes complicated by candidiasis, it is frequent (up to 85% of patients undergoing HNC radiotherapy (41)) and limits food intake due to the pain it causes. It can be spontaneous due to the cancer presence and peritumoral inflammation but is mainly iatrogenic, especially during radiotherapy, chemotherapy, and a fortiori chemoradiotherapy.
- Nausea-vomiting and anorexia, often iatrogenic (rapid enteral nutrition, chemotherapy, etc.), also limit food intake.
- Performing diagnostic (panendoscopy under general anesthesia, esophagogastroduodenoscopy) and/or therapeutic (surgery, gastrostomy) exams requires iterative fasting.
- Taste and/or smell disorders (31) are also frequent (up to 100% of patients undergoing HNC chemoradiotherapy (3)). These can involve changes in perception thresholds (hypergeusia or hyperosmia), or distortion of perception (dysgeusia or dysosmia). Taste and/or smell disorders are sometimes already present at the HNC diagnosis but are mainly aggravated by treatments, particularly irradiation (3, 42, 43). They lead to a decrease in food intake by reducing the pleasure of eating (31, 37, 44). Someone who enjoys eating eats more (45). This point is discussed further in the article.
- Psychological state of patients (46, 47) can also limit food intake. It can be both a cause (depressive state reactionary to the disease and/or general condition causing anorexia) and a consequence (eating disorders causing isolation, even from one's own family (48)).

3.2. Increased Energy Expenditure:

The main cause is iatrogenic due to treatments (surgery, radiotherapy, chemotherapy), which increase resting catabolism. Patients rarely increase their physical activity level sufficiently to affect their energy balance.

3.3. Metabolic Changes Induced by Cancer:

Generally, catabolism is increased and anabolism decreased by inflammatory cytokines (TNF- α , IL-6, IL-1) (49, 50). All metabolic pathways are affected:

- Protein metabolism: anabolism is reduced, catabolism increased, and inflammatory proteins are synthesized by the liver at the expense of anabolic proteins.

- Lipid metabolism: increased lipolysis.
- Carbohydrate metabolism: activation of gluconeogenesis and peripheral tissue insulin resistance.

Resting energy expenditure increases according to the degree of inflammation.

4. SPECIFIC TASTE AND SMELL DISORDERS IN HNC

4.1. Brief Reminder on Flavor Perception Physiology

i. Flavor perception begins before the direct stimulation of gustatory and olfactory sensory receptors. When we want to eat, the preoral phase (visual, orthonasal olfactory, and possibly other senses like hearing, touch) stimulates appetite and salivation. The typical example is feeling hungry when visiting a bakery, where all senses contribute to appetite: the sight of well-displayed pastries, the smell of fresh viennoiseries, the sound of a flaky pain au chocolat or pressing a loaf of bread. Everyone has their own "madeleine" moment...

ii. The oral phase, after food is placed in the mouth, allows the solubilization of sapid molecules through the insalivation and mastication of the food bolus. Mastication requires the effective function of cheeks, lips, teeth, masticatory muscles, palate bones, to ensure proper anterior, posterior, and lateral containment in the oral cavity. The tongue is particularly important at this stage as it distributes food in the oral cavity, brings it to the teeth, helps fragment it, and homogenizes the insalivated bolus. These molecules will stimulate gustatory receptors (sweet, salty, bitter, sour, umami) distributed throughout the oral cavity and, to a lesser extent, in the oropharynx.

iii. Molecules will also aerosolize to stimulate olfaction via retronasal pathway.

The surface of gustatory receptors is large and easily accessible. The transmission of gustatory information depends on several nerves: the facial nerve for the anterior two-thirds (in front of the lingual V) of the homolateral hemi-tongue, and the glossopharyngeal nerve for the posterior third (behind the lingual V) of the homolateral hemi-tongue. The surface of olfactory receptors is small and difficult to access as it is located at the top of the ethmoid. The transmission of olfactory information goes directly to the brain via the first cranial nerve.

4.2. Nosology of Flavor Perception Disorders

Flavor disorders are divided into supra-sensory disorders (disgust, conditioned aversions not covered in this chapter) and direct sensory impairments. Taste disorders can be quantitative (change in perception threshold: hypogeusia or ageusia, hypergeusia) and/or qualitative (change in normal taste perception modality or dysgeusia). Similarly, olfactory disorders are divided into quantitative (change in perception thresholds: hyposmia or anosmia, hyperosmia) and qualitative (change in normal olfactory perception modality or dysosmia). Some dysosmias have specific names: parosmia (perceived odor is not the expected one), phantosmia (olfactory hallucination in the absence of any stimulus), cacosmia (unpleasant odor, generally related to putrefaction), and osmophobia (fear of certain odors). Smell and taste perception disorders often coexist in the same patient but one may be predominant. When gustatory or olfactory function is evaluated through psychophysical tests, the prevalence of these disorders in HNC is very heterogeneous, with figures ranging from 4 to 92%.

4.3. Common causes of taste and smell disorders in HNC

They mainly affect the oral phase, prior to stimulation of the sensory receptors. They are illustrated in Table 1.

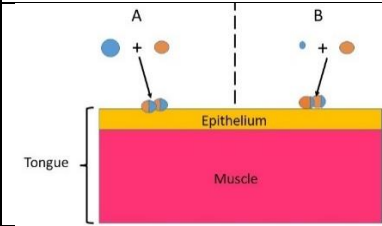
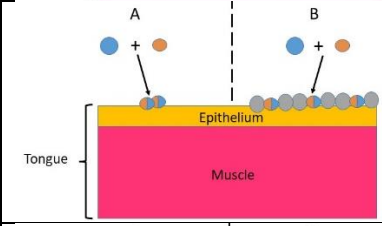
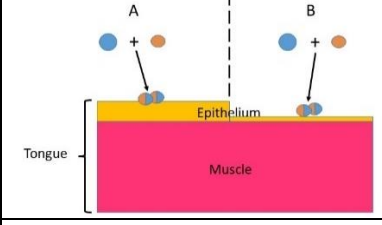
Mechanism	Causes	Scheme
Saliva	Xerostomia Change in salivary viscosity	
Flavour molecules not provided by the diet	Necrosis/inflammation Oral infection Medicaments Toxic	
Direct damage to taste	Reduction in the number of sensory cells and/or receptors (surgery, radiotherapy, chemotherapy)	
Direct damage to smell	Reduction in the number of sensory cells and/or receptors (surgery, radiotherapy, chemotherapy)	
Impairment of sensory transmission	Surgery: nerve section Radiotherapy – chemotherapy: polyneuropathy	

Table 1: Main mechanisms and causes of flavour perception disorders in head and neck cancer

A: normal situation. B: pathological situation as described in the "causes" column

 : saliva;  : food bolus;  : salivated food bolus;  : necrosis / inflammation / oral flora / cellular debris

i. Impairment of mastication

Mastication is essential, as it breaks down the food and releases its flavor molecules. Any impairment of mastication can therefore have an impact on taste and smell: disorders of oral continence (anterior due to damage to the lips, especially by surgery; posterior due to damage to the soft palate, especially by surgery and radiotherapy), disorders of masticatory motricity (damage to the tongue, cheeks, teeth, mandibular and maxillary bones by surgery and/or radiotherapy).

ii. Saliva

Saliva is essential for the lubricating capacity of its proteins (51), in particular mucins, large glycosylated proteins that lubricate and protect the epithelium, and for the perception of flavors: the five gustatory tastes (sweet, salty, bitter, acid, umami), fat, oral somesthesia via the trigeminal nerve, retro-olfaction (52).

It enables insalivation of the food bolus, facilitating mastication and particle agglomeration (role in food bolus granulometry), fragmentation of starch-containing molecules by α -amylase, and delivery to sensory receptors of the molecules that should stimulate them (liquid saliva delivers palatable molecules to taste receptors, aerosolized saliva delivers olfactory molecules to olfactory receptors via feedback-olfaction).

Changes in saliva following radiotherapy and/or surgery, both in terms of quantity (xerostomia) (53, 54) and quality (more viscous (55)), diminish the solubilization of sapid molecules, the pathway of these sapid molecules to taste receptors, and the aerosolization of olfactory molecules.

Xerostomia may also force the patient to drink in order to swallow (56), thus draining these sapid molecules from the oral cavity too quickly, in addition to causing an early feeling of satiety. Xerostomia may also force the patient to drink in order to swallow, thus draining these sapid molecules from the oral cavity too quickly, as well as contributing to an early feeling of satiety. The qualitative change in saliva (more acidic, increased sodium excretion (57)) can also directly stimulate taste receptors.

iii. Presence of odors and flavors not supplied by the diet

There are several clinical situations involving the presence of flavor molecules not supplied by the diet:

- o The presence of cancer, per se, provokes the presence of tumoral and peritumoral necrosis and inflammation.
- o Oral bacterial and fungal colonization changes (57). The secretions and debris of this new oral flora are also perceived (flavor).
- o Presence of oral and/or oropharyngeal mucositis, or even candidiasis (58, 59): inflammation creates an interposition between sapid molecules and receptors on the surface of taste cells, in addition to secretions and cellular debris (cf. previous clinical situation).
- o Drug treatments: these can have a direct effect on sensory receptors (60-63). Chemotherapy platinum (which is excreted in saliva after intravenous administration) and oral antifungal treatments are everyday examples in the treatment of CVADS. But other substances (antimicrobials, antifungals, antivirals, corticosteroids, psychoactive drugs, etc.) also impair flavor perception
- o Smoking
- o ...

iv. Impairment of taste cells and receptors

Surgical excision, particularly of the lingual region, permanently reduces the number of taste cells and their receptors.

Similarly, the number of taste cells and their receptors is reduced, early but generally transiently, by irradiation (63-68) and/or chemotherapy (63, 65, 69). This reduction in cell population is linked both to radiation- and chemo-induced apoptosis and to the alteration or non-renewal of sensory cells due to stem cell damage (70).

Micronutrient deficiencies (vitamin A, zinc, niacin) also reduce taste receptor sensitivity (70, 71).

v. Olfactory cell and receptor damage

The majority of causes of isolated olfactory disorders are defects in the transmission of olfactory substances to the olfactory epithelium: obstruction of the nasal cavity by crusts, post-surgical or radiotherapy fibrosis, loss of nasal airflow after laryngectomy, etc.

Excisional surgery involving the olfactory epithelium is extremely rare, due to the difficulty of access and the rarity of its involvement (olfactory aestheticeuroblastoma, outside the scope of CVADS).

On the other hand, as with gustatory involvement, the number of olfactory cells and their receptors can be reduced by irradiation of the olfactory epithelium (63-68) and/or chemotherapy for general neuropathy (63, 65, 69).

vi. Impairment of sensory information transmission

Nerves transmitting taste information (cranial nerves V, VII, IX, or X) may be affected by surgical nerve transection, or by neuropathy (direct or by deafferentation) linked to irradiation and/or chemotherapy (63).

vii. Impact of flavor perception disorders on nutritional status

Few studies have investigated the impact of taste disorders on nutritional status. McLaughlin (31) showed in his cohort of 92 patients that weight loss was significantly associated with taste disorders,

whereas this was not the case with type of treatment, tumor site and time since the end of treatment. Sanchez-Lara et al. (44) showed that when sugar detection thresholds were increased, calorie, protein, carbohydrate and zinc intakes were reduced, and weight loss was more frequent. Shi et al. (72) showed that an increase in umami perception thresholds was significantly associated with loss of appetite, dissatisfaction with current state, weight loss and serious impairment of mealtime hedonism. We can therefore see that the results of sensory tests diverge (sweetness impairment in Sanchez-Lara (44) and umami impairment in Shi (71)), but that gustatory sensory disorders have an unfavorable impact on nutritional status, confirmed by a number of literature reviews (37, 73, 74).

However, there are no data on the impact of olfactory disorders on nutritional status.

viii. Impact of flavor perception disorders on quality of life

Very few data are available (43, 72, 75). In addition, quality-of-life questionnaires incorporate disorders of flavor perception (e.g., questions 43 and 44 of the EORTC HN35 questionnaire, the most widely used to measure quality of life in CVADS (76)).

3. MT IN ENT

MT is widely under-reported in the literature. Its incidence in patients with CVADS is thought to vary between 24% (36) and 77% (77) (compared with between 9.7 and 78% in a population with cancer of any location (78)). To date, very few studies have focused on MT (incidence, potential causes, impact on quality of life, potential treatments).

The causes of MT are poorly established (79) and probably multiple, either concomitantly or sequentially:

3.1. Chemical mechanisms: two different mechanisms have been described, depending on the metal ion:

- Oral lipoperoxidation by Fe²⁺, with an important role for the retronasal pathway in the perception of metallic taste (16, 80-82). Malondialdehyde is the stable end product of lipid membrane peroxidation (82) and contributes to an olfactory stimulus. In addition, nasal occlusion and/or the use of chelators such as EDTA and lactoferrin can abolish lipoperoxidation-induced MT (82).
- Direct perception of the metal ion (Fe³⁺, Cu²⁺, Cu³⁺) as a taste stimulus (78, 82).

3.2. Electrical mechanism: anodic electrical stimulation of the longus causes MT, probably through local hydrolysis and the presence of H⁺ ions (16).

3.3. Neurological mechanism (Figure 2): anesthesia of the tympanic cord (a branch of the facial nerve responsible for the transmission of taste afferents in the anterior two-thirds of the homolateral tongue) causes ageusia in the relevant nerve territory, increased perception of certain sapid tastes in the territory of the glossopharyngeal nerve (responsible for the transmission of taste afferents in the posterior third of the homolateral tongue) and sometimes MT (13, 14).

The reduction or suppression of gustatory afferents from the tympanic cord would cause the facial nerve to lift its inhibition of the glossopharyngeal nerve: in pathological conditions (reduction of tympanic cord afferents), gustatory afferents mediated by the glossopharyngeal nerve would be considered a sensory afferent by the central nervous system, whereas they would not be in physiological conditions, thus causing MT (15).

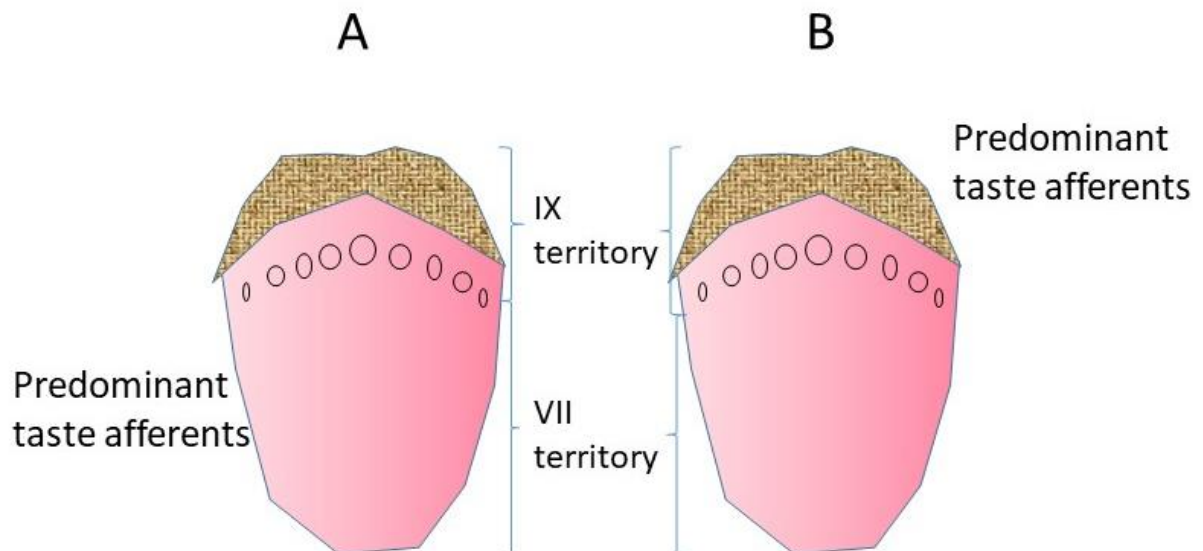


Figure 2: scheme of the hypothesis of lifting the inhibition of the facial nerve on the glossopharyngeal nerve

A: normal situation. B: Lifting of inhibition

VII: facial nerve (mediation of sensory information by the tympanic cord), IX: glossopharyngeal nerve

3.4. Proteomic mechanism: a strong link between bitter hypersensitivity and MT has been shown (35). In addition, hyperexpression of bitter receptor genes of the T2R family, particularly in the mouth, has been described during radiotherapy (18).

The study of this dysgeusia is therefore necessary in order to precisely establish its incidence in CVADS, the potential cause(s) and possibly one or more treatments.

4. MANAGEMENT OF FLAVOR PERCEPTION DISORDERS AND MALNUTRITION IN PATIENTS WITH HNCs

4.1. Prevention of flavor perception disorders

- The most “sparing” lingual excision surgery possible avoids the loss of taste organs, while ensuring complete excision of the cancer, the primary aim of surgery.
- With regard to radiotherapy, preference should be given to intensity-modulated radiotherapy (more precise, less damaging to taste organs), minimizing the dose of radiation to the tip of the tongue (area of maximum density of taste organs) and to the oral cavity in general.
- Avoiding toxic substances (tobacco, alcohol) is also essential.
- Zinc supplementation has shown no significant preventive effect on taste perception disorders (3)
- Prevention and treatment of mucositis: the use of low-intensity, high-energy lasers, oral hygiene checks before each radiotherapy or chemotherapy treatment, and good hygiene of prostheses all help to prevent the onset of oral mucositis and candidiasis. Specific treatment of these conditions should be started as soon as they are diagnosed. Particular attention should be paid to antifungal agents, which can cause dysgeusia.

4.2. Advice

Various advice can be given (84): chew and swallow slowly to increase insalivation and flavor perception, limit cooking odors, improve the visual appearance of dishes, adapt seasonings and food choices, increase fluid intake, increase saliva production (flavored or unflavored ice cubes or crushed ice, chewing gum, neutral glycerine sticks, artificial saliva, mister)...

Numerous culinary advice brochures are available for patients and professionals.

4.3. Rehabilitation

There are a number of ways to treat taste and swallowing disorders, and undernutrition in general, in patients with HNCs:

- Dental / prosthetic rehabilitation
- Speech therapy and self-education (85, 86). These methods are common sense, but we must not be dogmatic. Indeed, in the randomized phase 3 trial by Ahlberg et al (87), 190 HNC patients treated with rehabilitation during and 3 months after treatment (weekly speech and physical therapy, home exercises) were compared with 184 HNC control patients (no rehabilitation). There was no significant difference in weight or overall survival at 2 years. There was even statistically more consumption of oral nutritional supplements and less return to work in the rehabilitated group.

4.4. Specific treatments for flavor perception disorders

There are few treatments available specifically for flavor perception disorders. Zinc supplementation appears to reduce these disorders (3). Olfaction disorders can be treated with nasal +/- general corticosteroids (in the absence of contraindications).

4.5. Increasing oral intake

Care by a dietician is essential. Ravasco et al. showed a clear difference in nutritional status between simple advice given by doctors, oral nutritional supplements prescribed ad libitum by doctors, and dietetic advice by a dietician, which alone of the three arms ensured the best medium- and long-term nutritional status in patients treated for HNC (26). Ideally, patients should be referred to a dietician as soon as they are diagnosed, as they are frequently malnourished.

4.6. Enteral nutrition

The possibility of enteral nutrition at some stage in the treatment process should be discussed with the patient and his or her family as soon as the diagnosis is made.

The AFSOS, Association Francophone des Soins Oncologiques de Support, has published an inter-regional guideline on nutrition for patients with HNC (88). These guidelines are freely available. A decision tree, on p. 16, lists the indications for enteral nutrition according to the patient's status (no undernutrition, undernutrition, severe undernutrition or severity criteria) and oral intakes assessed by the Food Intake Assessment Tool (visual analog scale) (89). The SFNCM, Société Française de Nutrition Clinique et Métabolique, recommends daily intakes of 25 to 30 kcal/kg per day in perioperative care and 30 to 35 kcal/kg per day in medical oncology, and 1.2 to 1.5 g/kg of protein per day (90).

As the digestive tract of a CVADS patient is mostly functional, parenteral nutrition should be avoided. Similarly, patients should be monitored for the development of an inappropriate renutrition syndrome (88).

4.5. Adapted physical activity (APA)

Oral and/or enteral (or even parenteral) calorie intake is a necessity, but assimilation and synthesis of lean body mass requires APA.

Several randomized trials have shown statistically significant results in terms of improved quality of life (91-95), reduced asthenia (93), increased lean body mass (91, 92) and muscle strength (91, 92). Capozzi et al. carried out a literature review of 16 articles on the efficacy of APA in patients with HNC (96): benefit for physical activity, lean body mass, muscle strength, fatigue, absence of deterioration in quality of life during treatment, increase in quality of life. No safety problems have been reported if APA is supervised by a trained professional. Adherence seems better if APA is started after treatment. The authors therefore recommend that patients be assessed and made aware of APA before any treatment, and that APA be started afterwards. They also make specific recommendations on the conditions under which APA should be performed in patients with HNC:

- Rather indoor, as time (endurance) and intensity (strength) can be easily modulated (97, 98)
- Regularity: 3 times / week (97-100)
- Progressiveness: gradual increase in load (20% at start, up to 60-70% of maximum strength) (97-99)
- Global exercises (stretching, postural exercises, muscle-strengthening exercises with light weights and elastic bands) and specific exercises (shoulders) (98-100)
- Supervision, especially if elderly, BMI >30 and undergoing radiotherapy (101): Capozzi et al. found an adherence rate of 66-88% if supervised, 95-97% if supervised and at home, and 53% at home only (96).
- Initial assessment and information and initiation of APA after cancer treatment (96).

CONCLUSION

HNC are the archetype of cancer-induced undernutrition in general, taste and smell disorders due to their location and the treatments used, and MT in particular. Several areas of research, both clinical and fundamental, are currently underway: types of enteral nutrition, the benefits or otherwise of prophylactic placement of an enteral nutrition approach, the benefits of APA in patients with HNC, etc.

However, there is a clear lack of data on the impact of flavor perception disorders on nutritional status and quality of life in HNC. Finally, the causes of MT also need to be identified, and possible treatment(s) evaluated. Management of undernutrition must be early and multi-professional.

ANNEX 2. Traduction en anglais du chapitre sur la perception du goût métallique de l'Encyclopédie des Sciences

This chapter reviews the state of the art in metallic taste perception, prior to our own studies on the subject, in particular the GOUTMETALSAIN ancillary study.

Introduction

Flavor is the sum total of olfactory, gustatory and chemesthetic sensations (tactile, thermal, (astringency, menthol, molecules producing a pungent sensation)) perceived when consuming a food. This is an evolutionarily important mechanism, as it contributes to the survival of living organisms. It enables them to detect nutrients essential to their physiological needs and well-being, and to recognize their environment. It is also essential for the identification of compounds in food that could be potentially toxic for them, and which should therefore be avoided and not consumed. Five basic flavors have been described, i.e. salty, sweet, sour, bitter and umami (102), for some of which the taste buds of the tongue contain specific molecular receptors. It is suggested, however, that other flavors exist but are poorly characterized, such as fat or metallic taste (MT). For Omur-Ozbek (82), MT is a flavor, not a taste (role of taste, retronasal pathway, trigeminal sensitivity with astringency). Nevertheless, as the term “metallic taste” is well established in the general population, we will continue to use it.

This chapter presents the various causes of metallic taste in humans, with particular emphasis on physiological and pathological reasons.

1. The formation of metallic taste in food

MT is commonly reported as a defect in many foods containing polyunsaturated fats, such as oils, cereal products and dairy products, but also in mineral water. MT is caused, among other things, by contact with metal food packaging and processing equipment (80).

In the case of canned beer, for example, the use of pulsed electric fields to improve microbiological quality releases ferrous ions into the beverage, inducing an unpleasant MT (103). Similarly, the use of high-intensity ultrasound in various food processes enhances the metallic taste of food products containing crystallized fats such as margarine, ice cream or mayonnaise (104), but also meat (105). In the case of corn chips, the manufacturing process and the quality of the vegetable oil play an important role in the formation of the metallic taste and hence consumer acceptability (106).

MT is also frequently reported when sweeteners such as saccharin, aspartame, sorbitol, sucralose or stevia are used (107-109), which limits their use in processed foods. The same applies to potassium chloride (KCl), used as a salt substitute, whose metallic taste interferes with the organoleptic qualities of foods, as has been shown for tomato sauces (110).

The origin of this metallic taste is often linked to the presence of metal ions, particularly iron, either added to the food or by transfer of metals from the packaging to the contents. Metallic ions can lead to the formation of volatile compounds responsible for the metallic taste by oxidation of the fat present in the food, or directly in the mouth by oral lipoperoxidation (see §5 and **Erreur ! Source du renvoi introuvable.**). The formation of these metallic notes can affect the organoleptic quality of food products fortified with iron but also enriched with polyunsaturated fatty acids, as has been shown for milk powder (111) or cassava-based cookies (112)). However, the use of lactoferrin as an antioxidant in these foods can significantly limit this oxidation problem. Indeed, lactoferrin (**Erreur ! Source du renvoi introuvable.**), an iron-binding milk protein, reduces the production of lipid oxidation by-products and has been shown to reduce the formation of unpleasant flavors.

2. Causes of metallic perception

There are several competing hypotheses to explain metallic perception. None of them has yet been fully validated. Depending on the circumstances in which metallic perception is triggered (iatrogenic after middle ear surgery, during cancer treatment, etc.), one or more of these causes could explain it, either sequentially or concomitantly.

2.1. Hypothesis of perception by specific sensory receptors.

2.1.1. Are there specific receptors for metallic taste?

Stevens et al (113) applied their concept of multidimensional taste scales (locating a taste among the five usual flavors and aluminum) to iron sulfate in 47 healthy volunteers. The metallic taste was not grouped with the traditional sapid flavors. On the open nose, the flavors closest to iron sulfate were acidity and sweetness; on the occluded nose, bitterness and umami, while acidity and saltiness were the most distant. Perception of iron sulfate was more localized with aluminum, witness astringency, trigeminal. Are there specific receptors for metallic taste? Some authors think so, and attribute the perception of metallic taste to the T2R (79) or TRPV1 (79, 107) receptor families. Other, more numerous, authors refute this hypothesis. Indeed, there is a clear reduction in the perception of iron when the retro-olfactory pathway is eliminated (12, 114-116), with no change in the perception of other tastes, underlining the importance of the retronasal pathway (113). For them, the perception of metallic taste is mainly retroolfactory and linked to oral lipoperoxidation (see below).

2.1.2. Physiological circumstances of metallic taste perception

While the hypothesis of direct stimulation of specific receptors has not been confirmed, metallic perception can occur in physiological circumstances:

- the presence of metallic salts in the mouth (mainly iron-based: ferrous chloride, gluconate, sulfate, but also copper, zinc, magnesium, platinum), calcium salts (16, 82). The ferric ion (Fe^{3+}) is not detected (82)
- drugs (117), sweeteners as described in §2. (16)

2.1.3. Proteomic modification of sensory receptors hypothesis

This hypothesis is most often evoked in oncology, particularly medical oncology. Schematically, chemotherapies act on rapidly renewing cells and induce their apoptosis. In addition to chemotherapy-induced neuropathy, olfactory and gustatory sensorialities are directly altered:

- the number of sensory cells varies. These cells renew themselves, but in different ways depending on their precursors, leading to changes in the ratios of sensory cells to each other
- the number of receptors on the surface of these sensory cells varies. Hirai et al. (18), for example, showed variations in the expression of bitter T2R receptors during chemotherapy, three of which had significantly different levels of expression (TAS2R42, TAS2R43, T2R3).

These modifications induce changes in olfaction and gustation, either in their detection thresholds (hyper-hypoguesia / hyper-hyposmia), or a perversion of their perception (paragueusia, parosmia). Two hypotheses have been put forward to explain metallic taste: i. a ligand present in the mouth binds with an over-represented receptor; ii. a metallic taste is perceived in the absence of ligand in the mouth (spontaneous binding between the receptor and a G protein). In all cases, there is a decrease in the threshold of sensitivity to metal and thus an increased propensity to perceive a metallic taste (78).

2.2. Hypothesis of galvanism between dental crowns

Electrical stimulation of the tongue (12, 16), particularly anodic rather than cathodic (118), induces a metallic perception, probably through local hydrolysis and production of H^+ ions.

Ayres has shown that a spontaneous current, with a maximum intensity of $100\mu\text{A}$, can sometimes be spontaneously recorded (119). Sutow et al (120) have also shown the presence of a peak intensity followed by an exponential decay due to electrical contact between opposing dental crowns in the mouth.

However, this hypothesis of dental galvanism to explain metallic perception is unlikely. Indeed, Bergman et al. showed no significant difference in polarity in 16 people complaining of oral galvanism (four of whom complained of a metallic taste) compared with nine control patients (121) (even if the number of subjects is small and the study not very scientifically robust). Furthermore, many of the people complaining of a metallic taste have no amalgam or dental crowns. Finally, in the three-dimensional diagrams of Stevens et al. (113), the acidity (excess of H^+ ions) and metallic perception of iron sulfate were close nose open but far nose occluded. These variations are difficult to explain by the galvanism hypothesis.

2.3. Neuronal damage hypothesis

Damage to the chorda tympani (vegetative and sensory branch of the facial nerve anastomosing with the lingual nerve) during otological surgery is a well-known and long-standing circumstance that triggers the perception of a metallic taste. Neurological damage without oral involvement can therefore cause a metallic taste. This damage is most of the time of iatrogenic origin (surgical with section of a nerve, neuropathy induced by chemotherapy, radiotherapy, another treatment).

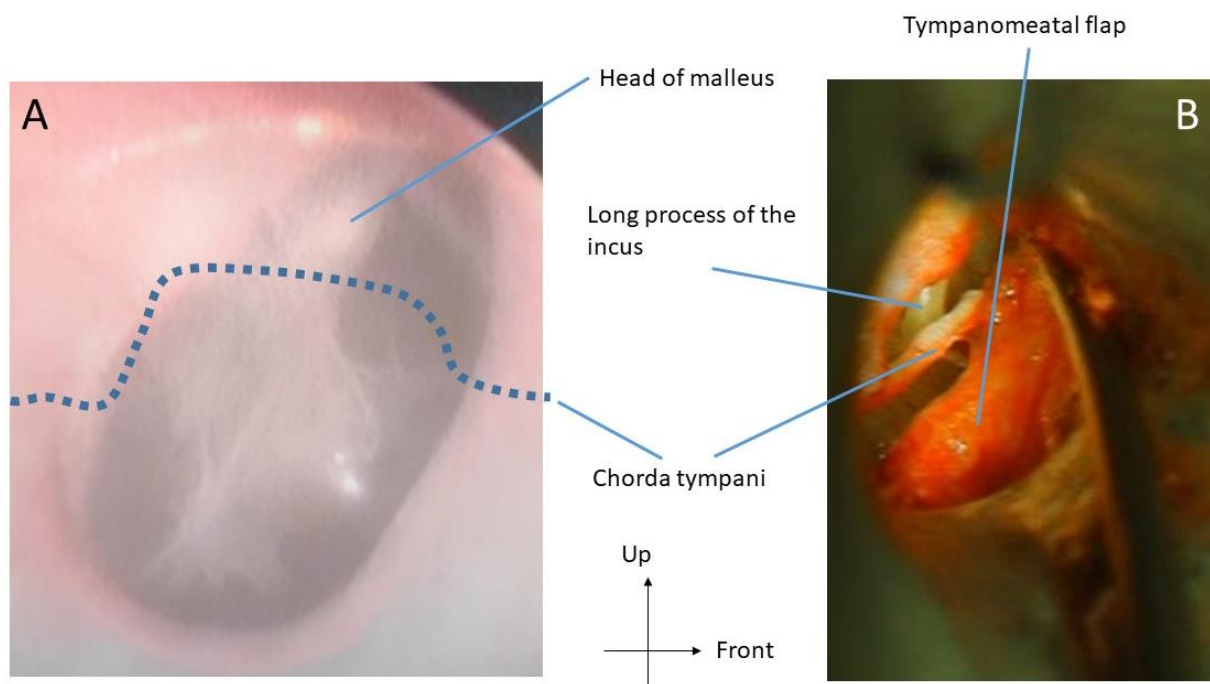


Figure 1: Relationships between the chorda tympani and the eardrum

A: projection of the chorda tympani path on right otoscopy. B: view of the chorda tympani during surgical approach to the middle ear.

4.3.1. Neuropathy and metallic taste

Direct stimulation or sectioning of the tympanic cord (**Erreur ! Source du renvoi introuvable.**) and glossodynia (lingual neuralgia) are known to trigger a metallic taste (16).

Metallic taste can also be encountered during pregnancy (16). This is known as phantoguesia, a sensory hallucination.

4.3.2. Removal of facial nerve inhibition on the glossopharyngeal nerve

As a reminder :

- the chorda tympani transmits taste information from the anterior two-thirds of the homolateral tongue
- the superficial petrous nerve, a branch of the facial nerve, transmits gustatory information from the homolateral hemi-palate.
- The glossopharyngeal nerve, essentially a motor and sensory nerve of the pharynx, transmits gustatory sensory information from the base of the tongue.

Kveton et al. (13) studied the sensory impact of tympanic cord involvement after vestibular schwannoma surgery (cochleovestibular nerve surgery in the internal auditory canal induces facial nerve involvement without glossopharyngeal nerve involvement), by performing supraliminal intensity tests of the four flavors at different locations in the oral cavity, dependent on the chorda tympani or glossopharyngeal nerve (Figure 2). They showed agueusia or hypogueusia in the chorda tympani territory and, in mirror image, hypergueusia in glossopharyngeal territories. Yanagisawa et al. have made similar findings when anesthetizing a tympanic cord in healthy volunteers (15). In their view, the sensory activity of the glossopharyngeal nerve, normally inhibited by that of the tympanic cord, is considered a predominant afferent by the central nervous system when sensory activity from the chorda tympani is impaired.

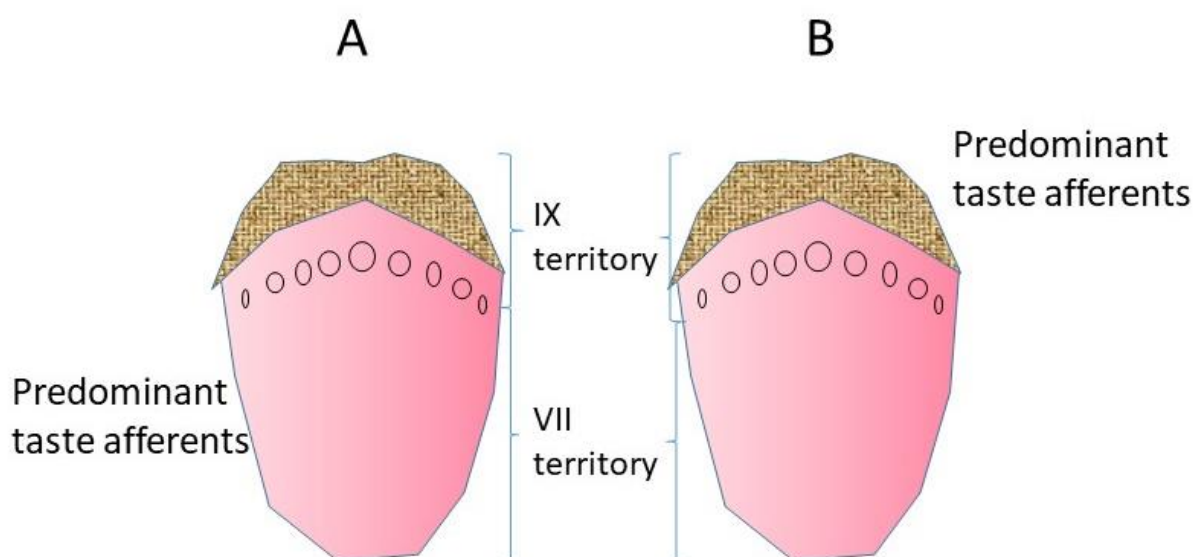


Figure 2: Scheme of the release of inhibition of the facial nerve on the glossopharyngeal nerve

From (19):

A: normal situation, B: removal of VII inhibition on IX. VII: facial nerve, IX: glossopharyngeal nerve

A neuronal impairment can therefore cause inhibition of the perception of one flavor, amplifying the perception of others (14, 122, 123). This finding is the basis for the hypothesis that the facial nerve lifts inhibition on the glossopharyngeal nerve in the genesis of MT (79). Indeed, the neuropathic effects of cancer treatments are highly complex (reduction in the number of sensory receptors, alteration of the cell structure or change in the receptor surface, interruption in neural coding, renewal of taste receptors and taste cells with irregular synaptic connections between the sensory cell and its nerve fiber (65)). Taste detection thresholds generally tend to increase (124). Work is in progress to confirm or refute this hypothesis.

4.4. Oral lipoperoxidation hypothesis

Oxidation of unsaturated lipids in the diet or in the mouth generates a metallic taste, with significant involvement of the retronasal pathway (78, 122, 125)), even if the gustatory or trigeminal component cannot be completely excluded for certain ions, copper in particular (126). In particular,

iron but also copper in solution can cause the most oral lipid oxidation, the marker of which is malondialdehyde, itself a generator of MT.

Studies of MT perception have clearly shown that this taste corresponds mainly to the instantaneous formation of volatile compounds upon taste stimulation by an iron or copper sulfate solution. These volatile compounds are generated in the oral cavity and pass into the nasal cavity to be perceived (retronasal perception). Indeed, Epke and Lawless (127), in studies of perceptual thresholds for iron and copper solutions, showed that these thresholds were significantly lower in the absence of nose-clips than in a test with nose-clips, where retronasal perception is abolished (Table 1).

Component	Detection threshold (μM) (min, max)	
	Open nose	Closed nose
Ferrous sulfate	29.9 (7.5, 120)	161 (60.1, 429)
Iron chloride	64.0 (21.2, 193)	227 (99.8, 515)
Copper sulfate	7.8 (3.2, 19.0)	24.6 (11.1, 54.4)
Copper chloride	8.2 (3.4, 19.5)	15.6 (6.5, 37.5)

Table 1: Thresholds for retronasal (open nose) or gustatory (closed nose) detection of iron and copper solutions

Adapted from Epke and Lawless, 2007 (127).

Although the reactions in the mouth that give rise to the formation of volatile compounds with a “metallic” note are not clearly identified, many authors suggest that this genesis is due to the instantaneous oxidation of polyunsaturated fatty acids contained in saliva or mucous membranes (82). This synthesis would lead to the formation of volatile compounds with very low detection thresholds, such as certain aldehydes, but also ketones (see Figure 3). These volatile compounds generated in the mouth contribute to altering the taste of the food consumed, but do not all have a specific metallic note. However, certain ketones, such as 1-octen-3-one and 1-nonen-3-one, and aldehydes, such as trans-4,5-epoxy-2(E)-decenal, are highly characteristic of this metallic taste. They also have a very low detection threshold in the ppb (parts per trillion) range (128). This hypothesis was reinforced by the work of Glindemann et al. (129), who showed that the metallic aroma formed on contact with iron on human skin was due, among other things, to the oxidation of skin lipids and the formation of these volatile compounds.

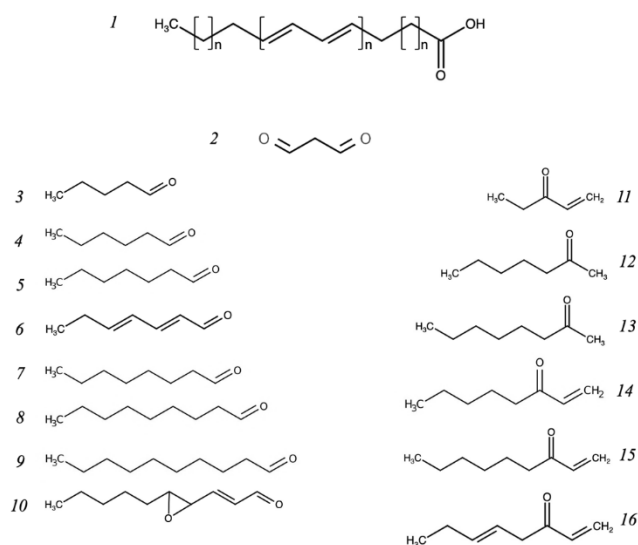


Figure 3: volatile components

(with the exception of malondialdehyde) liable to be formed, either in the food or in the oral cavity during consumption, by lipoperoxidation from polyunsaturated fatty acids contained in the food matrix, saliva or in the oral mucosa. 1: polyunsaturated fatty acid with two conjugated double bonds; 2: malondialdehyde; 3: pentanal 4:hexanal; 5:heptanal; 6: trans,trans-2,4-heptadienal; 7: octanal; 8: nonanal; : 9: decanal; 10: trans-4,5-epoxy-2(E)-decalal; 11: 1-penten-3-one; 12: 2-heptanone; 13: 2-nonanone; 14: 1-octen-3-one; 15: 1-nonen-3-one; 16: cis-1,5-octadien-3-one

Another hypothesis is that the odorant molecules responsible for the metallic taste are formed directly from the polyunsaturated fatty acids that make up the lipids contained in the food before it is consumed (see §1). When the food is placed in the mouth, the aroma compounds formed become more volatile as the temperature rises (130). It has also been shown that, unlike gold or stainless steel, the use of zinc or copper cutlery can also lead to a metallic taste in the mouth (131), linked to the metal's contact with the oral mucosa.

5. Incidence and repercussions of metallic taste

The incidence of metallic taste has been little studied, and exclusively in oncology. We identified 22 studies reporting the incidence of metallic taste, estimated at between 5.2% and 80% of patients. A meta-analysis (11) found an average incidence in 29% of patients treated for cancer. As a reminder, the French National Cancer Institute estimated the number of new cancers per year in metropolitan France at 382,000 in 2018. The prevalence of metallic taste is therefore far from negligible.

Similarly, the impact of metallic taste has only been “studied” in oncology. Only five studies have reported weight changes in their cancer patient populations (8, 132-135), in association with changes in taste and smell in cancer patients, but none were dedicated to MT. Consequently, it is not possible to assess the specific impact of MT on nutritional status. Similarly, two studies noted an alteration in quality of life due to taste and smell disorders, including MT (77, 136), but neither was specific to MT.



Figure 4: lactoferrine structure

For these populations, lactoferrin supplementation can significantly reduce the importance of metallic taste. Indeed, lactoferrin (Figure 4) is a protein found in many biological fluids (milk, saliva, tears) and serves to control iron levels in these fluids. When used for supplementation, lactoferrin complexes iron, reducing its concentration in saliva and thus limiting undesirable oral oxidation reactions (137).

ANNEX 3 : Mode opératoire des tests gustatifs

1. Objet

L'objectif est de développer un test psychophysique d'intensité gustative avec stimulation directe sur le nerf glossopharyngien et le nerf facial des deux côtés de la langue dans le cadre du projet TORCAD qui vise à évaluer les liens entre causes potentielles et apparition du goût métallique (torquegueusie) chez des personnes atteintes de CVADS.

2. Domaine d'application

Ce test peut s'appliquer à toutes personnes saines ou atteintes de pathologie (avant, pendant et après leur traitement) dont on veut déterminer la sensibilité gustative.

3. Matériel

- Blouses coton et jetable
- Charlotte
- Masque FFP2
- Visière de protection
- Gants en nitrile
- Virucide
- Poubelle classique
- Balance de précision 10^{-4} g
- Flaçon à prélèvement de 60 mL (VWR Réf 216-1808)
- Chronomètre
- Flacons pour composés sapides
- Eprouvette graduée
- Coton-tiges bébé
- Essuie-tout
- Bouteille d'eau Evian
- Gobelet
- Serviette
- Feuille descriptive des saveurs
- Feuille présentant l'ordre des dépôts
- Feuille de reconnaissance et notation d'intensité
- Feuille pesées salive
- Questionnaire Covid

4. Préparation des solutions sapides

Nous avons opté pour des préparations individuelles c'est-à-dire que chaque tube eppendorf contient la masse de composé nécessaire pour 1 patient (les quantités ont été volontairement surestimées de façon à ne pas manquer).

saveur	Composé	Masse composé / tube eppendorf (g)	Volume eau à ajouter (mL)	Concentration (g/L)
Amer	Caféine anhydre	0,5	24	20
Acide	Acide citrique	1,25	24	50
Salé	Chlorure de sodium	1,875	24	75
Sucré	Sucrose	21,25	12	850
Métallique	Sulfate ferreux	0,25	25	10

Dans le tableau ci-dessus sont indiquées la masse de chacun des composés et la quantité d'eau à ajouter.

- Verser le contenu du tube eppendorf dans un flacon d'environ 50 mL en verre de préférence brun
- Ajouter la quantité d'eau d'Evian nécessaire en rinçant à plusieurs reprises le tube eppendorf.
- Refermer les flacons et les agiter afin de solubiliser les solutions.

Attention ! pour les solutions « sucré » et « amer » la solubilisation est difficile, il est nécessaire d'utiliser un agitateur avec barreau magnétique, voire même de chauffer.

5. Préparation pour la collecte de salive

Tarer les pots à prélèvement avec leur bouchon et noter sa masse en gramme.

Déroulement de l'étude

► Avant l'arrivée du patient :

Côté expérimentateur : Mettre une blouse coton + blouse jetable + une charlotte + des gants en nitrile + un masque FFP2 + une visière de protection.

Côté patient, prévoir :

- un morceau d'essuie tout pour y déposer son masque pendant la séance,

- une bouteille d'eau Evian,
- un gobelet en carton, une serviette en papier
- les fiches d'information, de consentement et de reconnaissance + notation d'intensité des saveurs

► **Accueil du patient :**

- ① Demander au patient de se désinfecter les mains au gel hydro-alcoolique
- ② Lui demander de s'installer et de prendre connaissance de la fiche d'information et de signer la fiche de consentement
- ③ Lui dire de mettre son masque sur un essui-tout devant lui.
- ④ L'informer oralement du contenu de la séance :
 - vous allez réaliser une collecte de salive pour déterminer votre flux salivaire au repos.
 - des solutions de différentes saveurs telles que salé, sucré, amer, acide, métallique ainsi que de l'eau seront déposées sur votre langue à différentes localisations à l'aide un coton-tige bébé. Il vous sera demandé de fermer les yeux juste avant que le dépôt soit effectué.
- ⑤ 23 dépôts seront réalisés avec une pause de 1min entre chaque dépôt avec rinçage de la bouche à l'eau. Pour chaque saveur déposée, il vous est demandé de noter la saveur que vous percevez ainsi que son intensité sur l'échelle de « intensité faible » à « intensité forte ».

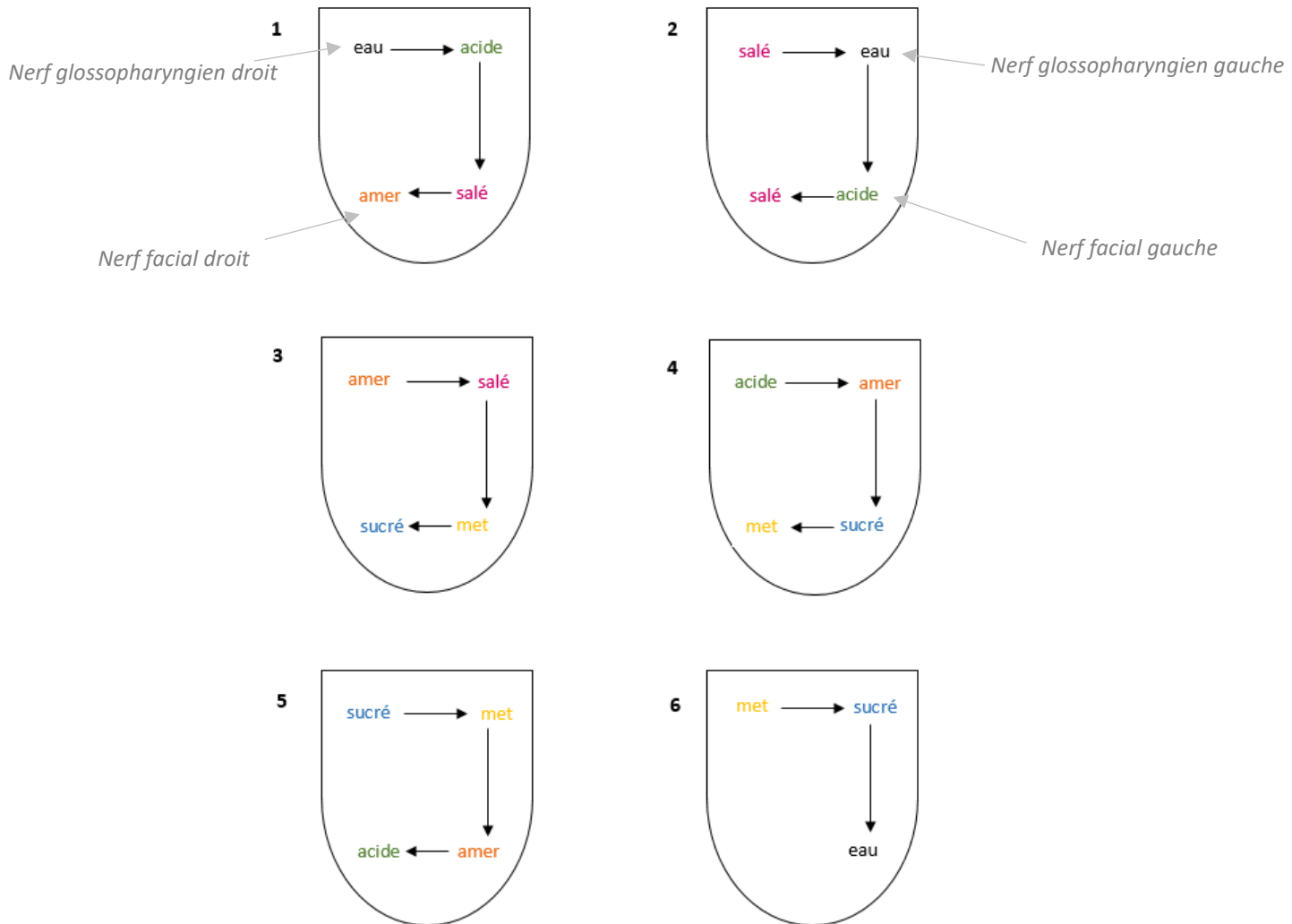
Mesure du flux salivaire

- Désinfecter le flacon de prélèvement avec une lingette imbibée de virucide devant le patient avant de lui donner.
- Demander au patient de déglutir avant de commencer le prélèvement et de ne pas faire de mouvements de bouche durant le prélèvement.
- Le patient déposera sa salive dans le flacon dès que celle-ci s'accumule en bouche et ce, pendant 10 minutes.
- Refermer le flacon à l'aide du bouchon et le désinfecter à l'aide d'une lingette.
- Peser le flacon avec le bouchon et noter sa masse en grammes.
- Détermination du flux salivaire au repos (mL/min) = (masse totale repos (g) – masse tare repos (g)) / 10

Test gustatif sur la langue

- Disposer, **à l'abri des regards du patient**, les flacons contenant les solutions sapides et l'eau, un chronomètre, les coton-tiges bébé, de l'essui-tout et la feuille présentant l'ordre des dépôts.
- Imbiber un des côtés d'un coton-tige bébé dans la solution indiquée durant environ 5 secondes. Un coton-tige servira deux fois (1 côté = 1 dépôt). Au besoin apposer le coton-tige sur du papier essui-tout pour enlever un excédent de solution
- Demander au patient de fermer les yeux.
- Poser le coton-tige au niveau de la zone de la langue indiquée durant environ 4 secondes.
- Demander au patient de fermer la bouche et de noter la saveur perçue ainsi que son intensité sur l'échelle discontinue correspondante à 5 points de « faiblement intense » à « fortement intense ».
- Enclencher le chronomètre pour 1 minute.
- Demander au patient de se rincer la bouche avec de l'eau Evian par plusieurs petites gorgées.
- Réaliser à nouveau cette manipulation pour chacun des 23 dépôts.

Les dépôts des solutions sont effectués selon l'ordre indiqué ci-dessous.



► A la fin de la séance :

Demander au patient de remettre son masque et de se désinfecter les mains au gel hydro-alcoolique.
 Aérer la salle pendant toute la durée du nettoyage et de la désinfection, et au minimum 10 minutes.
 Jeter le gobelet en carton, la serviette, l'essuie-tout et les coton-tiges dans une poubelle classique.
 Désinfecter les poignées de portes, l'espace patient (chaise, table, stylo, bouteille d'eau) au virucide.
 Mettre les EPI pour le nettoyage et la désinfection : gants pour tous les produits de nettoyage et lunettes de protection obligatoires.
 Jeter les gants, la charlotte et le masque, changer de blouse dès le nettoyage terminé et désinfecter la visière de protection au Septysan.
 Fermer et évacuer la poubelle.

ANNEX 4: final protocol of MDA dosage

Fluorometric Microplate Assay for 2-Thiobarbituric Acid Reactive Substances (TBARS) in microvolume of saliva samples

INTRODUCTION

This assay is generally part of a set of marker assays based on the same saliva sample collected from humans.

Substances reacting with thiobarbituric acid (TBA) are essentially malondialdehyde (MDA) (and HAE), a by-product of membrane peroxidation and one of the end products formed during oxidative stress. This method enables the determination of TBARS, which should be considered as a global test, allowing a global approach to lipoperoxidation. It is not totally specific for malondialdehyde.

The choice of direct calibration or spiking method depends on sample volume, and will be the same for the whole project (volumes of 450 or 2000 μL respectively required for each method). The **direct calibration method** has been chosen for the routine assay of salivary samples because of the low sample volume required, the latter usually being low-volume samples from clinical studies.

ABBREVIATIONS

MDA = Malondialdehyde

HAE = 4-hydroxyalkenal

TBA = Acide Thiobarbiturique

TBARS = Thiobarbituric Acid Reactive Substances

BHT = 2,6-di-tert-butyl-4-methylphenol

TMP = 1,1,3,3-Tetramethoxypropane

TCA = Trichloroacetic Acid

HCl = Chlorhydric Acid

NaOH = Sodium Hydroxyde

IQC = Internal Quality Control

PRINCIPLE OF THE PROCEDURE

The principle of the assay is based on the reaction between one molecule of MDA and two molecules of TBA in an acidic medium at high temperature, resulting in the formation of a fluorescent product (ex.532 nm/em.553 nm). To avoid artifactual MDA formation during the procedure, an antioxidant (BHT) is added to the reaction medium. Heating during incubation enables determination of total MDA, i.e. MDA present in free and bound form. Calibration of the measurement is performed using a range of MDA, a substance manufactured extemporaneously by acid hydrolysis of a solution of TMP.

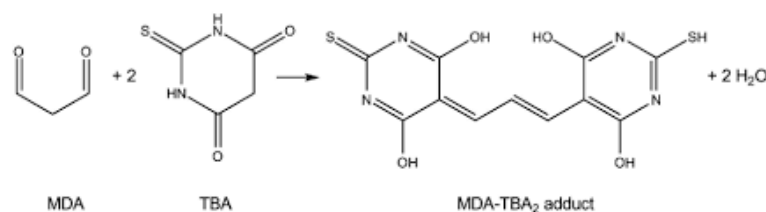


Figure : Formation of the fluorescent compound

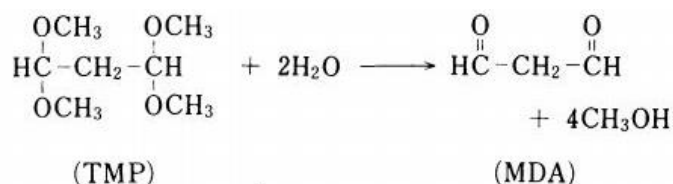


Figure : Transformation of TMP into MDA by acid hydrolysis (mole-by-mole reaction)

MATERIALS NEEDED

1. Analytical balance sensitive to 0.1mg
2. Fluorometric plate reader capable of exciting the sample at 532 nm and reading at 585 nm.
3. Polypropylene microcentrifuge tubes
4. Deionized Water (dH₂O)
5. Adjustable monochannel and multichannel micropipettes (10 – 1,000 µL) and tips
6. Heated bain-marie
7. Magnetic stirrer and magnetic bars
8. Classic laboratory glassware
9. 100 mL Reagent reservoirs
10. Non-treated 370µL working volume 96-well flat bottom black polypropylene wells
11. Reagents :

REAGENTS	N° CAS	REFERENCE
HCl	7647-01-0	CARLO ERBA 403871
TMP	102-52-3	SIGMA ALDRICH 108-383
TCA (4°C)	76-03-9	SIGMA ALDRICH T9159-100G
NaOH	1310-73-2	FLUKA 71692
TBA	504-17-6	SIGMA ALDRICH T5500-25G
BHT	128-37-0	SIGMA B1378-100G
Ethanol absolute anhydrous - RPE - For analysis -ACS	64-17-5	CARLO ERBA 4146072

STORAGE OF PREPARED REAGENTS

1. BHT solution : 4 hours
2. 1,25% TBA solution in 0,3% NaOH : 2 days at 4°C in dark
3. 9,8% TCA solution : 8 hours
4. TMP solutions : 8 hours
5. Reagent mixture (« F Solution ») : 4 hours

WARNINGS AND PRECAUTIONS

1. Wear gloves and safety glasses when performing this assay, as the acid used is corrosive.
2. Work with Biological Safety Cabinets to handle biological samples to protect the user and prevent him from being contaminated by eventual pathogeneus from the sample but no during the protocol steps with acid chemicals handling.

PROCEDURAL NOTES

1. A sample from a saliva pool (a mixture of saliva from several individuals), aliquoted and stored at -80°C , is introduced into all the plates, enabling inter-plate repeatability to be checked. It constitutes the IQC.
2. Samples, IQC and blanks should be run together on the same plate using the same standard curve.
3. Each sample is assayed in at least two replicates, and the concentration values obtained are averaged to determine the concentration of each sample. Each replicate is placed on a different plate. Example: if there are 3 plates, plate 1b is the replicate of plate 1, plate 2b the replicate of plate 2, etc.).

REAGENT PREPARATION

1. 0.3% NaOH solution: Weigh 1.5 g sodium hydroxide and dissolve in 500 mL ultrapure water.
2. Solution of 1.25% TBA in 0.3% NaOH: Weigh 312 mg thiorbarbituric acid and dissolve in 25 mL 0.3% NaOH. Stir until the compound is completely dissolved.
3. 1M HCl solution = 36.5 g/L: In a 10 mL flask, add 0.829 mL (i.e. 0.9865 g) of 37% HCl solution and make up to the mark with ultrapure water.
4. 9.8% TCA solution (for the reagent and a final concentration of 150mM in the reaction medium): Prepare on the same day, 0.98 g in 10 mL pure water
5. BHT solution 3.5 mM = 0.771 mg/mL: Prepare the same day, weigh out 15.42 mg BHT and dissolve in 20 mL 99% ethanol.
6. Reagent mixture: Solution F (for one plate): Mix 7.5 mL of 9.8% TCA solution + 5 mL of 1.25% TBA solution, then add 1 mL of 3.5 mM BHT solution to a beaker.
7. Standard range: MDA is prepared in the laboratory on the day of the assay from a commercial 6M TMP solution.
This preparation is then successively diluted to obtain two solutions at 6 and 0.6 μM , which are used to produce the range directly in the microplate.
 - a. Pre-dilution of TMP stock solution (6M):
 Solution A (60 mM): 100 μL commercial TMP + 9.9 mL pure water
 Solution B (1 mM): 200 μL of A + 11.2 mL ultrapure water + 600 μL 1M HCl in a water bath at 95°C for 5 minutes in a beaker.
 Solution C (60 μM): 1.2 of B + 18.8 mL pure water in a 20 mL flask
 - b. Dilution of MDA solution for calibration range:
 Solution D (6 μM): 2 mL C + 18 mL pure water in a 20 mL flask
 Solution E (600 nM): 2 mL D + 18 mL pure water in a 20 mL flask

SAMPLE STABILITY

1. Samples should be frozen at $-70^{\circ}\text{C}/-80^{\circ}\text{C}$ to prevent loss of MDA and HAE and sample oxidation. Samples should not be stored at -20°C .
2. Samples should not be refrozen several times and should be protected from light to avoid photooxidation.

SAMPLE PREPARATION

1. Thaw untreated saliva sample (whole saliva) at room temperature
2. Add BHT: 12 μL of 3.5mM BHT for 150 μL of sample (i.e. 1 well)

- Clarify this mixture in a 1.5 mL eppendorf tube (15,000g, 15 min, 4°C), recover the supernatant and freeze at -20°C overnight, or perform the assay extemporaneously.
- ICQ preparation: The salivary pool sample undergoes the same preparatory steps as the samples.

STANDARD CURVE PREPARATION

- MDA is prepared in the laboratory on the day of the assay from a commercial 6M TMP solution.
- This preparation is then successively diluted to obtain two solutions at 6 and 0.6 μM , which are used to produce the assay directly in the microplate (see REAGENT PREPARATION SECTION).
- The standard range and the reaction mixture are made directly in the microplates.

Table 1 : Fluorometric Standard Curve Preparation

MDA Conc. (μM)	nmole MDA/well	MDA Solution	$V_{\text{H}_2\text{O}}$ (μL)	V_{MDA} (μL)	$V_{\text{SOLUTION F}}$ (μL)	V_{TOTAL} (μL)
0	0	-	165	-	135	300
$2 \cdot 10^{-2}$	0,006	E	155	10		
$6 \cdot 10^{-2}$	0,018	E	135	30		
$1 \cdot 10^{-1}$	0,03	E	115	50		
$3 \cdot 10^{-1}$	0,09	D	150	15		
$9 \cdot 10^{-1}$	0,18	D	135	30		
$15 \cdot 10^{-1}$	0,3	D	115	50		
$22,5 \cdot 10^{-1}$	0,45	D	90	75		

ASSAY PROCEDURE

- Add ultra-pure water
- Add salivary samples and IQC with multichannel pipette
- Add MDA (solution D or E following **Table 1**)
- Then add solution F (TBA+TCA+BHT) using a multichannel pipette, mixing back and forth 5 times.
- Cover plate with cling film and aluminum foil, incubate 30 min at 60°C
- Stop the reaction by placing the microplate on ice for 10 min.
- Remove adhesive film from microplate
- Read fluorescence at ex.532 nm / em.553 nm in 10 minutes

Scheme 1: Sample Plate Layout if 2 plates in replicate

	1	2	3	4	5	6	7	8	9	10	11	12
A	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
B	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
C	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample

D	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
E	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
F	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
G	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
H	0	0.006	0.018	0.03	0.09	0.18	0.3	0.45	0	<i>CQI</i>	<i>CQI</i>	-

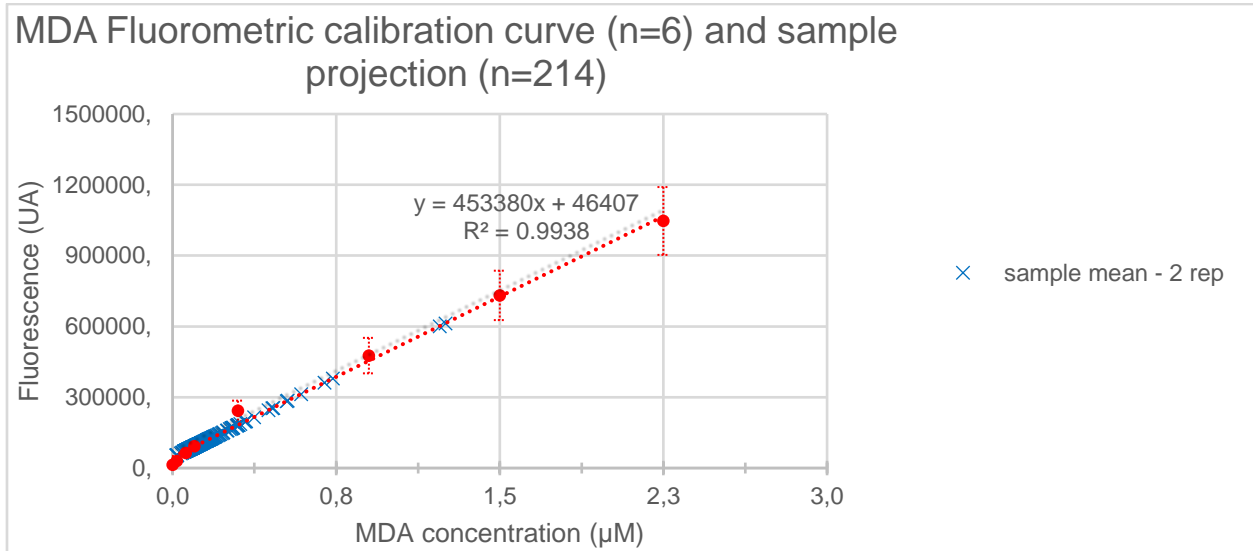
CALCULATIONS

1. Fluorescence values obtained for all duplicated wells.
2. Plot a standard curve using the fluorescence values for each Standard versus the MDA concentration for each Standard. The equation of the line can be found using a linear fit method.
3. Do not subtract the fluorescence value of the Blank from the Sample fluorescence value.
4. Calculate the MDA concentration for each Sample using the fluorescence value and the equation generated by the Standard Curve.
5. Assay quality control
 - a. Intra-plate coefficient of variation around the mean is <10% for range points
 - b. When plotting the fluorescence of the range points at different concentrations, the alignment of the points (and an $R^2 > 0.98$) attests to the smooth running of the reaction and the linearity (instrumental response) of the assay.
 - c. The TBARS concentration calculated for the IQC (salivary pool), within the concentration range of this protocol, should have a repeatability of this order (e.g.: 6 replicates):

	VOC (%) on concentration values calculated
intra-plate	<10%
Inter-plate	<15%

- d. The sample concentration value is valid if the fluorescence value is within the measuring range, i.e. for this assay: between the absorbance value of the point at 0.006 and that at 0.09 nmol/well of MDA. Otherwise, if the value is not within the measuring range, dilute the sample 2 times if the value is above the upper limit and repeat the assay under the same conditions. If the value is below the lower limit, indicate 0.02 μ M as the result.

Figure: Typical Standard Curve and samples projection



Discussion and Limitations

The chosen method for total MDA measurement is the method using TBA, with detection conducted through fluorescence. Upon addition of TBA in the presence of MDA, the addition of two TBA molecules onto the MDA is observed (82, 147, 162). The mechanism of the reaction between TBA and MDA is provided in Figure 6.

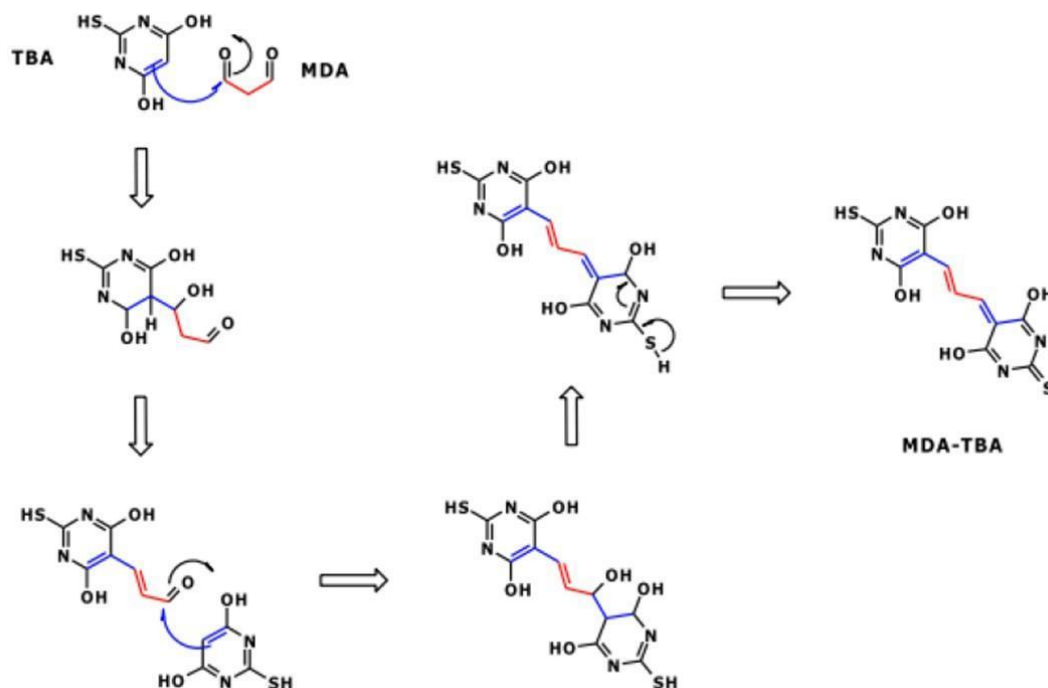


Figure 6: mechanism of the reaction between TBA and MDA

The fluorescence of the formed complex can be measured at 553 nm (emission) and 532 nm (excitation) (161).

It is important to note that this method allows for the measurement of TBARS and not solely MDA. It is non-specific because TBA can react with other molecules (282). Indeed, as discussed in the previous section, saliva contains numerous molecules that could interfere with the measurement of salivary

MDA (172), such as other oxidation products like 4-HNE. Furthermore, the challenging experimental conditions (high acidity + high temperature) can lead to additional oxidation of available biological compounds in the sample, resulting in an overestimation of MDA quantification (172).

However, despite controversies regarding the specificity of TBA towards compounds other than MDA, it remains the most widely used method as an index of lipid peroxidation

Moving forward, it is acknowledged that MDA is measured with the inherent imprecision it entails.

Several methods can be employed to control these artifacts:

- Since saliva contains numerous molecules capable of reacting with TBA, it is necessary to precipitate the lipoprotein fractions to minimize the formation of interfering substances. The use of precipitating agents enables the assay to be specific to MDA (148).
- Additionally, the use of BHT (an antioxidant) helps prevent additional formation of MDA during the assay (173).

The effect of storage over 18 months, a common timeframe in the context of research during clinical studies, has not been evaluated.

ANNEX 5: questionnaires

1. EORTC QLQ 30 (187)

	Pas du tout	Un peu	Assez	Beaucoup
1. Avez-vous des difficultés à faire certains efforts physiques pénibles comme porter un sac à provision chargé ou une valise?	1	2	3	4
2. Avez-vous des difficultés à faire une longue promenade?	1	2	3	4
3. Avez-vous des difficultés à faire un petit tour dehors?	1	2	3	4
4. Etes-vous obligé(e) de rester au lit ou dans un fauteuil pendant la journée?	1	2	3	4
5. Avez-vous besoin d'aide pour manger, vous habiller, faire votre toilette ou aller aux toilettes?	1	2	3	4
Au cours de la semaine passée :	Pas du	Un	Assez	Beaucoup
		tout	peu	
6. Avez-vous été gêné(e) pour faire votre travail ou vos activités de tous les jours?	1	2	3	4
7. Avez-vous été gêné(e) dans vos activités de loisirs?	1	2	3	4
8. Avez-vous eu le souffle court?	1	2	3	4
9. Avez-vous ressenti de la douleur?	1	2	3	4
10. Avez-vous eu besoin de repos?	1	2	3	4
11. Avez-vous eu des difficultés pour dormir?	1	2	3	4
12. Vous êtes-vous senti(e) faible?	1	2	3	4
13. Avez-vous manqué d'appétit?	1	2	3	4
14. Avez-vous eu des nausées (mal au cœur) ?	1	2	3	4
15. Avez-vous vomi?	1	2	3	4
Au cours de la semaine passée:	Pas du	Un	Assez	Beaucoup
	Tout	peu		
16. Avez-vous été constipé(e)?	1	2	3	4

17. Avez-vous eu de la diarrhée?	1	2	3	4
18. Etiez-vous fatigué(e)?	1	2	3	4
19. Des douleurs ont-elles perturbé vos activités quotidiennes?	1	2	3	4
20. Avez-vous eu des difficultés à vous concentrer sur certaines choses, par exemple, pour lire le journal ou regarder la télévision?	1	2	3	4
21. Vous êtes-vous senti(e) tendu(e)?	1	2	3	4
22. Vous êtes-vous fait du souci?	1	2	3	4
23. Vous êtes-vous senti(e) irritable?	1	2	3	4
24. Vous êtes-vous senti(e) déprimé(e)?	1	2	3	4
25. Avez-vous eu des difficultés pour vous souvenir de certaines choses?	1	2	3	4
26. Votre état physique ou votre traitement médical vous ont-ils gêné(e) dans votre vie familiale?	1	2	3	4
27. Votre état physique ou votre traitement médical vous ont-ils gêné(e) dans vos activités sociales (par exemple, sortir avec des amis, aller au cinéma...)?	1	2	3	4
28. Votre état physique ou votre traitement médical vous ont-ils causé des problèmes financiers?	1	2	3	4

Pour les questions suivantes, veuillez répondre en entourant le chiffre entre 1 et 7 qui s'applique le mieux à votre situation

29. Comment évalueriez-vous votre état de santé au cours de la semaine passée?

1	2	3	4	5	6	7
Très mauvais					Excellent	

30. Comment évalueriez-vous l'ensemble de votre qualité de vie au cours de la semaine passée?

1	2	3	4	5	6	7
Très mauvais					Excellent	

2. EORTC HN 35 (76)

Les patients rapportent parfois les symptômes ou problèmes suivants. Pourriez-vous indiquer s'il vous plait, si durant la semaine passée, vous avez été affecté(e) par l'un de ces symptômes ou problèmes. Entourez s'il vous plait, le chiffre qui correspond le mieux à votre situation

Au cours de la semaine passée:	Pas du tout	Un peu	Assez	Beaucoup
31. Avez-vous eu mal dans la bouche ?	1	2	3	4
32. Avez-vous mal à la mâchoire ?	1	2	3	4
33. Avez-vous eu des douleurs dans la bouche ?	1	2	3	4
34. Avez-vous mal à la gorge ?	1	2	3	4
35. Avez-vous eu des problèmes en avalant des liquides ?	1	2	3	4
36. Avez-vous eu des problèmes en avalant des aliments écrasés ?	1	2	3	4
37. Avez-vous eu des problèmes en avalant des aliments solides ?	1	2	3	4
38. Vous êtes-vous étouffé(e) en avalant ?	1	2	3	4
39. Avez-vous eu des problèmes de dents ?	1	2	3	4
40. Avez-vous eu des problèmes à ouvrir largement la bouche ?	1	2	3	4
41. Avez-vous eu la bouche sèche ?	1	2	3	4
42. Avez-vous eu une salive collante ?	1	2	3	4
43. Avez-vous eu des problèmes d'odorat ?	1	2	3	4
44. Avez-vous eu des problèmes de goût ?	1	2	3	4
45. Avez-vous toussé ?	1	2	3	4
46. Avez-vous été enroué(e) ?	1	2	3	4
47. Vous êtes-vous senti(e) mal ?	1	2	3	4
48. Votre apparence vous a-t-elle préoccupé(e) ?	1	2	3	4

Au cours de la semaine passée:	Pas du tout	Un peu	Assez	Beaucoup
49. Avez-vous eu des difficultés à manger ?	1	2	3	4
50. Avez-vous eu des difficultés à manger devant Votre famille ?	1	2	3	4
51. Avez-vous eu des difficultés à manger devant D'autres personnes ?	1	2	3	4
52. Avez-vous eu des difficultés à prendre plaisir aux repas ?	1	2	3	4
53. Avez-vous eu des difficultés à parler à d'autres personnes ?	1	2	3	4
54. Avez-vous eu des difficultés à parler au téléphone ?	1	2	3	4
55. Avez-vous eu des difficultés à avoir un contact social avec votre famille ?	1	2	3	4
56. Avez-vous eu des difficultés à avoir un contact social avec vos amis ?	1	2	3	4
57. Avez-vous eu des difficultés à sortir en public ?	1	2	3	4
58. Avez-vous eu des difficultés à avoir un contact physique avec votre famille ou vos amis?	1	2	3	4
59. Avez-vous éprouvé moins d'intérêt aux relations sexuelles?	1	2	3	4
60. Avez-vous éprouvé moins de plaisirs sexuels ?	1	2	3	4
Au cours de la semaine passée:		Oui		Non
61. Avez-vous pris des anti-douleurs ?		1		2
62. Avez-vous pris des compléments nutritionnels (à l'exclusion de vitamines) ?		1		2
63. Avez-vous utilisé une sonde d'alimentation ?		1		2
64. Avez-vous perdu du poids ?		1		2
65. Avez-vous pris du poids ?		1		2

3. Questionnaire sur le goût métallique (35)

1. Avez-vous eu un changement du goût depuis le diagnostic de cancer ?
 - a) Non (*si c'est le cas, vous avez fini, merci de ne pas remplir la fin du questionnaire*)
 - b) Oui

2. Quelle serait la cause des changements du goût à votre avis ?
 - a) Le goût a changé à cause de la maladie
 - b) Le goût a changé à cause du traitement
 - c) Le goût a changé à la fois à cause de la maladie et du traitement
 - d) Le goût a changé à cause de quelque chose d'autre
 - e) Je n'ai pas de réponse à cette question

Les questions suivantes concernent le goût durant la semaine passée. Si vous considérez que votre goût est normal cette semaine (comme avant le diagnostic) vous avez fini, merci de ne pas remplir la fin du questionnaire.

Pourriez-vous choisir quel état l'énoncé ci-dessous s'applique à vous ?

Entourer le chiffre qui correspond au mieux à votre état.

	Pas du tout	Un peu	Moyennement	Beaucoup	Je ne sais pas
3. Je me sens nauséeux	1	2	3	4	5
4. L'odeur de la nourriture m'incommode	1	2	3	4	5
5. J'ai du mal à digérer la nourriture	1	2	3	4	5
6. J'ai moins d'appétit	1	2	3	4	5
7. J'ai la bouche sèche	1	2	3	4	5
8. J'ai des difficultés pour avaler certains aliments	1	2	3	4	5
9. Le sucre me dérange	1	2	3	4	5
10. Le salé me dérange	1	2	3	4	5
11. L'acidité me dérange	1	2	3	4	5
12. L'amertume me dérange	1	2	3	4	5
13. Mon appétit est meilleur	1	2	3	4	5
14. J'aime manger des bonbons	1	2	3	4	5
15. Le chocolat me dérange	1	2	3	4	5
16. J'ai des difficultés à manger de la viande rousse (boeuf, cheval, porc, ...)	1	2	3	4	5
17. Le gras me dérange	1	2	3	4	5
18. La nourriture chaude me dérange	1	2	3	4	5

Pourriez-vous indiquer, saveur par saveur, comment vous percevez cette saveur par rapport à avant votre traitement ?

Entourer le chiffre qui correspond au mieux à votre état.

	Je ne ressens plus rien	J'ai beaucoup de difficultés à percevoir la saveur	J'ai quelques difficultés à sentir la saveur	La saveur est normale	La saveur est plus forte	Je ne sais pas
19. La nourriture en général	1	2	3	4	5	6
20. Le sucré	1	2	3	4	5	6
21. Le salé	1	2	3	4	5	6
22. L'acidité	1	2	3	4	5	6
23. L'amertume	1	2	3	4	5	6

Pourriez-vous préciser quel état s'applique à vous ?

Entourer le chiffre qui correspond au mieux à votre état

	Pas du tout	Un peu	Moyennement	Beaucoup	Je ne sais pas
24. Je ne suis pas capable de percevoir l'odeur de la nourriture	1	2	3	4	5
25. Tout a mauvais goût	1	2	3	4	5
26. La nourriture n'a pas le goût qu'elle devrait avoir	1	2	3	4	5
27. J'ai un goût amer dans la bouche	1	2	3	4	5
28. J'ai un mauvais goût dans la bouche	1	2	3	4	5
29. Tout a un goût amer	1	2	3	4	5
30. Je préfère manger froid que chaud	1	2	3	4	5
31. Tout a bon goût	1	2	3	4	5
32. Le mauvais goût a un impact négatif sur ma qualité de vie	1	2	3	4	5

33. Certaines personnes peuvent avoir des perceptions différentes concernant le goût de certains aliments.

Dans ce cas, le goût de ces aliments peut être différent de ce qu'il était avant le traitement. Avez-vous eu l'impression que le goût de certains aliments était différent de celui avant le diagnostic ?

a) Je n'ai pas eu cette impression de goût différent

b) J'ai eu cette impression de goût différent pour certains aliments, ce goût ressemblait au goût (*choix multiples possibles*) :

- De sang
- Amer
- D'une substance chimique
- D'une substance moisie
- D'un médicament
- De métal
- Sucré
- Salé
- Acide
- Autres, nommez-le goût :

34. Certains patients peuvent avoir un goût continu dans la bouche.

Avez-vous eu un goût continu dans la bouche que vous n'aviez pas avant votre diagnostic ?

- a)** Je n'ai pas eu cette impression de goût continu dans la bouche
- b)** J'ai eu cette impression de goût continu dans la bouche. Ce goût ressemblait à (*choix multiples possibles*) :

- De sang
- Amer
- D'une substance chimique
- D'une substance moisie
- D'un médicament
- De métal
- Sucré
- Salé
- Acide
- Autres, nommez-le goût :

Répondez aux questions suivantes uniquement si vous avez eu une impression de goût métallique.

Si vous n'avez pas eu cette impression de goût métallique, vous avez terminé le questionnaire.

Pourriez-vous préciser quel état s'applique à vous ?

Entourer le chiffre qui correspond au mieux à votre état

	Pas du tout	Peu	Moyennement	Beaucoup	Je ne sais pas
35. Le goût métallique est intense	1	2	3	4	5
36. Le goût métallique me dérange avec la nourriture en général	1	2	3	4	5
37. Le goût métallique me dérange avec seulement quelques aliments	1	2	3	4	5
38. Le goût métallique devient plus fort quand je mange	1	2	3	4	5
39. Le goût métallique est présent toute la journée	1	2	3	4	5

40. Considérez-vous ce goût métallique comme l'aspect le plus négatif des modifications de votre goût ?

- a) Non
- b) Oui
- c) Je ne sais pas

41. Combien de temps avec-vous eu ce goût métallique dans la bouche ?

- a) Moins d'une semaine
 - b) Entre une semaine et un mois
 - c) Entre un et trois mois
 - d) Plus de trois mois
 - e) Je ne peux pas répondre à cette question
- Vous avez terminé de remplir le questionnaire. Merci de votre coopération.*

ANNEX 6: Supplementary appendix to Support Care Cancer 2022;30:5691-702 (11)

Table S1: PRISMA 2020 statements on abstract

Section and Topic	Item #	Checklist item	Reported (Yes/No)
TITLE			
Title	1	Identify the report as a systematic review.	Yes
BACKGROUND			
Objectives	2	Provide an explicit statement of the main objective(s) or question(s) the review addresses.	Yes
METHODS			
Eligibility criteria	3	Specify the inclusion and exclusion criteria for the review.	Yes
Information sources	4	Specify the information sources (e.g. databases, registers) used to identify studies and the date when each was last searched.	Yes
Risk of bias	5	Specify the methods used to assess risk of bias in the included studies.	Yes
Synthesis of results	6	Specify the methods used to present and synthesise results.	Yes
RESULTS			
Included studies	7	Give the total number of included studies and participants and summarise relevant characteristics of studies.	Yes
Synthesis of results	8	Present results for main outcomes, preferably indicating the number of included studies and participants for each. If meta-analysis was done, report the summary estimate and confidence/credible interval. If comparing groups, indicate the direction of the effect (i.e. which group is favoured).	Yes
DISCUSSION			
Limitations of evidence	9	Provide a brief summary of the limitations of the evidence included in the review (e.g. study risk of bias, inconsistency and imprecision).	Yes
Interpretation	10	Provide a general interpretation of the results and important implications.	Yes
OTHER			
Funding	11	Specify the primary source of funding for the review.	Yes
Registration	12	Provide the register name and registration number.	NA

From: Page MJ et al. (283)

For more information, visit: <http://www.prisma-statement.org/>

Table S2: PRISMA Checklist on full paper

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Page 1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Table s1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Page 2
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Page 2&3
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Page 3
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Page 3
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Page 3 & 4
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Page 4
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Page 4
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Page 4
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Page 4
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Page 4
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Page 4
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Page 4
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Page 4

Section and Topic	Item #	Checklist item	Location where item is reported
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Page 4
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	Page 4
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	Page 4
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Page 4
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	NO
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Page 5 Figure 1
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Page 5
Study characteristics	17	Cite each included study and present its characteristics.	Page 5 Table 1
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Page 5&6 Figure 2 Table 2 Figure S1
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Table 1
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Page 5
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Page 6 Figure 3
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	Page 6 Figures S2 to S5
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Page 6 Figure S6
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Page 6 Figure 4
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	-

Section and Topic	Item #	Checklist item	Location where item is reported
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Page 6
	23b	Discuss any limitations of the evidence included in the review.	Page 7
	23c	Discuss any limitations of the review processes used.	Page 8
	23d	Discuss implications of the results for practice, policy, and future research.	Page 8
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Page 2
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Page 2
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	Page 2
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Page 8
Competing interests	26	Declare any competing interests of review authors.	Page 8
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Table 1 and Supplementary materials

From: Page MJ et al. (283)

For more information, visit: <http://www.prisma-statement.org/>

		B.L.T. randomization process	B.L.T. deviations from intended intervention	B.L.T. missing outcome data	B.L.T. measurement of the outcome	B.L.T. selection of the reported results			Overall
RCT	Cusnir et al. (206)	-	-	X	X	-			X
	Halyard et al. (136)	-	+	X	-	-			X
	Soares et al. (135)	-	-	+	X	-			X
		B.L.T. confounding	B.L.T. selection of participants	B.L.T. classification of interventions	B.L.T. deviation from intended interventions	B.L.T. missing data	B.L.T. measurement of outcomes	B.L.T. selection of the reported results	Overall
Non RCT	Al-Taie et al. (202)	X	X	-	X	-	X	+	X
	Alvarez et al. (203)	X	X	-	-	+	X	+	X
	Amezaga et al. (204)	-	+	+	+	+	-	+	-
	Bernardhson et al. (205)	-	+	+	+	-	-	+	-
	Coa et al. (8)	X	X	-	X	+	X	+	X
	Derksen et al. (207)	X	-	-	-	X	-	X	X

Genvresse et al. (208)								
Ijpma et al. (132)								
Ijpma et al. (35)								
Lindsey et al. (134)								
Logan et al. (122)								
McDaniel et al. (210)								
McGreevy et al. (211)								
Newell et al. (189)								
Ponticelli et al. (212)								
Rehwaldt et al. (213)								
Ui Dhuibir et al. (214)								

















Wickham et al. (77)								
Wilken et al. (215)								

Table S3: Review authors' judgments about each risk of bias item, presented for each included study.

 : low;
  : moderate;
  : serious;
  : critical

B.L.T.: Bias Linked To

Doc S1: R code used for meta-analysis

```

rm(list=ls())
setwd("~/OneDrive/Documents/Publis/SLR Torgueusie/Manuscript/traitements")
library(readr)
library("meta", lib.loc=~ /Library/R/3.5/library")
Forest_MTB <- read_delim("Forest-MTB.csv", ";", escape_double = FALSE, trim_ws = TRUE)
View(Forest_MTB)

#Meta-all studies
mp_tot<-metaprop(Forest_MTB$MT,Forest_MTB$N,studlab=c(Forest_MTB$Study),method =
"Inverse")
quartz("tot", 10,7,dpi=100)
forest(mp_tot,col.diamond.random="red", col.diamond.lines.random = "darkred",col.random="red",
comb.fixed = FALSE,print.Q.subgroup=TRUE,test.subgroup.random = TRUE, weight.study =
"random")

#funnel
quartz("tot", 10,7,dpi=100)
funnel(mp_tot, col="red", bg="red", comb.fixed = FALSE, col.random="red", cex=1.5,
studlab=TRUE,pos.studlab=3, xlim=c(-3.5,2.5),lwd=2)

metabias(mp_tot)

#Leave one out analysis

metainf(mp_tot, pooled = "random")
quartz("tot", 10,7,dpi=100)
forest(metainf(mp_tot, pooled = "random"))

#Meta senso
mp_sens<-metaprop(Forest_MTB$MT,Forest_MTB$N,studlab=c(Forest_MTB$Study),
byvar=c(Forest_MTB$Senso),bylab="Senso risk of bias",title=gs("metallic"),
outclab = "Metalic taste prevalence", method = "Inverse")
mp_sens

quartz("Senso", 10,7,dpi=100)
forest(mp_sens,col.diamond.random="red", col.diamond.lines.random =
"darkred",col.random="red",
comb.fixed = FALSE,print.Q.subgroup=TRUE,test.subgroup.random = TRUE, weight.study =
"random",col.by="grey40")

#Meta Treatment
mp_treat<-
metaprop(Forest_MTB$MT,Forest_MTB$N,studlab=c(Forest_MTB$Study),byvar=c(Forest_MTB$Trea
tment),bylab="Treatment",method = "Inverse")
mp_treat
quartz("Treatment",10,7,dpi=100)
forest(mp_treat,col.diamond.random="red", col.diamond.lines.random =
"darkred",col.random="red",
comb.fixed = FALSE,print.Q.subgroup=TRUE,test.subgroup.random = TRUE, col.by="grey40")

```

#Meta Cancer type

```
mp_canc<-
metaprop(Forest_MTB$MT,Forest_MTB$N,studlab=c(Forest_MTB$Study),byvar=c(Forest_MTB$Cancer),bylab="Cancer",method = "Inverse")
mp_canc
quartz("Cancer",10,7,dpi=100)
forest(mp_canc,col.diamond.random="red", col.diamond.lines.random = "darkred",col.random="red",
      comb.fixed = FALSE,print.Q.subgroup=TRUE,test.subgroup.random = TRUE, col.by="grey40")
```

#meta Risk of Bias

```
mp_risk<-
metaprop(Forest_MTB$MT,Forest_MTB$N,studlab=c(Forest_MTB$Study),byvar=c(Forest_MTB$Overall),bylab="Overall risk of bias",method = "Inverse")
mp_risk
quartz("Risk",10,7,dpi=100)
forest(mp_risk,col.diamond.random="red", col.diamond.lines.random = "darkred",col.random="red",
      comb.fixed = FALSE,print.Q.subgroup=TRUE,test.subgroup.random = TRUE,col.by="grey40")
```

#Meta wo critical

```
Forest_MTB_Crit<-Forest_MTB[-c(15:16),]
mp_tot<-
metaprop(Forest_MTB_Crit$MT,Forest_MTB_Crit$N,studlab=c(Forest_MTB_Crit$Study),method = "Inverse")
quartz("tot-Critical", 10,7,dpi=100)
forest(mp_tot,col.diamond.random="red", col.diamond.lines.random = "darkred",col.random="red",
      comb.fixed = FALSE,print.Q.subgroup=TRUE,test.subgroup.random = TRUE, weight.study = "random")
```


Figure S1a: metallic taste sensory related method authors' judgments about each risk of bias item, presented for each included study

Study	Risk of bias			Overall
	D1	D2	D3	
Al-Taie	⊗	⊗	⊕	⊗
Amezaga	⊗	-	⊕	-
Alvarez	⊗	⊗	⊗	⊗
Bernhardson	⊗	⊗	-	⊗
Coa	⊗	⊗	⊗	⊗
Cusnir	?	-	⊗	⊗
Derksen	⊕	-	?	-
Genvresse	⊕	⊗	?	⊗
Halyard	-	⊗	⊗	⊗
Ijpma 2016	⊕	⊕	-	⊕
Ijpma 2017	-	⊕	⊕	⊕
Lindsey	⊕	⊗	?	⊗
Logan	⊕	⊕	?	-
Mc Greevy	⊕	-	?	-
McDaniel	⊗	⊗	⊕	⊗
Newell	-	-	⊕	-
Ponticelli	?	⊗	⊕	⊗
Rehwaldt	-	-	⊕	-
Soares	?	⊕	⊗	⊗
Ui Dhuibhir	⊗	-	⊗	⊗
Wickham	⊗	⊗	⊕	⊗
Wilken	⊗	⊗	⊕	⊗

D1: Bias due to time of evaluation
D2: Bias due to specificity of evaluation
D3: Bias due training of patients

Judgement
⊗ High
- Unclear
⊕ Low
? No information

Figure S1b: metallic taste sensory related method authors' judgments about each risk of bias item, presented as percentages across all included studies.

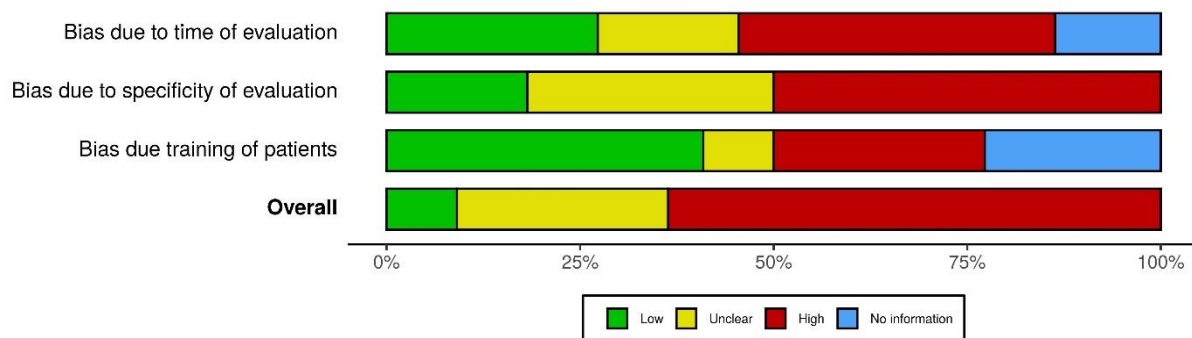


Figure S2: Random effect meta-analysis of metallic taste prevalence in cancer patients based on type of cancer.

Red dotted line represents the pooled random effects estimate. HNC: Head and Neck Cancer, NI: No Information

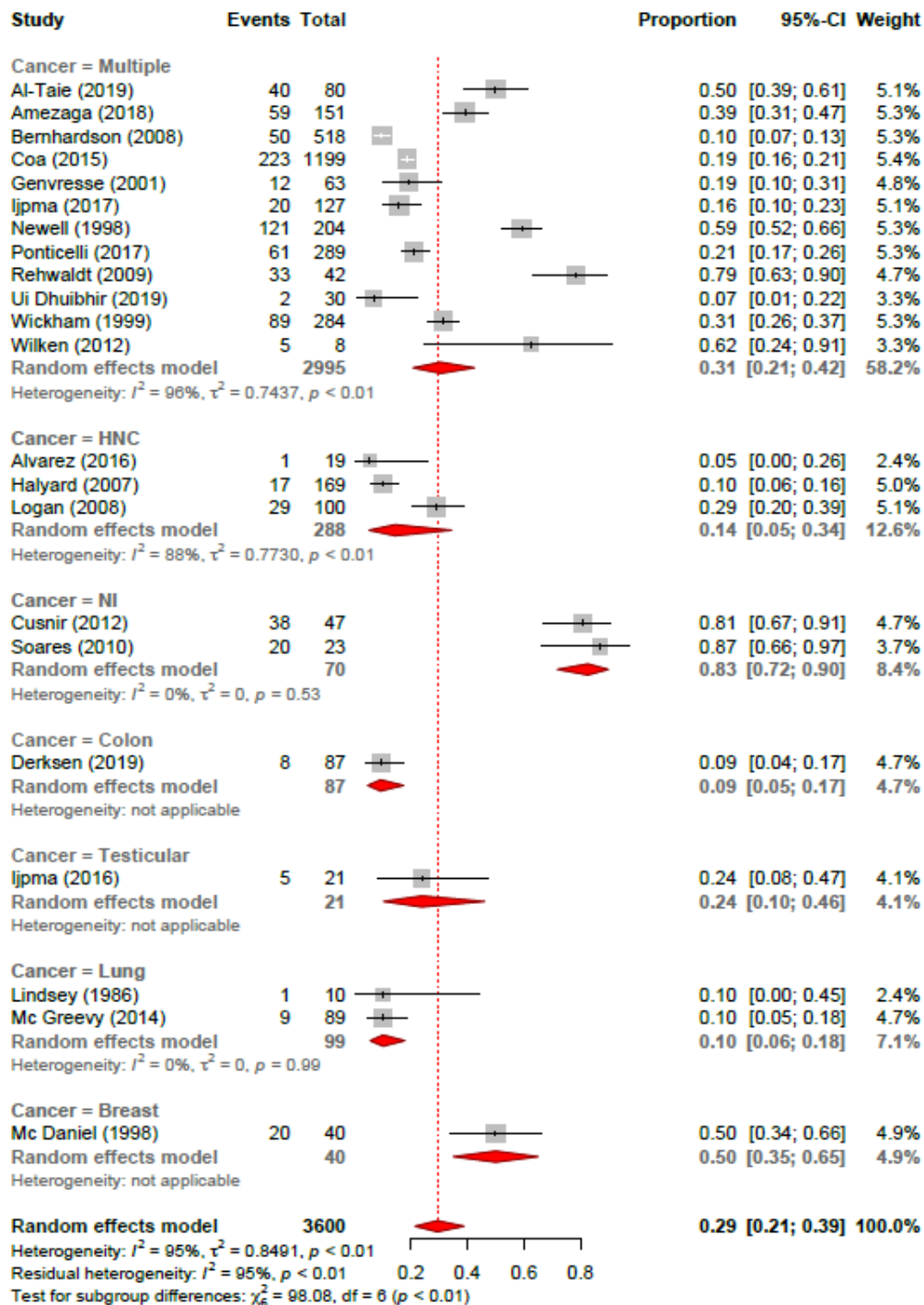


Figure S3: Random effect meta-analysis of metallic taste prevalence in cancer patients based on type of treatment.

Red dotted line represents the pooled random effects estimate. RT: radiotherapy, CT: chemotherapy

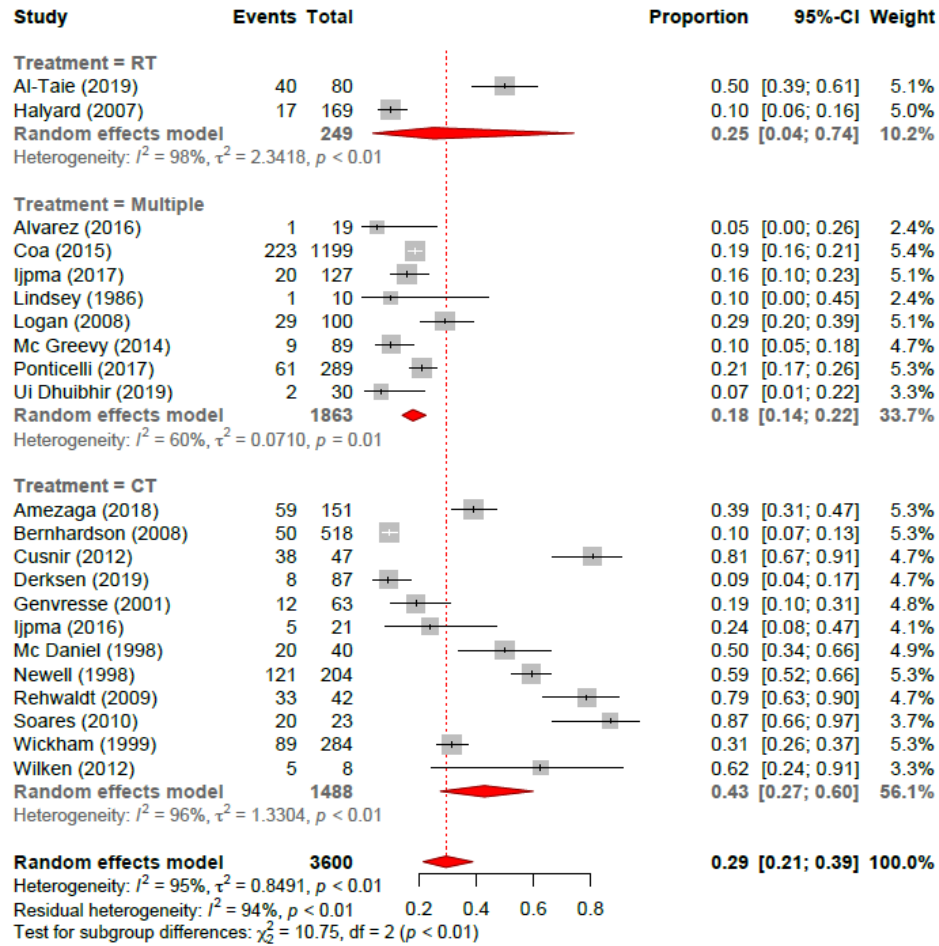


Figure S4: Random effect meta-analysis of metallic taste prevalence in cancer patients based on overall risk of bias level.

Red dotted line represents the pooled random effects estimate.

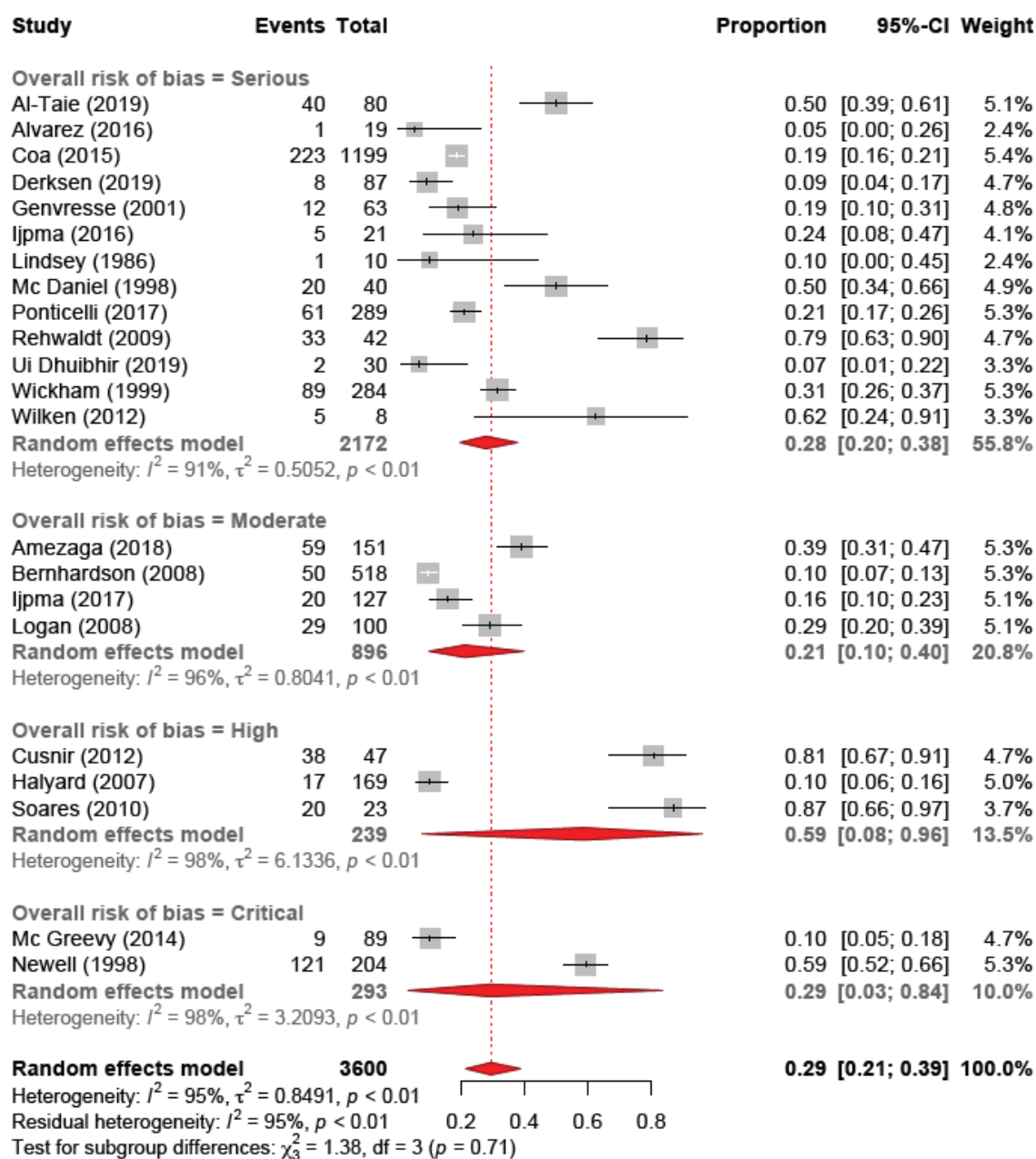


Figure S5: Random effect meta-analysis of metallic taste prevalence in cancer patients based on sensory risk of bias level.

Red dotted line represents the pooled random effects estimate.

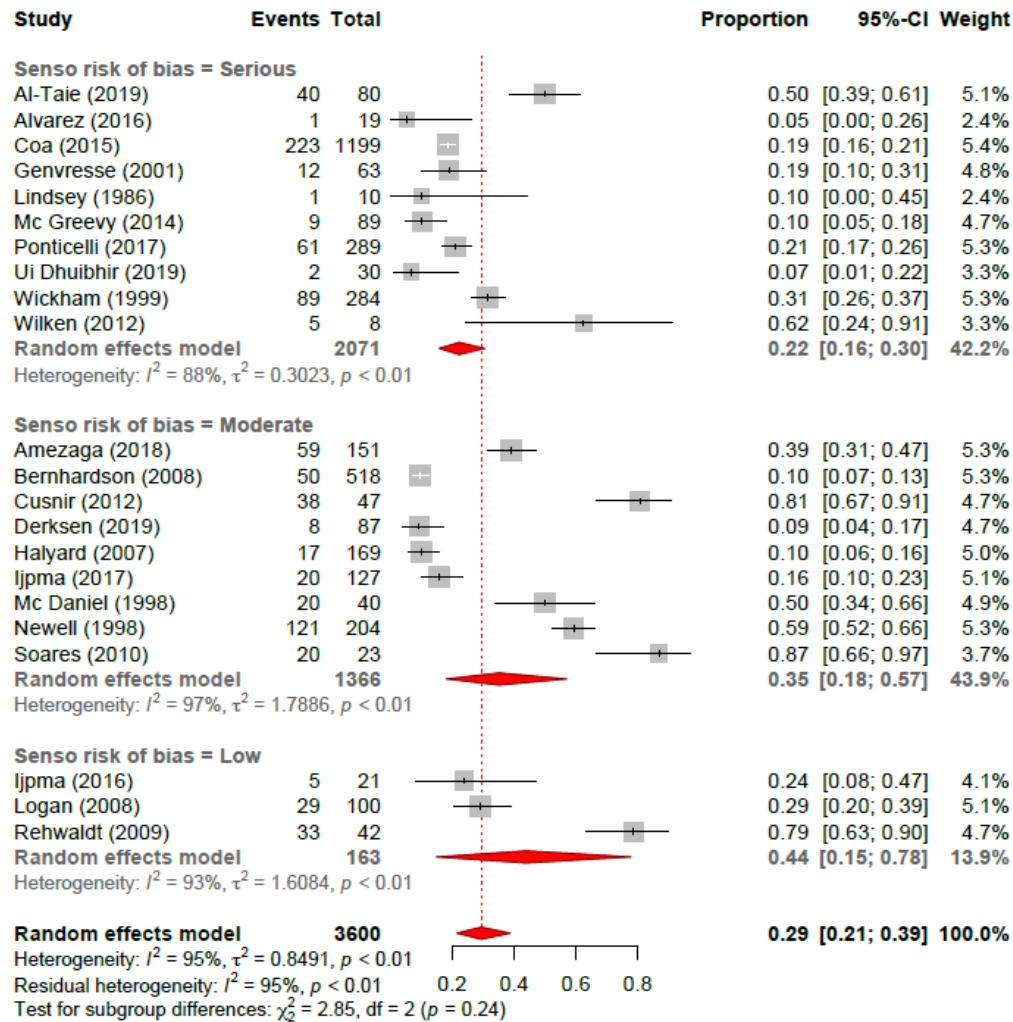


Figure S6: Random effect meta-analysis of metallic taste prevalence in cancer patients: leave-one-out analysis.

Red dotted line represents the pooled random effects estimate.

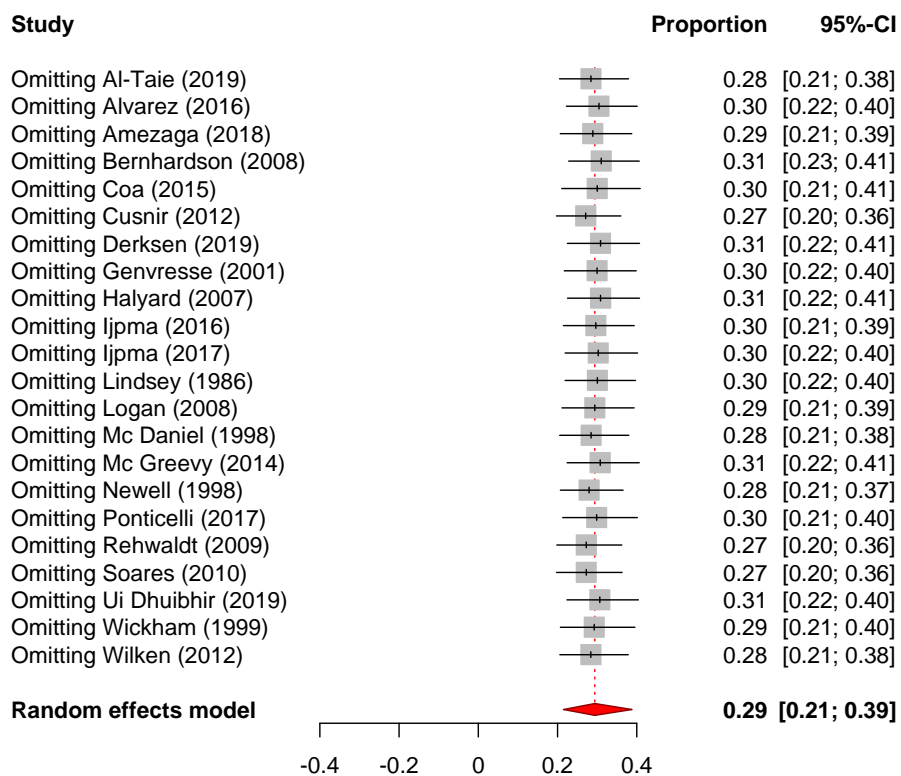
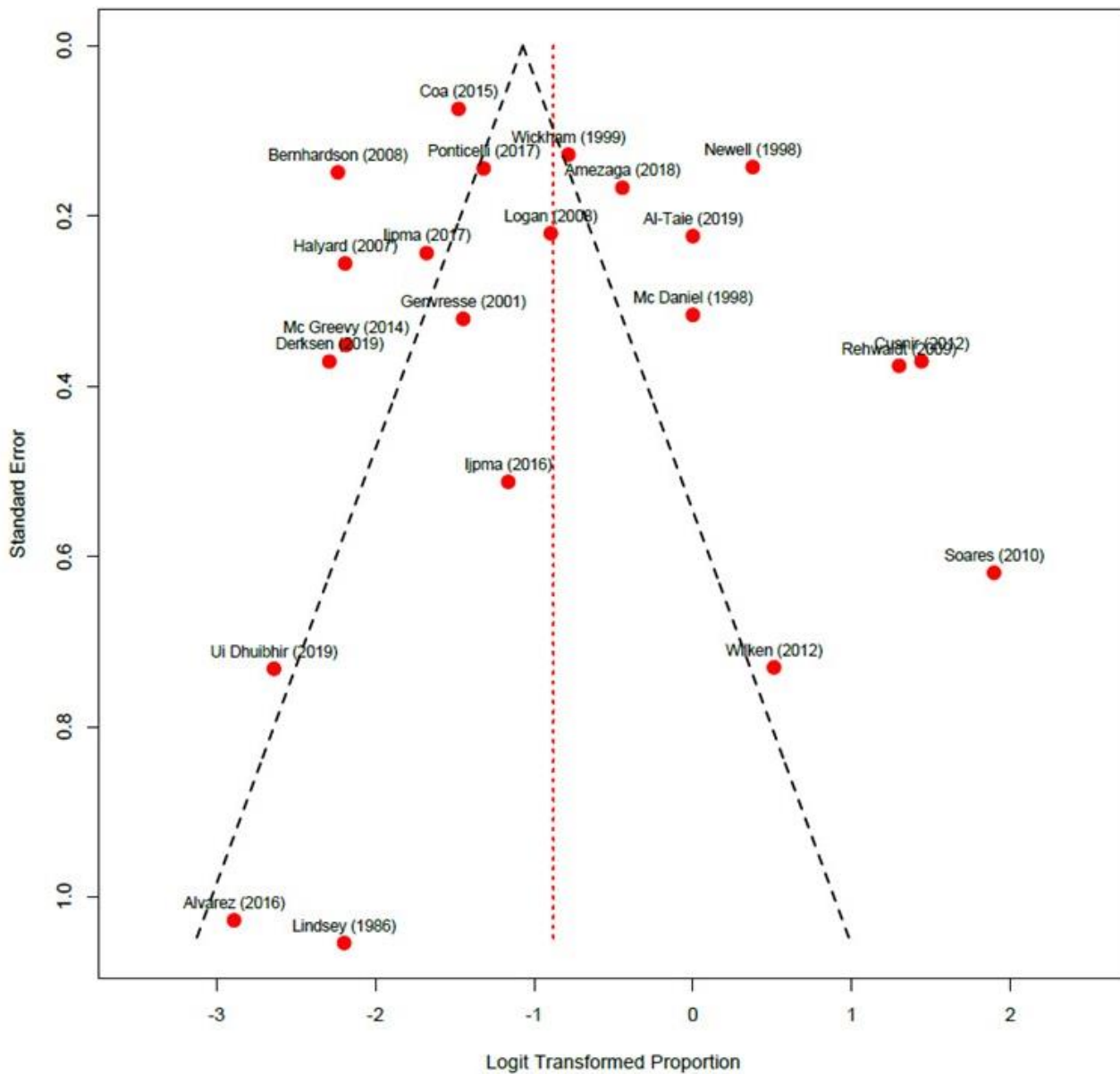


Figure S7: Funnel plot for random effects meta-analysis of MT prevalence rate versus standard error. The dotted black triangle line represents the 95% confidence interval. The red dotted line represents the pooled random effects estimate.



ANNEX 7: Supplementary files to the manuscript “Evaluating the Etiology of Metallic Taste During Head and Neck Cancer Treatments: A Study of Facial and Glossopharyngeal Nerve Interactions”

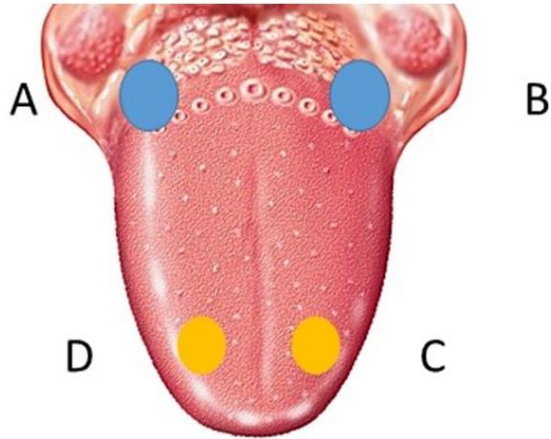


Figure S1: tests location on the tongue

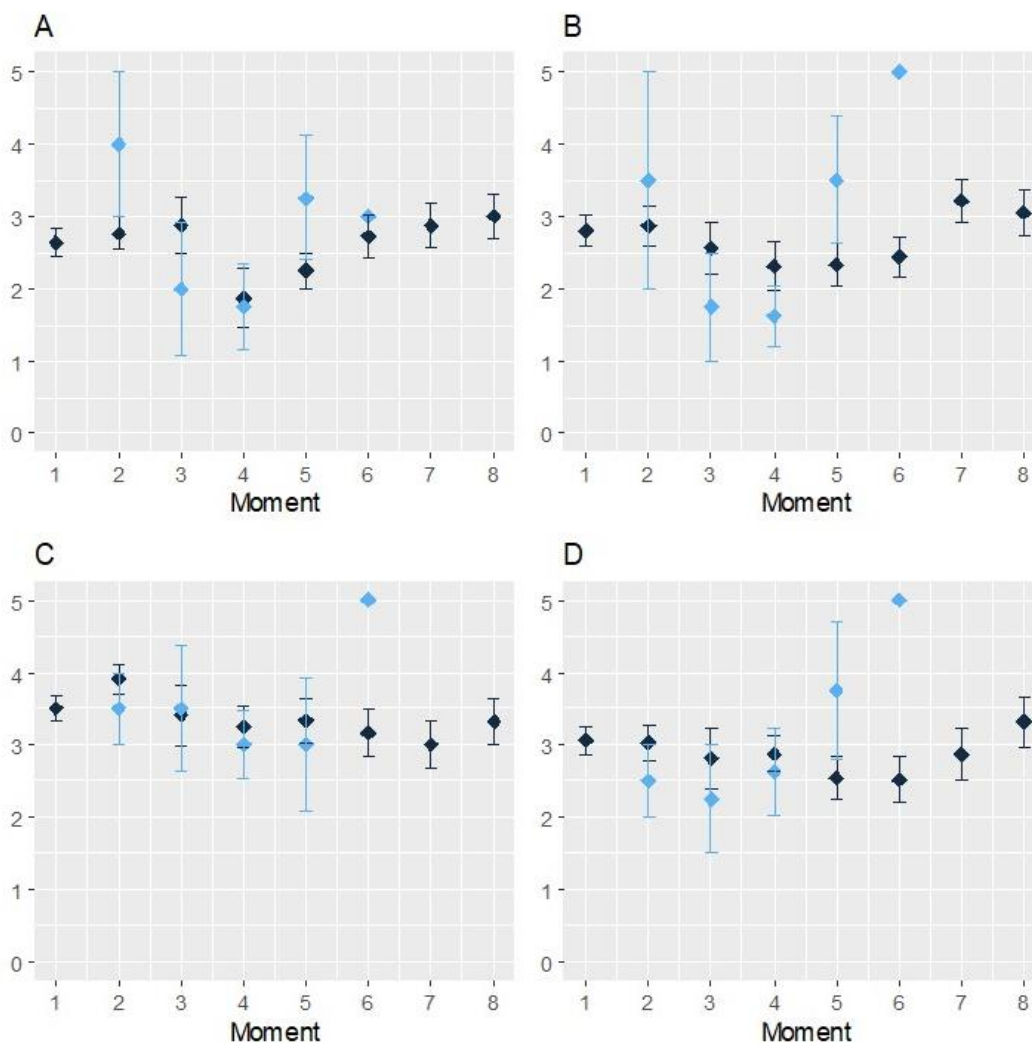


Figure S2: evolution of taste results for metallic according to the precise location on the tongue
 Blue = metallic taste, black = no metallic taste. A = right tongue base, B = left tongue base, C = left tip of the tongue, D = right tip of the tongue
 Moments: (m) moment 1); after surgery if any (m2); in the middle (m3) and at the end (m4) of the radiotherapy if any; at 3 (m5)-6 (m6)-9 (m7) and 12 (m8) months.

Taste	Location	Moment 2	Moment 3	Moment 4	Moment 5
Water	A	0.4058	0.6524	0.3743	1
	B	0.5489	1	0.2664	1
	C	0.4929	1	0.3743	0.4863
Salty	A	1	1	0.1111	0.3912
	B	0.6673	0.6604	0.8836	0.9358
	C	0.8188	1	0.0506	1
	D	0.4145	0.8442	1	0.1603
Sour	A	1	0.1973	0.8783	1
	B	1	1	0.4440	1
	C	1	0.8098	1	0.2679
	D	1	1	1	0.6532
Sweet	A	1	0.9067	0.6547	1
	B	1	0.7144	0.1797	0.6542
	C	1	1	0.8738	1
	D	1	0.7109	1	1
Bitter	A	1	0.8442	1	1
	B	1	1	0.6171	1
	C	0.4389	1	0.4689	0.8151
	D	0.7535	1	0.6171	1
Metallic	A	1	1	1	0.3045
	B	0.6422	0.9067	0.2664	0.2272
	C	1	1	0.6171	1
	D	1	1	0.6650	0.8151

Tableau S1 : results of chi-square tests of taste recognition according to the moment

Taste	Location	Moment 2	Moment 3	Moment 4	Moment 5
Water	A	0.6804	0.0015	0.2842	0.6942
	B	0.5268	0.8089	0.0458	0.8342
	C	0.4929	0.7732	0.8971	0.4178
Salty	A	0.783	0.5858	0.5898	0.4084
	B	0.3613	0.8802	0.184	0.9611
	C	0.8165	0.7148	0.4862	0.285
	D	0.8450	0.6354	0.8428	0.9687
Sour	A	0.7601	0.4616	1	0.8173
	B	0.6195	0.7873	0.0120	0.9412
	C	<10⁻⁵	0.7565	0.4705	0.8245
	D	0.5628	0.7766	0.2152	0.7688
Sweet	A	0.4043	0.7601	0.6956	0.3810
	B	0.0162	0.6230	0.6578	0.5651
	C	0.7734	0.0412	0.5830	0.8342
	D	<10⁻⁵	0.6059	0.8339	0.8818
Bitter	A	0.9523	0.5097	0.8134	0.5423
	B	0.4881	0.7663	0.8645	0.6472
	C	0.0012	0.7510	0.8763	0.1678
	D	0.5621	0.8245	0.8495	1
Metallic	A	0.4262	0.4245	0.8641	0.3316
	B	0.7459	0.3774	0.2262	0.2758
	C	0.5604	0.9309	0.6566	0.7479
	D	0.4648	0.5414	0.7082	0.2968

Tableau S2 : results of t-tests of tastes intensities according to the moment

ANNEX 8: Supplementary files to the manuscript “Exploring the Role of Oxidative Stress in Metallic Taste During Head and Neck Cancer Treatment: A Study of Salivary Malondialdehyde and Therapeutic Interventions”

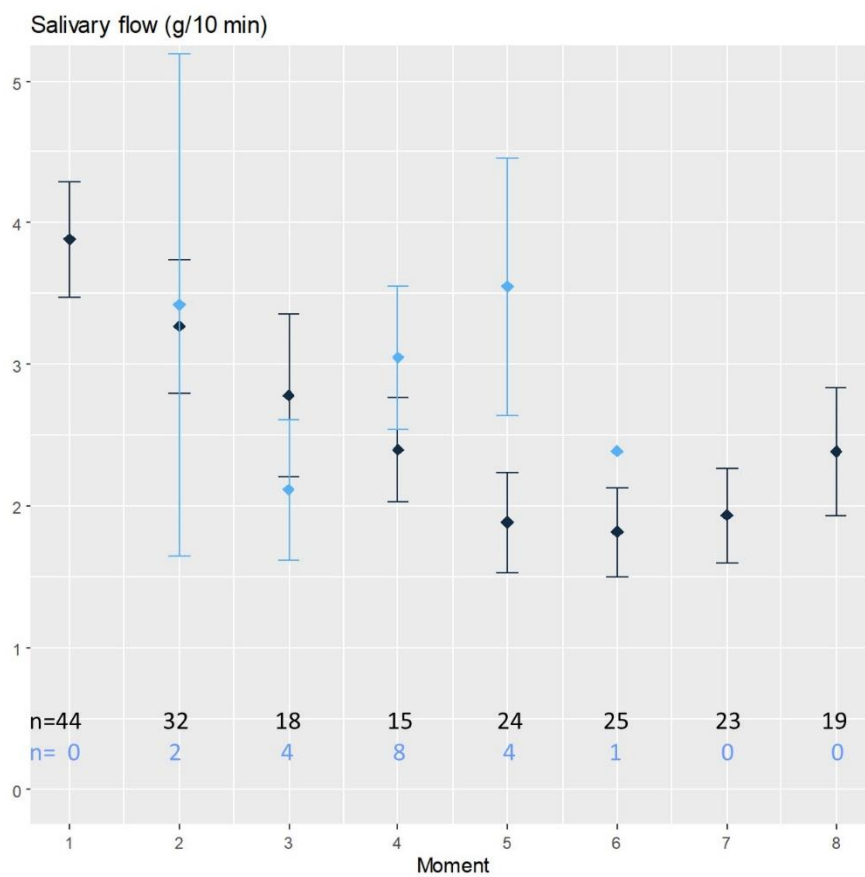


Figure S1: evolution of the salivary flow according to the different moments and the MT status.

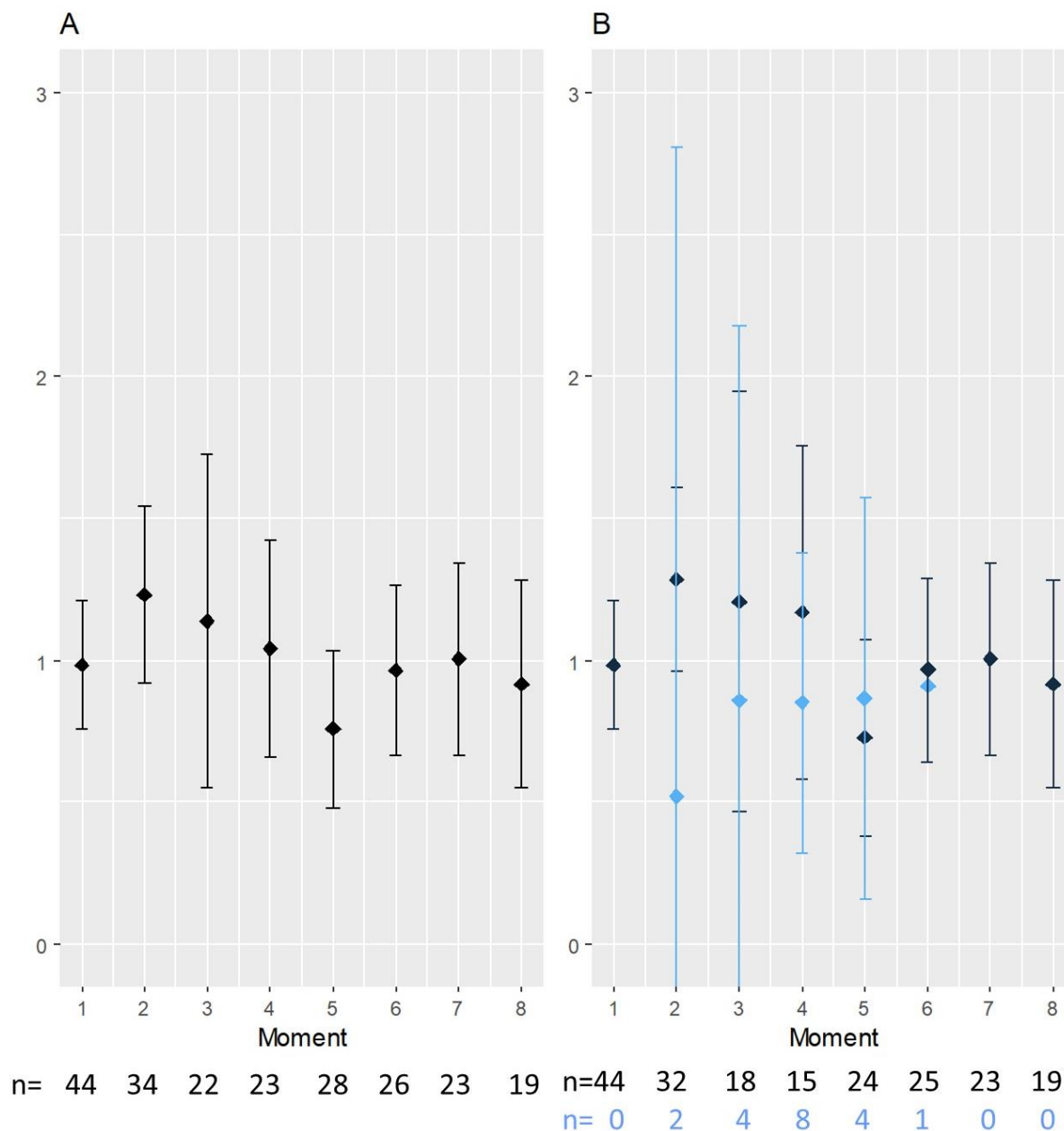


Figure S2: evolution of salivary protein concentration

A: global results of protein concentration (mg/ml) over time. B = protein concentration over time in two groups (Blue = metallic taste, black = no metallic taste)

Moments: (m) moment 1); after surgery if any (m2); in the middle (m3) and at the end (m4) of the radiotherapy if any; at 3 (m5)-6 (m6)-9 (m7) and 12 (m8) months.

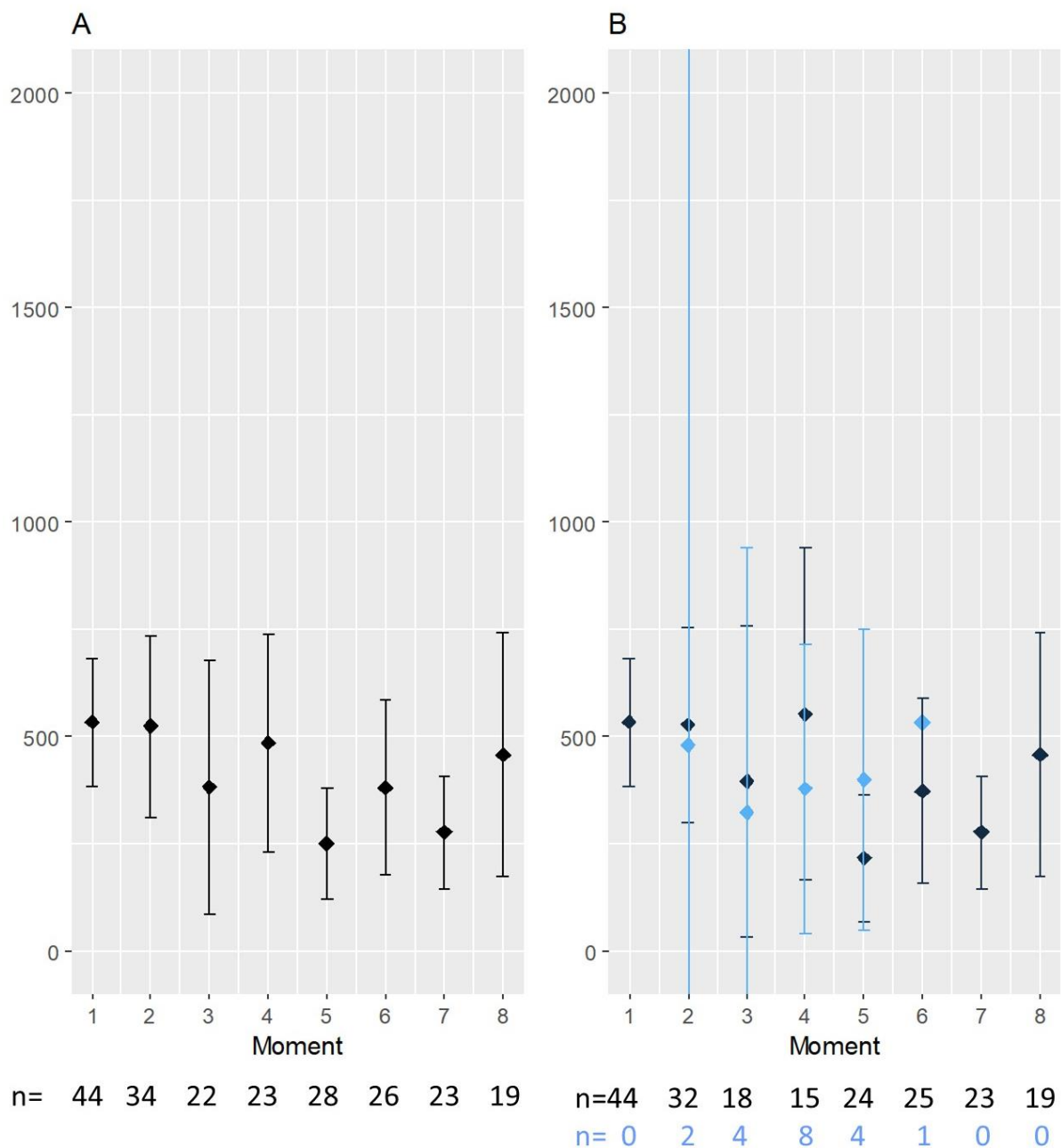


Figure S4: salivary catalase concentration

A: global results of salivary catalase concentration (IU/10 min) over time. B = salivary catalase concentration (IU/ml) over time in two groups (Blue = metallic taste, black = no metallic taste)

Moments: (m) moment 1); after surgery if any (m2); in the middle (m3) and at the end (m4) of the radiotherapy if any; at 3 (m5)-6 (m6)-9 (m7) and 12 (m8) months.

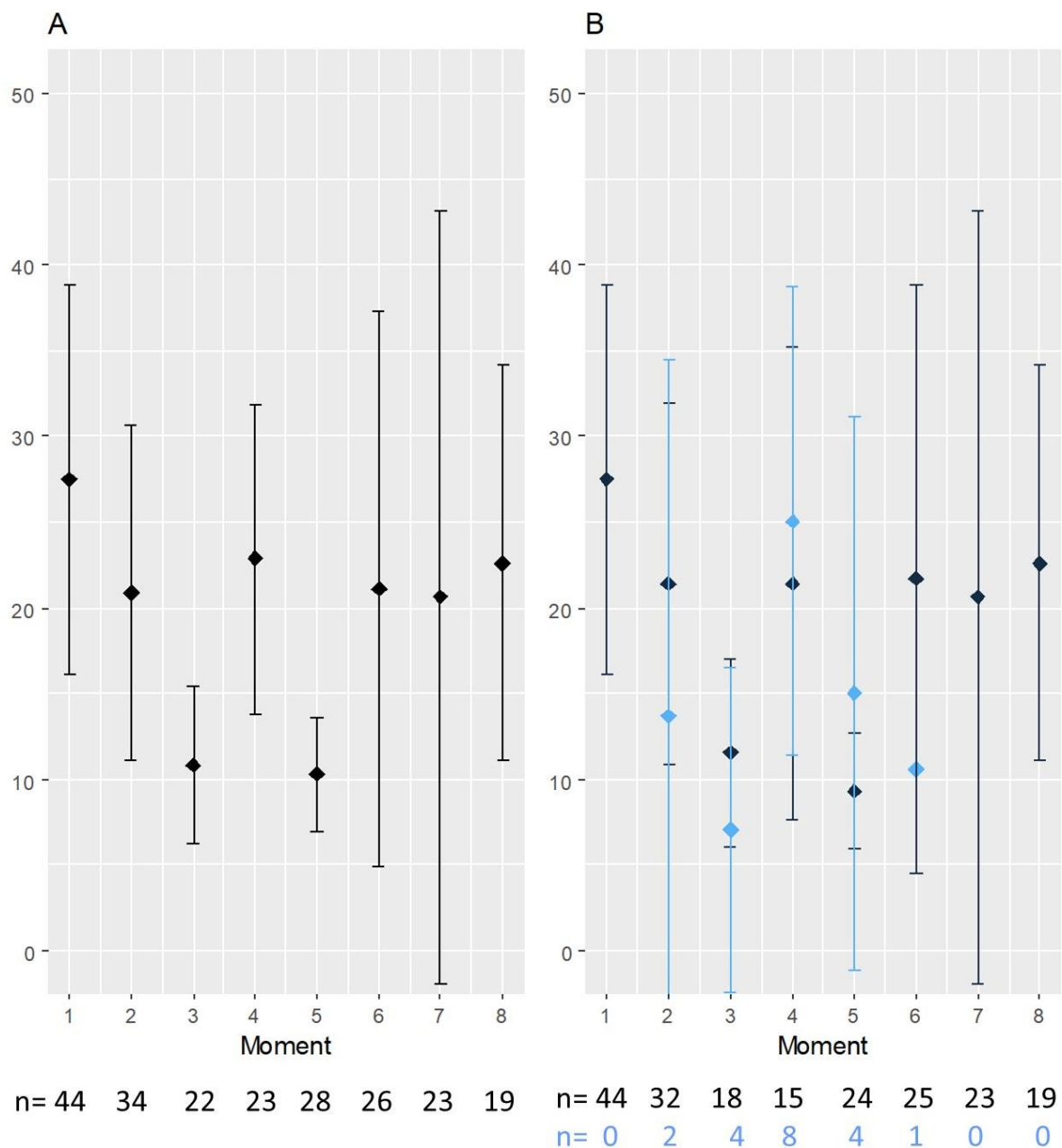


Figure S5: salivary superoxide dismutase concentration

A: global results of salivary superoxide dismutase concentration (IU/ml) over time. B = salivary superoxide dismutase concentration (IU/10 min) over time in two groups (Blue = metallic taste, black = no metallic taste)

Moments: (m) moment 1); after surgery if any (m2); in the middle (m3) and at the end (m4) of the radiotherapy if any; at 3 (m5)-6 (m6)-9 (m7) and 12 (m8) months.

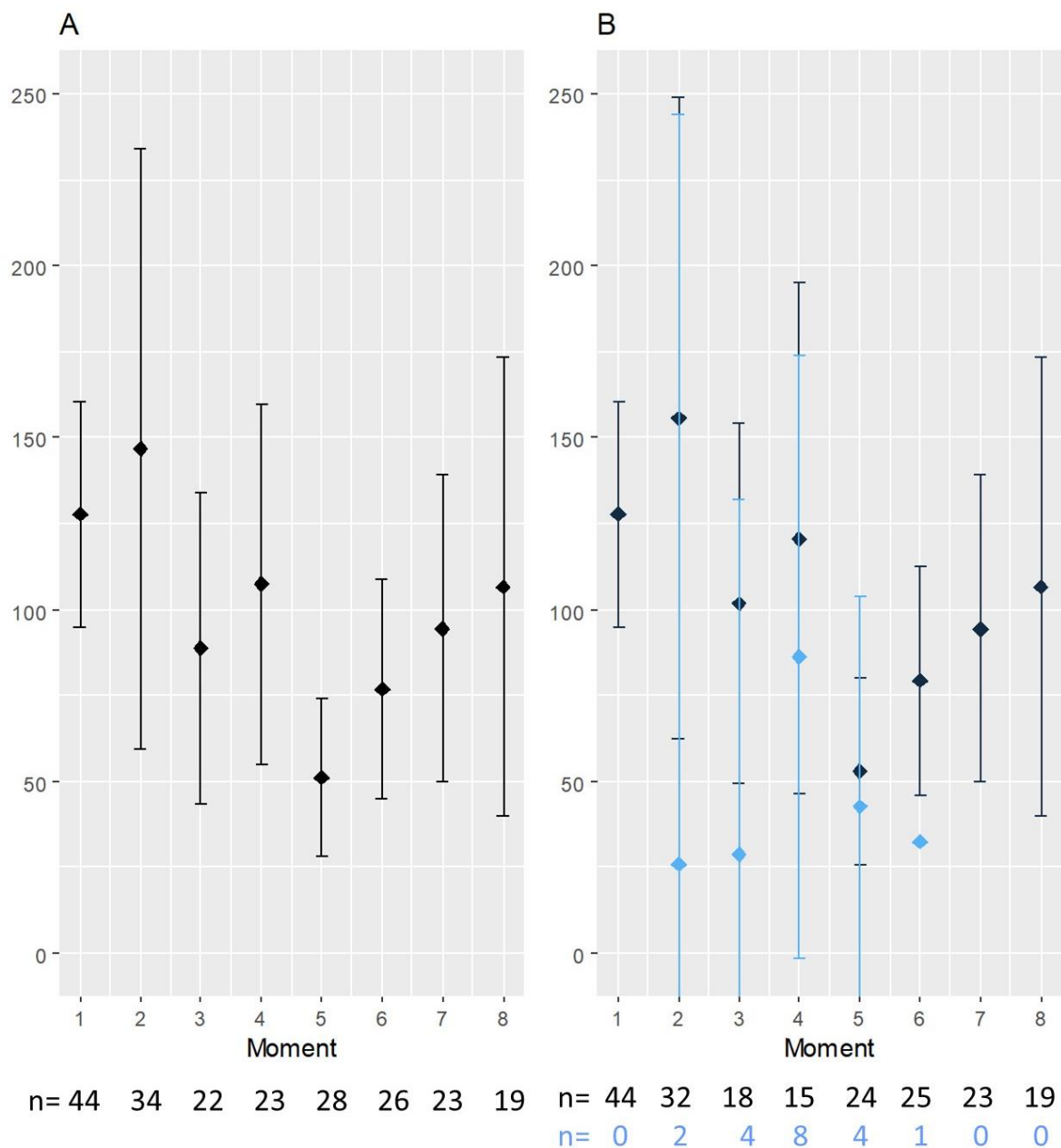


Figure S6: salivary GSTP1 concentration

A: global results of salivary GSTP1 concentration (IU/10min) over time. B = salivary GSTP1 concentration (IU/ml) over time in two groups (Blue = metallic taste, black = no metallic taste)
 Moments: (m) moment 1); after surgery if any (m2); in the middle (m3) and at the end (m4) of the radiotherapy if any; at 3 (m5)-6 (m6)-9 (m7) and 12 (m8) months.

ANNEX 9 : Traduction en anglais de l'article sur l'évolution des capacités de reconnaissance et d'intensité gustatives sur différentes régions de la langue chez des patients atteints de cancer des voies aérodigestives supérieures

Title : evolution of taste recognition and intensity in different regions of the tongue in patients with head and neck cancer

ABSTRACT

Background: this study examined the evolution of subjective and objective taste alterations in patients suffering from Head and Neck Cancer (HNC), a condition often overlooked but which significantly impacts patients' quality of life and diet. The research focuses on the temporal analysis of changes in different areas of the tongue before, during and after cancer treatment.

Method: a prospective, longitudinal follow-up of subjective and objective taste alterations was carried out in 44 patients diagnosed with HNC. It consisted of supraliminal sensory recognition and intensity tests performed at different locations on the tongue and at several key time points before, during and up to one year after treatment. Questionnaires were used to assess quality of life. Results were analyzed according to the different stages of treatment and patient demographics.

Results: Subjective and objective taste alterations were already present before treatment in some patients, deteriorated significantly over time and then improved while persisting up to one year after treatment. Impairment was significantly more pronounced at the base of the tongue than at the tip. In addition, a trend between subjective taste impairment and the type of HNC treatment (more frequent in the case of radiotherapy or radiochemotherapy) was demonstrated ($p=0.0360$).

Conclusion: the study highlights the frequency and importance of taste alterations in patients with HNC. It is therefore important to provide prior information, early assessment, follow-up and appropriate management to improve their quality of life and tolerance to treatment.

INTRODUCTION

Head and Neck Cancers (HNC) remain frequent, although their incidence is declining (243). In addition to a still high mortality rate, they also have a significant impact on patients' quality of life (43, 239, 255), both through the cancer itself and through treatment, which is generally multimodal. Among the many physical and psychological sequelae caused by these cancers and their treatments, taste alterations occupy an important but often neglected place, like supportive care in general, to the benefit of overall survival. Indeed, HNC patients are often confronted with significant gustatory alterations due to the effects of treatments, notably chemotherapy and/or radiotherapy (3, 220, 242). Taste alterations can impact nutritional intakes by reducing ingesta in already malnourished individuals (11, 37, 44, 241). Irune et al (43) and Buiret et al (19) describe different, possibly concomitant, mechanisms: mucosal damage, direct damage by external radiotherapy (reduced number of taste cells, neurotoxicity), chemotoxicity (reduced number of taste cells and their receptors, intra-salivary passage), xerostomia, surgery (reduced number of taste cells, neurological damage). Studies reported in Hovan's meta-analysis (3) showed high prevalences of dysgeusia in patients undergoing chemotherapy (56.3%), radiotherapy (66.5%) and combined radiochemotherapy (76%). Abendstein (255) and Asif (223) showed significant impacts on quality of life and overall taste during and after treatment, although interventions such as the use of amifostine, a cytoprotective agent against chemotherapy and radiotherapy, active on healthy cells but not on cancer cells through effects on transcription factors, gene expression, chromatin stability and enzyme activity (256), could reduce these symptoms (257).

As part of the TORCAD research project into the potential causes of metallic taste, we prospectively and longitudinally followed the evolution of taste alterations in HNC patients, analyzing the changes

observed for four tastes (sweet, salty, sour and bitter) before, during and up to one year after treatment. These data may contribute to the development of more appropriate management strategies to minimize the impact of treatment on taste perception and, consequently, on patients' quality of life.

METHODS

All patients diagnosed with HNC between April 2021 and March 2023 were invited to take part in the TORCAD study of metallic taste before, during and after HNC. Few patients accepted, as data collection was time-consuming and somewhat tedious.

Inclusion criteria were that patients had reached the age of majority and had HNC with a life expectancy of more than one year. Non-inclusion criteria were: not to have been offered total laryngectomy (the orthonasal sense of smell had to be preserved), not to have had previous cancer, HNC or not (previous treatment could have altered the individual's ability to taste), not to be pregnant (pregnancy disrupts taste). Exclusion criteria were cancer recurrence during the year of post-treatment follow-up. In this case, the data acquired up to the recurrence were retained. Similarly, in the event of withdrawal of consent, data acquired up to the time of withdrawal were retained.

The proposed treatment was discussed at a multidisciplinary consultation meeting. In the event of radiotherapy or radiochemotherapy, a prophylactic radiological gastrostomy was systematically performed.

Patients had several assessments: T1: before any treatment; T2: after surgery before radiotherapy if applicable; T3: in the middle of radiotherapy if applicable; T4: at the end of radiotherapy if applicable; T5: three months after the end of treatment; T6: six months after the end of treatment; T7: nine months after the end of treatment; T8: 12 months after the end of treatment.

At each time point, sensory recognition and intensity tests were performed, and quality-of-life questionnaires were completed. Sensory tests of flavor recognition and supraliminal intensity assessments were performed with cotton swabs, soaked in gustatory solution and applied to different regions of the tongue: the right tongue base (A), the left tongue base (B), the left tongue tip (C) and the right tongue tip (D). The test technique is shown in the video. For the taste solutions, the following compounds were used: sucrose solution (Sigma-Aldrich ref 16104) at 850 g/l for sweetness, table salt solution at 75 g/l for saltiness, anhydrous caffeine solution (Sigma-Aldrich ref W222402) at 20 g/l for bitterness and citric acid solution (Sigma-Aldrich ref W230618) at 50 g/l for acidity. Evian water, with its neutral organoleptic properties, was used to dilute the sapid compounds and to rinse the mouth between each test. At each position on the tongue, patients were asked to recognize one of four possible flavors (sweet, salty, bitter, sour), then rate the intensity of their perception on a Likert scale from 0 to 5. The order of flavors and the location of the cotton swab application were fixed in advance in the protocol and identical for all individuals. However, the multiplicity of tests (four flavors applied to the four locations on the tongue) and several weeks between each test meant that individuals were unable to anticipate test responses. The questionnaires used were the EORTC QLQ30 (187) and HN35 (76) for quality of life. Other parameters were measured: weight, a visual analog scale for ingesta (221), salivary flow assessed via the amount of saliva spat out in 10 min without stimulation (258), the presence of mucositis and/or candidiasis, smoking (active or not).

Ethics and statistics

The study was validated by the CPP Est I under number 2017-A03641-52. It is referenced on clinicaltrial under number NCT03558789.

Means and standard deviations were calculated for quantitative data. Frequencies were calculated for qualitative data.

Repeated-data ANOVAs were calculated for quantitative data. Interactions between qualitative criteria were tested by chi-2 tests.

Responses to taste tests were processed by a generalized linear model (binomial for flavor recognition, Gaussian for perceived intensity), on repeated data. Results were plotted in a U-shape. A degree 2 polynomial was therefore introduced to take this temporal evolution into account. The R software (version 4.3.1) was used.

RESULTS

Forty-four patients were recruited. Figure 1 reports the flow chart.

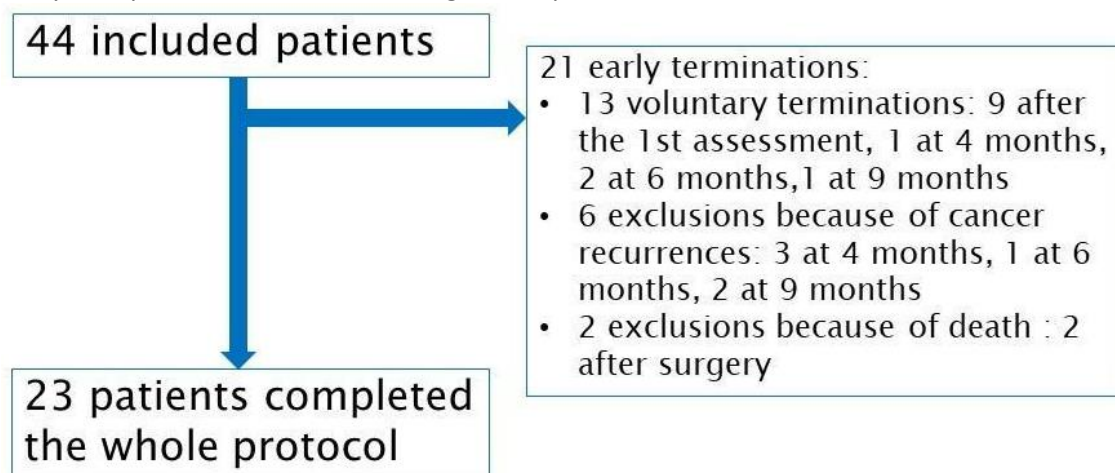


Figure 1: study flow-chart

Table 1 shows patient demographics and treatments.

Sex: males n(%) ; females n(%)	34 (77.3%), 10 (22.7%)
Mean age at diagnosis \pm SD (year)	63.1 \pm 9.8
Location n (%): Hypopharynx Larynx Oropharynx Oral Cavity Unknown primary	3 (6.8%) 8 (18.2%) 14 (31.8%) 14 (31.8%) 5 (11.4%)
Tumor stage n (%): 1 2 3 4 x	9 (20.4%) 18 (40.9%) 8 (18.2%) 4 (9.1%) 5 (11.4%)
Node stage n (%): 0 1 2a 2b 2c 3a	20 (45.5%) 10 (22.7%) 4 (9.1%) 5 (11.4%) 2 (4.5%) 3 (6.8%)

Smoking at inclusion n (%):	
Yes	20 (45.5%)
No	5 (11.4%)
Quit	19 (43.1%)
Treatment	
Withdrawal of consent before treatment	5 (11.4%)
Chemotherapy, surgery, radiotherapy	1 (2.2%)
Surgery	14 (31.8%)
Surgery, radiotherapy	12 (27.3%)
Surgery, radiochemotherapy	12 (27.3%)

Table 1: patients demographics and treatments.

Figure 2 shows the subjective evolution of dysgeusia reported in question 14 of the EORTC HN35 questionnaire. Subjective taste impairment varied significantly with time of assessment ($p < 10^{-5}$). As a reminder, moments 3 and 4 were obtained only in the case of radiotherapy and radiochemotherapy. Sixteen people had no dysgeusia reported in question 14 of the EORTC HN35 questionnaire (36.4%). After removing the nine people who had only had an initial assessment and had withdrawn their consent after the first assessment, seven people had reported no dysgeusia (15.9%), six had been treated by surgery alone and one by surgery and radiotherapy for laryngeal cancer. There was a trend in the relationship between type of treatment and subjective assessment of dysgeusia, reported in question 14 of the EORTC HN35 ($p = 0.0360$).

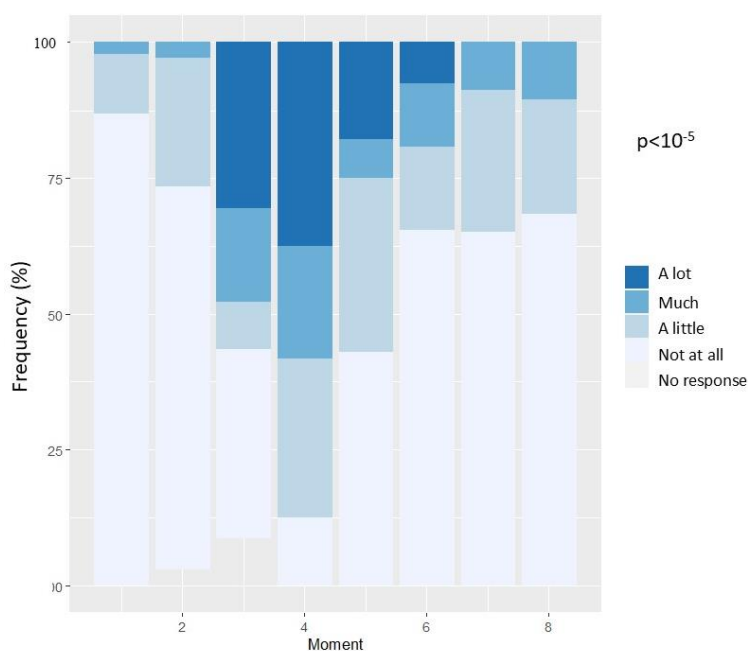


Figure 2: change over time in response to the question "Over the past week, have you had any taste problems?" (EORTC HN35 n°14)

Changes in salivary flow, weight, visual analog scale of ingesta and smoking at the time of assessment are presented in Figures S1 to 4 in Appendix 7. Salivary flow decreased significantly over time ($-0.31\text{g}/10\text{min}/\text{moment}$, $p < 10^{-5}$), as did weight ($-0.85\text{kg}/\text{moment}$, $p = 0.0012$).

Figure 3 shows the evolution of flavor recognition as a function of time and location on the tongue, and Figure 4 shows flavor intensity as a function of evaluation time and location on the tongue. For the vast majority of tests, there was a significantly different distribution or trend in the results of taste recognition and intensity tests according to the time of testing, with decreases in both

parameters essentially at times 3 and 4, corresponding respectively to the middle and end of radio(chemo)therapy, followed by a gradual return to baseline.

The results of the taste recognition tests were then compared according to whether they were performed on the base of the tongue (points A and B) or on the tip (points C and D). For all flavors, the impairment was significantly greater at the base of the tongue than at the tip ($p < 10^{-5}$ for salty, bitter and sweet, $p = 0.004$ for acid). For the intensity taste tests, impairment was also significantly greater at the base of the tongue than at the tip ($p < 10^{-5}$) for each flavor.

The size of the study did not provide sufficient power to investigate, for each flavor and location, the impact on identification and intensity according to the type of HNC treatment.

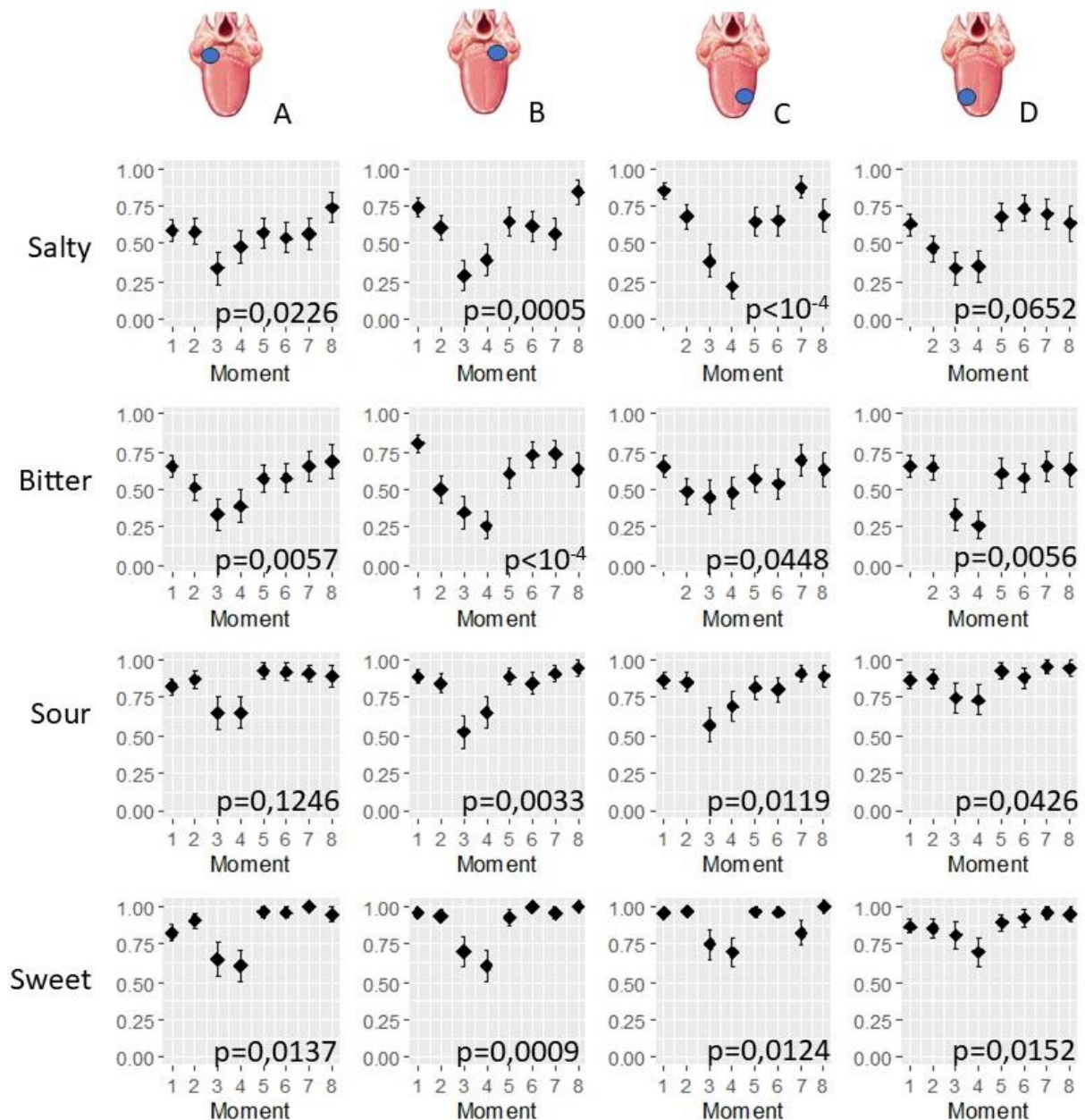


Figure 3: Evolution of flavor recognition as a function of evaluation time and location on the tongue

The value of p on each graph is the result of modeling flavor recognition (0: incorrect flavor recognition, 1: correct flavor recognition) for the different evaluation moments.

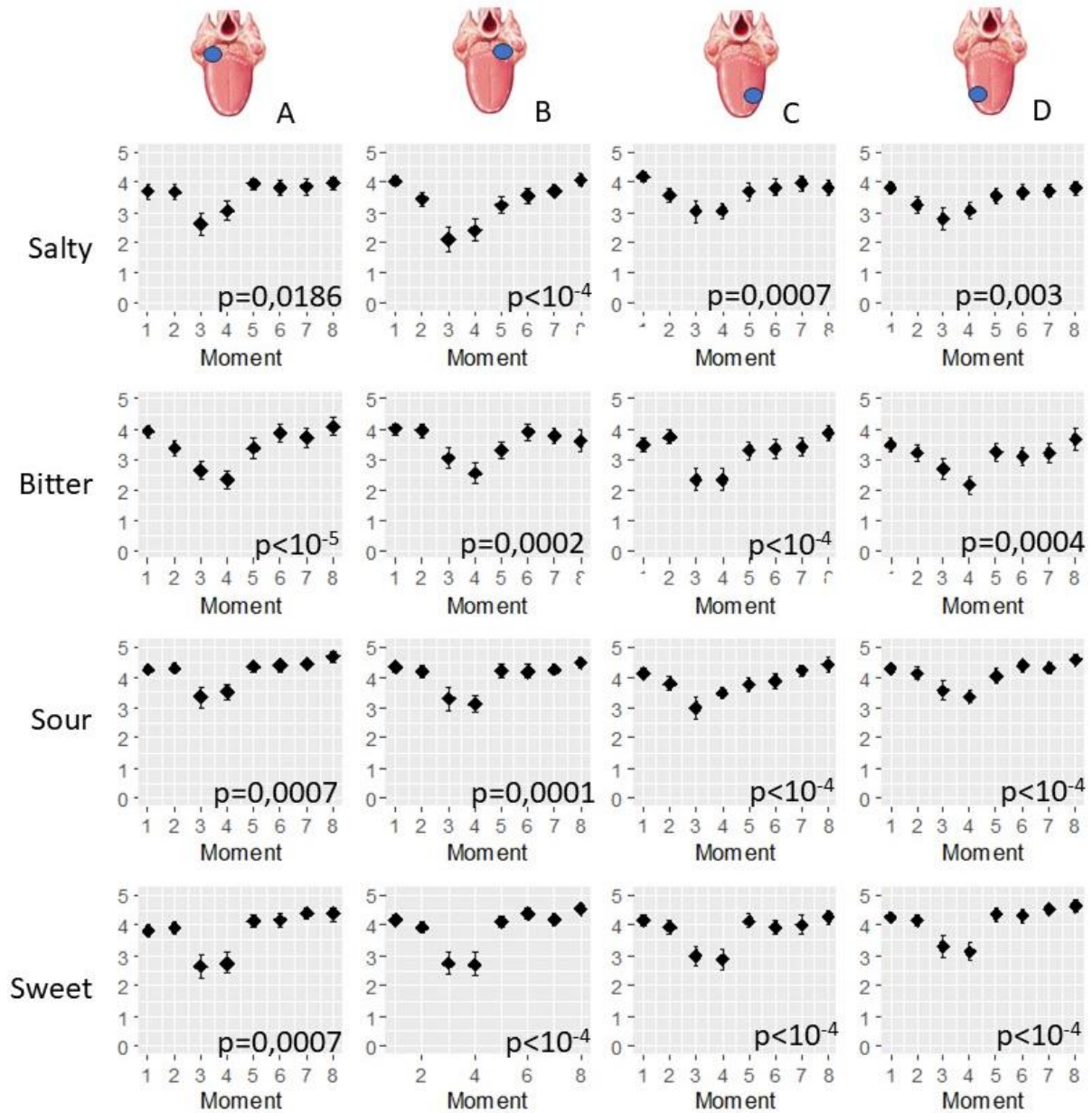


Figure 4: Flavor intensity as a function of evaluation time and location on the tongue

The p-value on each graph is the result of modeling the intensity of the flavor evaluated on a Likert scale from 0 to 5 for the different moments of evaluation.

DISCUSSION

The results of this study highlighted the frequency and intensity of taste alterations in patients with HNC. We showed that taste impairment, assessed both subjectively (questionnaire) and objectively (identification and intensity of tastes), already existed in some patients prior to cancer treatment, a finding echoed by other authors (43, 259). Both modalities (subjective and objective) were mainly affected during radiotherapy or radiochemotherapy (moments 3 and 4), again in line with the literature (53, 54, 75, 223, 241, 260-263), with a return to baseline beyond 6 months after the end of treatment. Finally, damage was significantly greater at the base of the tongue than at the tip. There were probably two reasons for this last finding. Firstly, as the oropharynx is the most common cancer site (on a par with the oral cavity), a higher dose of radiation was delivered to the oropharynx. On the other hand, radiotherapists used intensity modulation to spare the tip of the tongue and the incisivocanine block in cancers other than those of the oral cavity, so a lower dose was delivered to the tip of the tongue. Chen et al (264) had shown that irradiation of the tongue at more than 50Gy was a risk factor for dysgeusia. Moreover, the prolonged (more than one year) subjective and objective taste impairment observed in our study was also observed by other authors (30, 224). Lastly, although the lack of power due to our size did not allow us to verify whether the type of treatment had a significant impact on alterations by flavor and location, the trend observed between the type of treatment and responses to question 14 of the EORTC HN35 ($p=0.0360$) suggests a possible relationship. Moreover, the fact that objective and subjective dysgeusia were mainly present at times 3 and 4 (middle and end of irradiation) reinforces this hypothesis of treatment imputability. Kamprad et al. (260) and Ogama et al. (265) showed an alteration in taste recognition thresholds starting at 20Gy and peaking at 40 to 60Gy. For Kamprad et al. (260), taste perception thresholds were much more impaired if the whole tongue was irradiated, and recovery varied according to the volume of the tongue irradiated (8 weeks on average if two-thirds of the tongue was irradiated, and 6 months if the whole tongue was irradiated). Overall, the perception of all flavors was affected in our study. Other authors have obtained similar results. Some even demonstrated a differential impact on the flavors. For instance, Baharvand et al. (266), in a study with 22 patients with irradiated HNCs, showed a difference in the timing of sensory impairments (first salty and bitter flavors, then sour, followed by sweet) through taste threshold tests. In Conger et al. (267), bitterness and sourness were the first flavors to be affected (at 2.4 and 4 Gy, respectively). Mossman et al. (225) and Fernando et al. (268) observed that bitterness and saltiness were primarily affected. According to Kamprad et al. (260), all flavors were impacted as early as 20 Gy of irradiation. Maes et al. (224) demonstrated impairment of bitter, salty, and sweet flavors before any treatment (but not sour), followed by impairment of all flavors during irradiation (in descending order of impact: bitter, salty, sweet, sour). Mirza et al. (63) did not show significant differences in gustatory impairment over time, but their sample size was small (8 patients with HNCs), and the taste recognition thresholds were at the limit of significance. Ruo-Reda et al. (34) conducted a literature review on the impact of irradiation on taste. Although the results sometimes varied from one study to another, a more frequent impairment of bitterness, sourness, and saltiness, and less so of sweetness, was observed. The concordance observed in our study between subjective gustatory evaluation (EORTC HN35 14 question) and objective evaluation (recognition and intensity), particularly at moments 3 and 4, was also reported by Maes (224) and Baharvand et al. (266). However, several authors noted discrepancies between subjective and objective responses (17, 31, 32, 34, 269), either in one direction (major subjective impairment reported with no anomalies in objective tests) or in the other (significant changes in recognition or intensity thresholds in objective tests with minor subjective impact reported).

It is important to recognize that gustatory alterations were not only physiological but could also have significant psychological and social repercussions. Patients might experience frustration, sadness, or anger due to the loss of pleasure associated with eating, or face difficulties in social interactions (e.g., loss of perception of body odors) or environmental awareness (e.g., loss of perception of olfactory

danger markers), which could affect their overall quality of life. Therefore, it is essential to provide psychological and emotional support to help patients cope with these challenges. While it is crucial to inform patients about the impact of treatments on taste, particularly radiotherapy and chemoradiotherapy, few studies have been conducted on ways to prevent and/or treat dysgeusia. Nonetheless, Buntzel et al. demonstrated in a randomized placebo-controlled study that amifostine significantly reduced dysgeusia (38.1% vs 46.4%, $p=0.04$) (257). Najafizade et al. (270), in a prospective randomized study comparing preventive zinc sulfate supplementation (20 patients) vs placebo (15 patients), showed less impairment in taste perception in the supplemented group, as did Buntzel et al. with selenium supplementation (271). Jham et al. (272) showed in a randomized study that bethanechol vs placebo did not better prevent dysgeusia ($p=0.26$).

It is important to acknowledge the limitations of our study, which were mainly due to the small sample size. Indeed, it was challenging to motivate patients to participate in lengthy sessions of repeated tests, which resulted in a high attrition rate. Finally, the statistical tests conducted to evaluate the relationship between recognition and time (Figure 3) or between time and flavor intensity (Figure 4) were based on the assumption of independence between each value, as the time intervals were sufficiently long (three months) for successive evaluations to be relatively independent.

CONCLUSION

The subjective and objective gustatory alterations observed in the patients in our study with HNCs were common and prolonged complications. The recognition and intensity evaluation of all flavors were either significantly reduced ($p \leq 0.005$) or showed a trend toward reduction ($0.05 \leq p < 0.005$), more frequently in cases of radiotherapy or chemoradiotherapy.

Early assessment and appropriate management of these alterations are essential to improve patients' quality of life and support better treatment tolerance. Further research is needed to better understand the underlying mechanisms of gustatory alterations in patients with HNCs and to develop more effective strategies for managing these complications.

ANNEX 10: supplementary files to the manuscript “evolution of taste recognition and intensity in different regions of the tongue in patients with head and neck cancer”

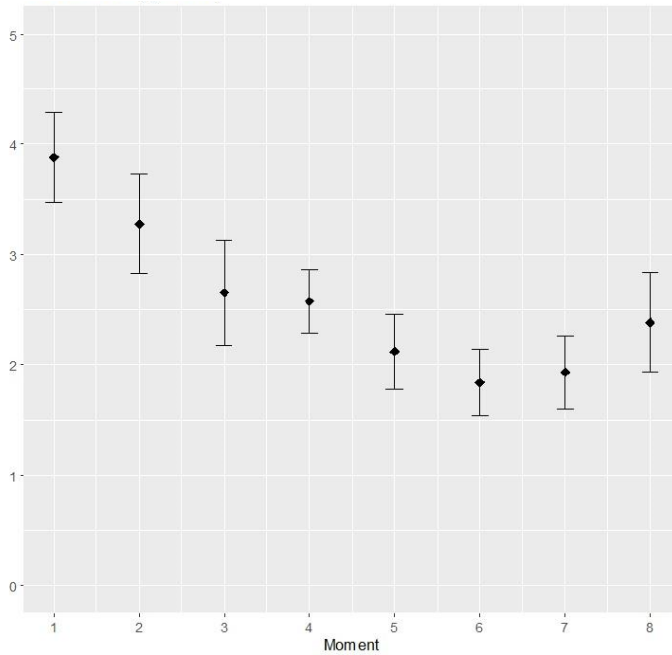


Figure S1 : évolution temporelle du débit salivaire (g/ 10 min) / salivary flow evolution with time (g / 10 min)

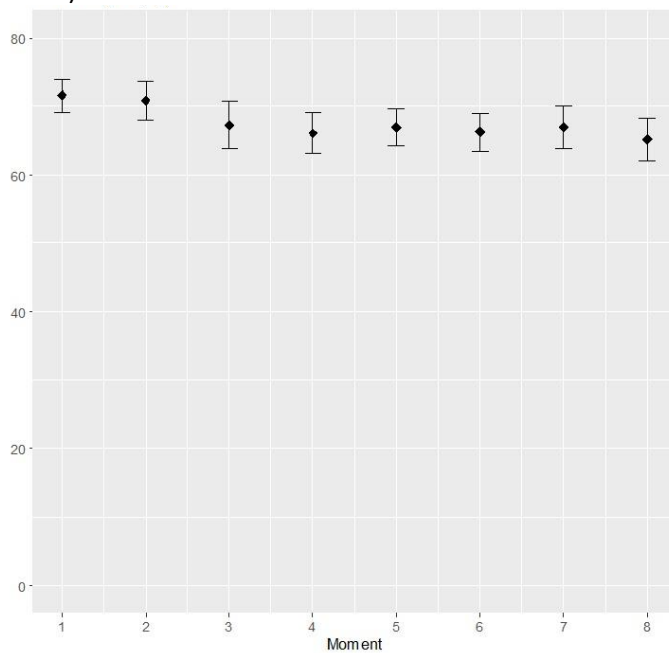


Figure S2 : évolution temporelle du poids corporel (kg) / weight evolution with time (kg)

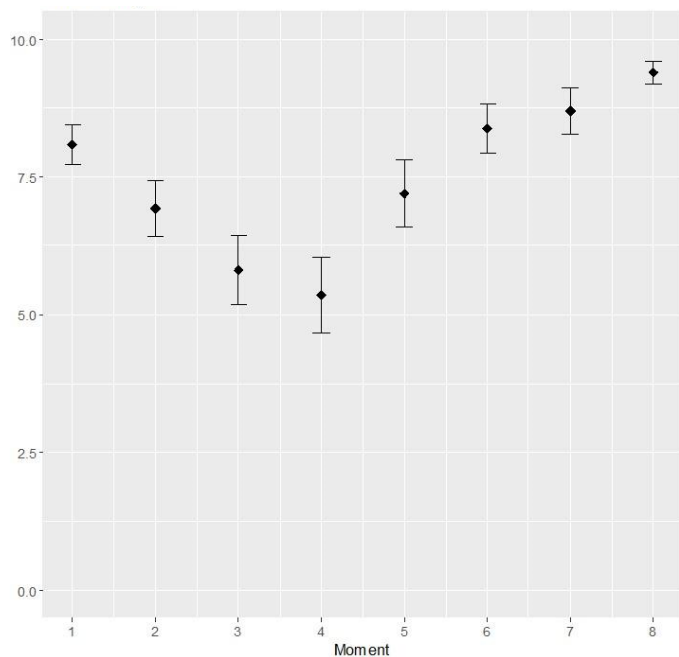


Figure S3 : évolution temporelle de l'échelle visuelle analogique des ingestas / evolution with time of visual analogic scale of oral intakes

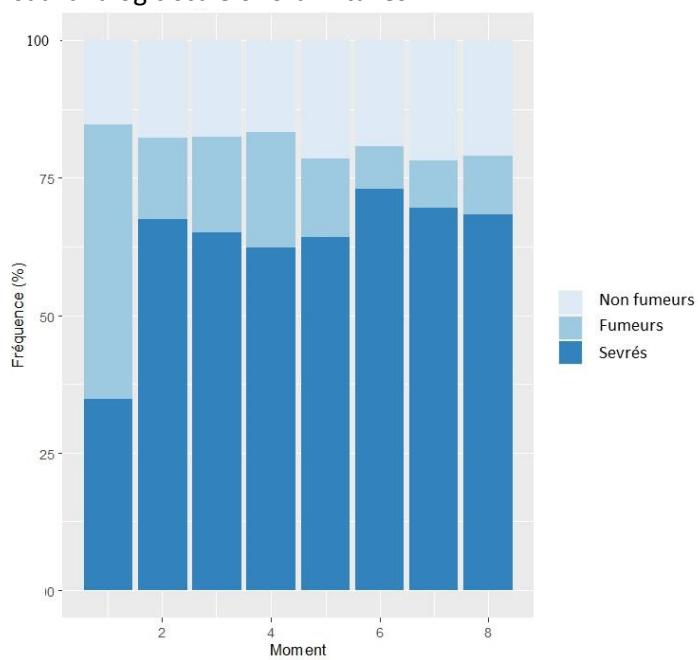


Figure S4 : évolution temporelle du tabagisme / evolution with time of tobacco consumption (non-smokers / smokers / quitters)