

## Original papers

# Signs, symptoms and the prevalence of fungi detected from the oral cavity and pharynx of radiotherapy subjects with head and neck tumors, and their susceptibility to chemotherapeutics

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**ABSTRACT.** Radio- and chemotherapy for malignant neoplasms, especially in head and neck region, is associated with a greater risk of fungal infections due to secondary alterations in the mucous membranes. The study had three aims: 1. to determine the signs and symptoms which occur among patients undergoing radiotherapy; 2. to determine the fungi prevalence in the mouth and throat of patients before, during and after radiotherapy; 3. to examine the sensitivity of strains to antimycotic drugs. The study comprised 44 patients (11 female, 33 male) with head and neck cancers, examined at the following stages: before radiotherapy (44 patients – batch 1), 3rd week of therapy (30 of the 44 patients – batch 2), last day of therapy (28 of batch 2 – batch 3) and the 6th week after completion of radiotherapy (10 of batch 3 – batch 4). Clinical examination was performed and mycological status was estimated from an oral rinse on a selected medium. The fungal strains were isolated and sensitivity to antifungal drugs was determined. The most common symptoms were pain, dysphagia, and dysgeusia. Physical examination revealed signs of mucositis mainly among patients from batches 2 and 3. The presence of fungi in the mouth and throat was noted in over 2/3 (66.2%) of the patients from batch 1, and in 4/5 (80%) of batch 2. The fungi were detected in over half (57.1%) of patients from batch 3 and also in patients from batch 4. In all cases, fungi of various *Candida* species were identified: 6 species in batch 1, 8 in batch 2, 6 in batch 3 and 5 in batch 4. The most frequently detected species was *C. albicans*, constituting 40–60%; the other species detected are known to be resistant to antimycotic drugs. The isolated strains were the most sensitive to nystatin and miconazole, and the least to ketoconazole and fluconazole. Conclusions: 1. Patients undergoing radiotherapy complain of pain, dysphagia, and dysgeusia; in most cases mucositis is diagnosed. 2. The high prevalence of fungi in the mouth and throat of patients treated by radiotherapy reinforces the need to perform mycological examinations in this group of patients to detect fungi, identify their species and determine of their sensitivity to drugs in order to prevent complications. 3. The species most frequently isolated from the patients are *C. albicans* and *C. glabrata*. The latter is characterized by resistance to the majority of antimycotic medications. 4. Most of the isolated strains are sensitive to nystatin and miconazole (applied locally) and to itraconazole (absorbed from the gastrointestinal tract).

**Key words:** fungi, tumors of head and neck, radiotherapy

## Introduction

In recent years, great progress has been made in the treatment of neoplasms and in fact, the most serious complications are those which occur as a result of the treatment itself. The literature reports

that the prevalence of fungi has increased several times among patients with immunodeficiencies, particularly those undergoing chemo- and radiotherapy, and may affect 3/4 of patients. As complications associated with the neoplasm by the presence of a mycosis may be life threatening, it is

important to determine the occurrence of fungi in particular phases of radiotherapy to allow a prognosis of the course and treatment of these patients [1,2]. Chemo- and radiotherapy, especially in cases of head and neck tumours, can promote the development of dryness of the oral and pharynx mucosa, facilitate infections caused by different pathogens [3]. Various species of fungi present in oral cavity and pharynx, such as *Aspergillus*, *Candida*, *Geotrichum*, *Mucor* or *Penicillium*, can cause systemic mycoses as a consequence of fungemia, whose prevalence during the last 10 years has increased fivefold and, in Europe, affects between 0.17 and 20.0 of 1000 hospital admissions [4].

The objectives of this study were as follows: 1. to determine the signs and symptoms which occur among patients undergoing radiotherapy; 2. to determine the fungi prevalence in the mouth and throat of patients before, during and after radiotherapy; 3. to examine the sensitivity of strains to antimycotic drugs.

## Materials and Methods

The study comprised patients (11 women, 33 men) 45–83 years old (average 63.1±9.46) who were undergoing radiotherapy for head and neck neoplasms. In total, 44 persons were assessed at the start of therapy (batch 1), 30 of these were assessed in week 3rd of therapy (batch 2), 28 of these on the last day (batch 3) and 10 of these in week 6th after completion therapy (batch 4). Cancer of the larynx (39.5%) was the most often mentioned reason to qualify patients for irradiation, while cancer of the tongue or the oropharynx were less frequent (11.6% each). Detailed data is presented in Table 1.

All patients were selected by interview and physical examination. The fungi were then collected by oral rinsing from the mouth and throat. The material was seeded directly into liquid Sabouraud medium, incubated at 37°C for 24 h, and the agar culture was then left at room temperature for a further 48 h. Direct specimens were then made of all the grown colonies in 0.9% solution of sodium chloride and examined under a microscope (×100, ×400 or ×800) for fungal structures. If such structures were detected then the culture was subcultured on Sabouraud agar. Subsequent control specimens were performed in the same manner after 5–10 days and then after further 10 days, and left for observation for up to 8 weeks. The transfer of the

Table 1. Location of neoplasm

Location	n	%
larynx	18	39.5
language	5	11.6
oropharynx	5	11.6
tonsills	4	9.3
laryngopharynx	3	7.0
nasopharynx	2	4.7
lymph nodes	2	4.7
lip	1	2.3
gingival / oral bottom	1	2.3
unspecified parts of the mouth	1	2.3
large salivary glands	1	2.3
skin	1	2.3
Total	44	100.0

colonies on fresh media enabled the isolation of pure bacteria-free strains (axenic cultures).

The strains were differentiated on the bases of morphological and biochemical features, according to protocols designed by the Department of Biology and Parasitology, Medical University of Lodz [5]. Selected biochemical features of individual strains were examined by performing auxanograms, measuring the ability to assimilate carbon, with API 20C and API 20C AUX tests (bioMerieux). On the basis of these tests, yeast-like fungi were classified into adequate genera and species based on the numerical classification described by the manufacturer (Analytical Profile Index, bioMerieux, Lyon, 1990).

The sensitivity of strains to antimycotic drugs (nystatin, natamycine, miconazole, ketoconazole, fluconazole, itraconazole) was performed by the disc-diffusion method. The degree of sensitivity was determined according to the manufacturer, based on the size of the zones of inhibition (BioMaxima SA). Patients with mycosis were cured by the drug in accordance with the mycogram results.

## Results

The most common symptoms were pain, dysphagia, and dysgeusia. It was found that while pain was reported by 3 patients (6.98%) in batch 1 at the start of therapy, it was reported by 18 and 21 patients (58.1% and 82.1%) in batches 2 and 3 respectively; differences statistically significant between results obtained for batch 1 and 2, batch 1

and 3 and batch 3 and 4 ( $p < 0.05$ ). While 2 patients in batch 1 (4.65%) complained of dysphagia, 7 and 9 (22.6% and 33.3%) reported it in batches 2 and 3 respectively. However, this increase was not significant ( $p > 0.05$ ). It was much less common to report taste disturbance, with one patient reporting this in batch 1 (2.33%), and 3 and 9 patients (9.68% and 32.1%) in batches 2 and 3 ( $p > 0.05$ ). More detailed information is presented in Fig. 1.

The anamnesis carried out among subsequent batches revealed that, in the course of radiotherapy, only a single patients received antibiotics. In contrast, antifungals and steroids were administered to 10 and 8 patients (33.3% and 26.7%), respectively, in the second batch, and 21 and 19 patients (75.0% and 67.9%) in the third batch. Between the frequency of use of each drug according to the batch are differences statistically significant ( $p < 0.001$ ). The results are shown graphically in Fig. 2.

Physical examination revealed signs of mucositis in one patient from batch 1, in 24 from batch 2, 27 from batch 3 and 7 from batch 4; median results in batches 2 and 3 were considerably higher ( $Me = 1$ ) than those of batches 1 and 4 ( $Me = 0$ ). These results obtained for particular batch are significantly different ( $p < 0.001$ ). More detailed information is presented in Fig. 1.

Table 2. The prevalence of different fungi in particular batch

Species	Batch			
	1.	2.	3.	4.
<i>C. albicans</i>	60.0	50.0	56.3	40.0
<i>C. krusei</i>	6.7	4.2	0	0
<i>C. glabrata</i>	10.0	12.5	12.5	20.0
<i>C. humicola</i>	3.3	4.2	0	0
<i>C. tropicalis</i>	16.7	4.2	12.5	0
<i>C. kefir</i>	3.3	4.2	0	10.0
<i>C. lusitaniae</i>	0	4.2	6.3	10.0
<i>C. guilliermondii</i>	0	8.3	0	20.0
<i>C. pelliculosa</i>	0	0	6.3	0
<i>C. albicans</i> + <i>C. tropicalis</i>	0	0	6.3	0

The presence of fungi in the mouth and throat was noted in over 2/3 (66.2%) of the patients from batch 1, and in 4/5 (80%) of the patients in batch 2. Fungi which were then found in over half (57.1%) of patients from batch 3 were also detected in patients from batch 4.

In all cases, the fungi of various *Candida* species were identified: 6 species in batch 1, 8 in batch 2, 6 in batch 3 and 5 in batch 4. The most frequent species to be found was *C. albicans*, constituting 40–60%; the other species detected are known to be

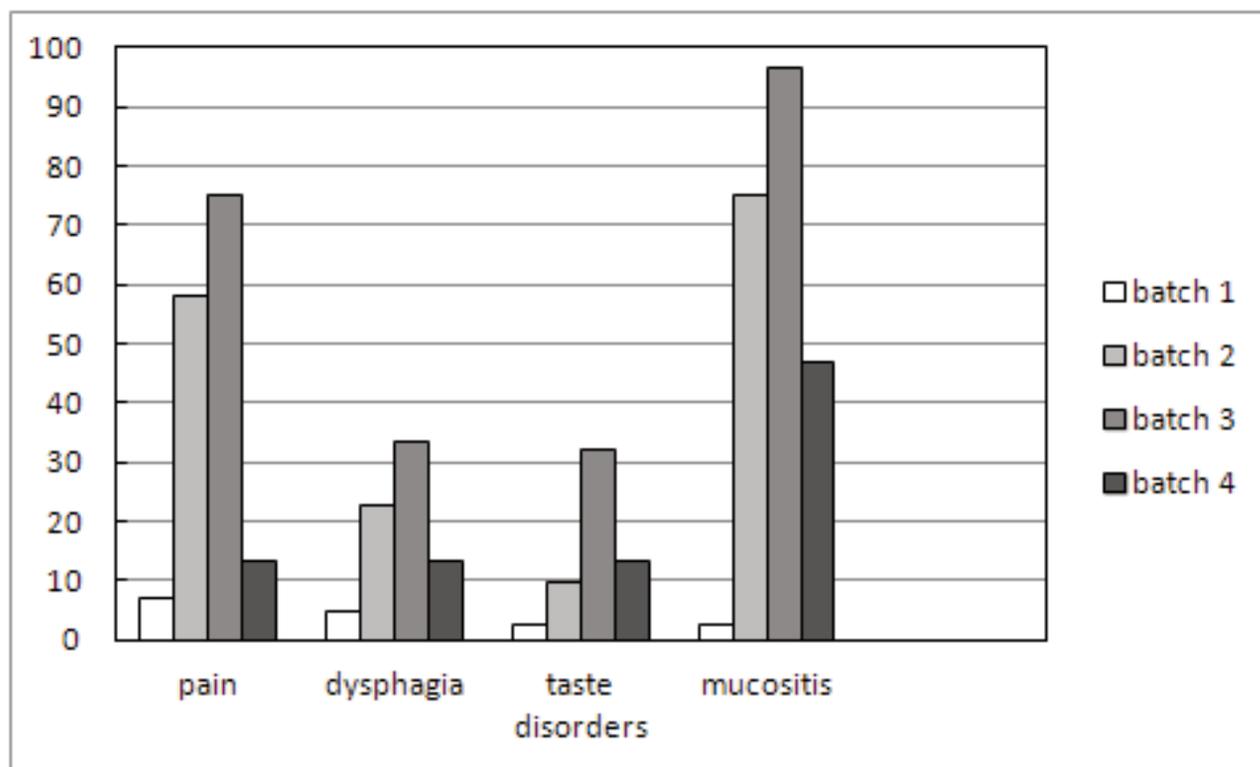


Fig. 1. Signs and symptoms observed in patients from different batch

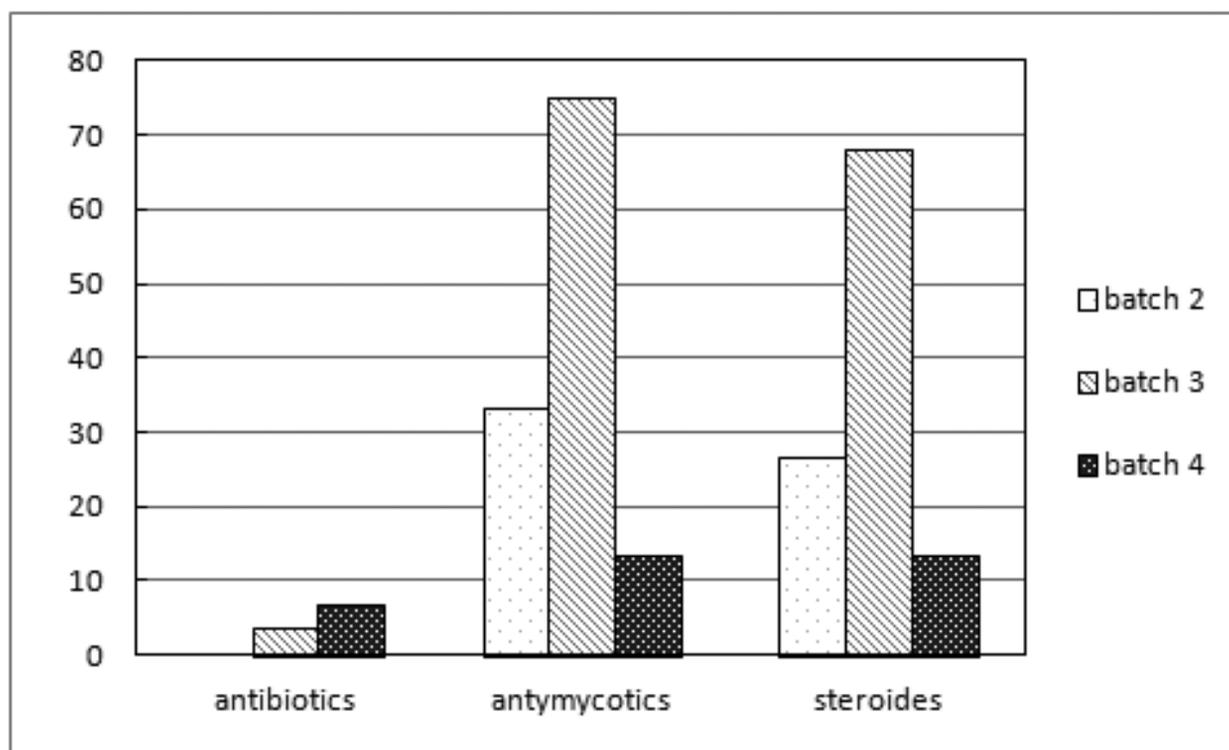


Fig. 2. Antibiotics, antifungal drugs and steroids taken by patients from different batch

resistant to antimycotic drugs. In addition, no significant statistical differences ( $p > 0.05$ ) were found in the incidence of individual species among patients of any batch, regardless of the species of fungus. Detailed results are shown in Table 2.

The sensitivity to medication was not found to be significantly different between strains, regardless of the medication used ( $p > 0.05$ ). However, statistically significant differences ( $p < 0.05$ ) were found between each of the medicines in batch 1; most strains of fungi were sensitive to nystatin and miconazole, and the least to ketoconazole and fluconazole. While the strains in batch 2 were significantly more sensitive to nystatin and miconazole than other drugs ( $p < 0.05$ ), no such significant difference was observed for ketoconazole, itraconazole or fluconazole. Similar results were obtained in batch 3. It was observed that the strains are significantly more sensitive to nystatin and miconazole than other drugs ( $p < 0.05$ ). No differences were found with respect to susceptibility to ketoconazole, itraconazole and fluconazole. Only in batch 4 were strains found to be significantly more sensitive to nystatin and miconazole than itraconazole ( $p < 0.05$ ). However, differences between the other medications were statistically insignificant ( $p > 0.05$ ) (Fig. 3).

## Discussion

Patients who undergo chemotherapy and/or radiotherapy are at increased risk of developing fungal infection. In the case of changes in the mucous membrane, fungi can move into the blood (fungemia) and develop into disseminated fungal infection, often leading to death. Hence, it is important to determine the presence of fungi in this group of patients before the beginning of chemo- or radiotherapy to enable early treatment.

In the present study with regard to the symptoms felt by the patients, between 3 and 21 of the examined patients reported pain, between 2 and 9 reported dysphagia and between 1 and 9 noted dysgeusia, depending on the batch. At the same time, mucositis symptoms were found in between 1 and 27 patients.

In comparison, 16 of 25 patients examined by Borysewicz-Lewicka et al. [6] complained of dry mouth and fungi of *Candida* genus were detected in 19 cases after radiotherapy. However, before radiotherapy, only 6 patients complained of dry mouth and *Candida* were detected in only 9. Similarly, Stryjski [7] showed that after radiotherapy, patients more often report symptoms such as burning (24% before, 66% after treatment), xerosthomia (respectively 36% and 76%),

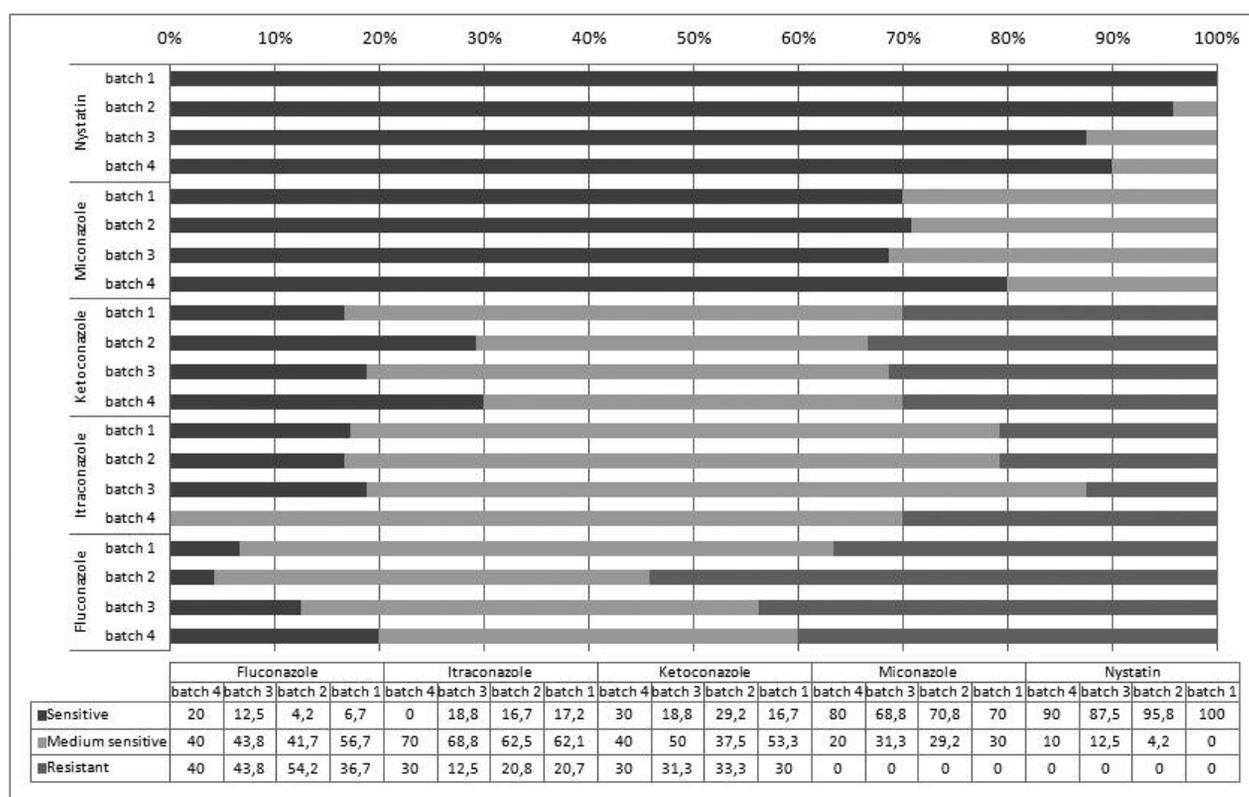


Fig. 3. The sensitivity of fungi from different batch to drugs

dysgeusia (22% and 62%), sensory disturbances (20% and 54%), spontaneous pain (2% and 30%) and provoked pain (18% and 58%). After the radiotherapy increased, increases were also noted in the incidence of redness mucosa (increased from 70% to 100%), swelling of the mucous membrane (24% to 70%), the presence of ulcers (2% to 24%) and deposits (18% to 62%). Belazi et al. [8] diagnosed mucositis in only 9 of 39 patients, the remaining mucositis accompanied pseudo-membranous candidosis, which developed after about 2 weeks after the start of radiotherapy and persisted for up to 6 months after its completion.

In the present study, more than two thirds (68.2%) of the patients were found to have fungi present in the mouth or throat before radiotherapy (batch 1), compared to 4/5 (80%) patients undergoing therapy (batch 2). Fungi were found to be present in more than half (57.1%) tested on the last day of therapy (batch 3), and these were all found in patients tested from batch 4.

Wisniewski et al. [9] studied 30 patients with mouth cancer. Fungi were detected in 17 of them by mycological and histopathological examination based on Grocott staining; in 12 by hematoxylin and eosin staining, and 14 by PAS staining. Although Čankovice et al. [10] identified *Candida* in 30% of

patients with oral cancer, 55% of the identified species being *Candida* species other than *C. albicans*, *Candida* was only found in 6.7% of patients with benign oral tumors.

Krajewska-Kulak et al. [11] revealed fungi in oral cavity of 24.4% of healthy subjects, 55.9% of patients with cancer and 70.0% of patients with gastrointestinal tract diseases: acute or chronic gastritis, peptic ulcer disease. The dominant identified species was *C. albicans*. However, Nucci et al. [12], found *C. glabrata* to be more commonly isolated during the course of radiation therapy for cancer of the head and neck outside of *C. albicans*.

Lalla et al. [13] showed that oral fungal infections were observed before treatment in 7.5% of patients undergoing radio- and chemo-therapy due to cancers of the head and neck, in 39.1% during treatment, and 32.6% after treatment. In contrast, Ramirez-Amador et al. [14] reported that the respective prevalences of detected fungi to be 43%, 62% and 75% (in 16% - mycoses).

Dwornicka [15] determined the growth of *C. albicans* isolated from the cheeks and tongue epithelium of patients before (study 1), in a 2–3 week course (study 2) and 3–4 months after completion of radiation therapy (study 3). Moderate fungal growth from the cheeks was 5.6%,

respectively, 38.9% and 25%, and the tongue – 5.6%, 19.4% and 16.7%; while growth was abundant in study 1 has not been observed, and from the cheeks in subsequent studies accounted for 27.7% and 25%, and from the tongue 52.8 and 61%, respectively.

Pytko-Polończyk et al. [16,17] studied 41 patients with cancer of the head and neck organs (mainly the larynx and tonsil) based on clinical and microbiological examination of oral mucosa before, in the 2nd and 4th week and also in 4–6 week after radiotherapy; they revealed the presence of different *Candida* species respectively in 46.3%, 68.3%, 70.7 and 43.9% of patients. The most frequently were isolated *C. albicans* (72.3%), much less *C. krusei*, *C. glabrata*, *C. kefyr* and *C. pseudotropicalis*. Recently cases of the mouth and throat mycosis among patients undergoing radiation therapy caused by *C. dubliniensis* and *C. glabrata* were presented [18,19].

As is seen from the above literature data, examined by us patients showed a higher prevalence of fungi.

The results obtained in this study for the drug sensitivity can't be compared with the literature data because such information has not been found.

## Conclusions

1. Patients undergoing radiotherapy complain of pain, dysphagia, and dysgeusia; in most cases mucositis is diagnosed.

2. The high prevalence of fungi in the mouth and throat of patients treated by radiotherapy reinforces the need to perform mycological examinations in this group of patients to detect fungi, identify their species and determine their sensitivity to drugs in order to prevent complications.

3. The species most frequently isolated from the patients are *Candida albicans* and *C. glabrata*. The latter is characterized by a resistance to the majority of antimycotic medications.

4. Most of the isolated strains are sensitive to nystatin and miconazole (applied locally) and to itraconazole (absorbed from the gastrointestinal tract).

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