Early detection in head and neck cancer – current state and future perspectives

Abstract

Survival and quality of life in head and neck cancer are directly linked to the size of the primary tumor at first detection. In order to achieve substantial gain at these issues, both, primary prevention and secondary prevention, which is early detection of malignant lesions at a small size, have to be improved. So far, there is not only a lack in the necessary infrastructure not only in Germany, but rather worldwide, but additionally the techniques developed so far for early detection have a significance and specificity too low as to warrant safe implementation for screening programs. However, the advancements recently achieved in endoscopy and in quantitative analysis of hypocellular specimens open new perspectives for secondary prevention. Chromoendoscopy and narrow band imaging (NBI) pinpoint suspicious lesions more easily, confocal endomicroscopy and optical coherence tomography obtain optical sections through those lesions, and hyperspectral imaging classifies lesions according to characteristic spectral signatures. These techniques therefore obtain optical biopsies. Once a "bloody" biopsy has been taken, the plethora of parameters that can be quantified objectively has been increased and could be the basis for an objective and quantitative classification of epithelial lesions (multiparametric cytometry, quantitative histology). Finally, cytomics and proteomics approaches, and lab-on-the-chip technology might help to identify patients at high-risk. Sensitivity and specificity of these approaches have to be validated, yet, and some techniques have to be adapted for the specific conditions for early detection of head and neck cancer. On this background it has to be stated that it is still a long way to go until a population based screening for head and neck cancer is available. The recent results of screening for cancer of the prostate and breast highlight the difficulties implemented in such a task.

Keywords: quantitative histology, human cytome project, hyperspectral imaging, optical coherence tomography

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1 Introduction

Recent data of the Robert Koch-Institute show an increase of newly diagnosed cancer in Germany to 425,000 cases in 2002 equaling 8% or 30,000 more cases than 2000. About 220,000 patients have died of cancer in 2002 [1]. The DRG-statistic 2006 shows that 82,401 patients have been treated as in-patients with ICD-codes C00–C14, C30–C33, C76, and C77 in German hospitals [2]. This sums up all cases from initial staging to curative treatment and palliative care.

This highlights that oncological prevention is a task for the entire society. The aim of prevention is to reduce the number of patients affected by cancer or succumbing to cancer too early. This concept is based on primary prevention (preventing the initiation of cancer) and on secondary prevention (early detection of cancer). An estimate of 50% might be prevented or cured by primary and secondary prevention [1]. However, the value of prevention is

under discussion and only 1% of the resources of the general health insurance in Germany are spent on prevention [3]. It certainly is wrong that prevention in principle is always safer and cheaper than treatment [4]. Implementing screening and prevention without thorough validation into population based programs is problematic, and the only screening program introduced so far (for breast cancer) is discussed contrarily [5]. Screening mammography for women aged 50 to 69 years reduces mortality of this age group by 15%: out of 2,000 women at this age 1 woman is saved from breast cancer death in a 10 years period; but 10 women get overdiagnosis or overtherapy, and 20% of women get at least 1 false-positive report within 10 years [6]. Most relevant are false-negative cases, such called interval-carcinoma which go undetected for a long time since they were non-pathological at screening. However, this phenomenon is hard to pinpoint in numbers. For other malignancies which are under discussion for screening (malignant melanoma,

colon cancer) there are no hard facts on negative side effects. On top, there is no generally accepted histological classification for epithelial neoplastic lesions, as it can also be observed in malignant melanoma where even experts hardly come to the same histological diagnosis [7].

Reacting to the unsatisfied situation in Germany members of the working group "Krebsepidemiologie" of the German Society of Epidemiology have published a paper together with the German Cochrane Centre [8]. They conclude that Germany completely lacks a sufficient infrastructure for conducting and evaluating of screening programs with the only exemption of breast cancer screening. In that case for the first time a specific test is applied centrally and the process quality is monitored. The most important aim of any screening ultimately is a reduction of mortality without screening side effects. In contrast of present "opportunistic" screening the targeted population of a true screening is healthy and has no symptoms yet. The goals for such a screening are: 1. reduction of incidence; 2. reduction of mortality; 3. reduction of overall mortality; and 4. improvement of quality of life. Ineffective screening just allows making the correct diagnosis earlier without altering the course of the disease. True net gain in lifetime can only be achieved by shifting death to later periods of life yielding to an overall longer life with known diagnosis (Figure 1). However, even optimal screening programs bear the risk of overdiagnosis and -therapy: minimal invasive and in situ carcinomas are detected and treated which would have never been clinically relevant in the patient's lifetime.

In addition, the public discussion focuses on the cost factor. According to the Fritz-Beske-Institute the overall cost for the health system will increase from 218 billiards today to 270 billiards Euro by the year of 2050 only due to the increase of expected life time. The cost per year per inhabitant in professional productive age for the treatment of cancer will therefore increase from 172.- € in 2008 to 280.- € in 2050. This calculation does not include extra costs for innovative diagnostics and therapeutics. But just due to a lack of competent health care professionals a hidden rationalization will occur [9]. This will yield to a health care collapse [10].

Up to 30% of cancer is estimated to be associated with avoidable risk factors (smoking, alcohol, occupational toxics, but also obesity, loss of mobility) [1]; nevertheless, so far the positive influence of a "healthy" lifestyle on cardiovascular disease, colon and breast cancer could not be proven [11], [12], [13]. Substitution with specific nutritients which seem to be sensible at first sight turned out to be devastating on a closer look: addition of calcium and vitamin D did not reduce the number of fractures but instead increase the number of nephroliths [14], and the "preventive" addition of estrogens and gestagens in postmenopausal women lead to significant additional health problems and massive health costs [15]. This underlines the necessary thorough evaluation of any screening program to prove that there is a positive overall cost-risk-ratio. The evidence must be clearly and object-

ively documented, and there must not be a negative consequence for not attending a program.

As early as 1984 Steiner showed that patient groups at high risk for head and neck cancer can be defined, that an adequate screening method is available [16], that endoscopy can be combined with cytology [17] albeit at slightly higher costs [18]. However, an anachronistic paradox still holds true unchanged: although cervix cancer has lower incidence and mortality in that anatomical location exfoliative cytology and PAP-staining are part of the routine screening but an equivalent approach for head and neck cancer is still absent. A colposcopic PAP-swab seems to be more readily accepted than a look into the oral cavity [19]. Indeed there is no high acceptance for screening of head and neck cancer throughout the population: even if directly invited in written form only 30–60% take part in screening; especially smokers have a low compliance [20], [21].

This phenomenon can be explained by the concept of cognitive dissonance [22], [23], [24]. According to that concept every individual tries to avoid non-congruent cognitive elements in order to reduce cognitive dissonance. Cognitive dissonance for example is produced by the clash of the attitude "I like smoking" with the fact "Smoking causes cancer". In order to reduce this intraindividual dissonance smokers fade the uncomfortable information out, thinking that it is not relevant for them and not realizing the warnings printed on the cigarette packs [25]. Cognitive dissonance has direct influence on the attitude and the acceptance of screening schemes [26]. On the national level screening for head and neck cancer has not been included into the national plan on "Early Detection of Cancer" of the Deutsche Krebshilfe following a national hearing in 2005 [27]. Therefore it can be claimed a great success to have head and neck cancer included into the Nationale Onkologische Präventionskonferenz 2007 in Essen [28]. According to the Robert-Koch-Institute the number of new cases of oral and pharyngeal cancer in 2006 is 7,800 and 2,600 patients, and 2,800 and 450 for laryngeal cancer (male and female, resp.) [29]. Including the cases of cancer of nose and paranasal sinuses, salivary glands, and other rare cases altogether 18–20,000 new head and neck cancer cases per year can be estimated in Germany. The DRG-statistics of the Statistisches Bundesamt details the in-patient cases in 2006 [2]: there have been 39,080 cases of cancer of the lips, oral cavity, and pharynx (C00–C11, C14), 9,296 cases of cancer of the hypopharynx (C12, C13), and 15,108 cases of cancer of the larynx and trachea (C32, C33). These cases have been associated with a mean stay as an in-patient of 8–10 days [2]. It is estimated that worldwide 615,000 new cases of head and neck cancer occur per year with a strong male preponderance (10–15:1). However, there are strong regional differences in some anatomical sublocalisations [30].
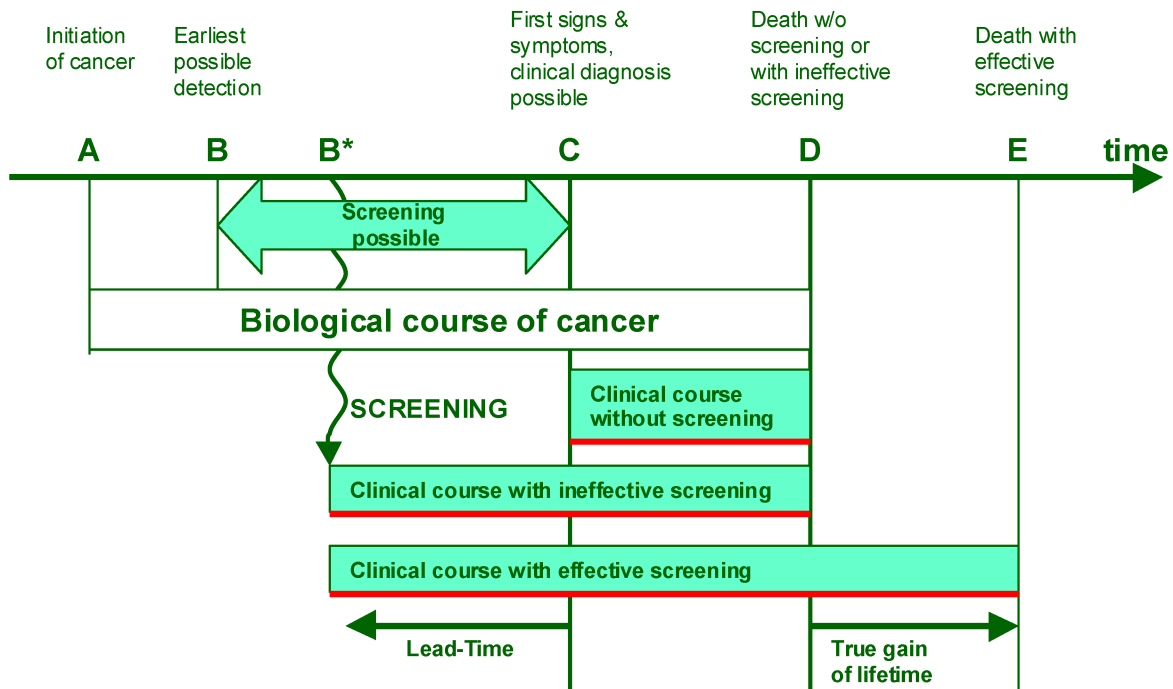
Only with effective screening a true gain in lifetime (B*–E) can be achieved. Ineffective screening just yields a prolonged life with known diagnosis (B*–D) as compared to the spontaneous course (C–D). From [8] with permission.

Figure 1: Effect of screening on lifetime with cancer

2 Risk factors

In order to prevent cancer or to detect it early potential and known risk factors have to be evaluated. In general and also for head and neck cancer the socioeconomic status of the patient has impact on his or her treatment. A retrospective analysis based in Georgia, USA, showed that the insurance status had significant influence on the survival of patients with head and neck cancer [31]. The observed delay time in making the diagnosis is mainly due to patient factors but there are different gaps reported depending on the anatomical sublocalisation ranging from 5 to 19 weeks [32], [33]. In oral cavity and pharyngeal cancer the delay was worst in non-smokers, large tumors, and singles [34].

2.1 Smoking

Tobacco smoking is a known risk factor for head and neck cancer. The Heidelberger case-control-study showed that >30 pack years increase the risk for head and neck cancer by the factor 4.8 [35], and >60 pack years by the factor 23.4 [36]. This information is relevant in the context of a study about smoking habits of the young in Germany: for those aged 12–17 years the percentage of smokers had a historical maximum in 1970 with 30%, decreased to 20% in 1993, had another rise until 1997 to 28%, and from then on showed a decrease to recent 18% of which each 9% assign themselves as occasional and permanent smokers, respectively [37]. In addition, the novel trend of shisha-smoking has to be mentioned,

which 14% name to be consumed at least once per month without assigning it as smoking nor as dangerous.

2.2 Alcohol

Alcohol is another known risk factor for head and neck cancer. The odds ratio ranges from 9.4 for 75 g ethanol per day [35] and 11.7 for >100 g ethanol per day [38]. There is a controversy about a hypothesized influence of the kind of alcohol consumed (wine vs. liquor etc.). However, there is a gender dependence: in females a consumption of >30 g ethanol per day increases the relative risk for head and neck cancer to 29; 40% of female head and neck cancer patients consumed >30 g ethanol per day [39]. In this group the amount of ethanol was lowest in cancer of the lips and highest in hypopharyngeal cancer [40].

Again, the attitude of the prospective new cancer patients, i.e. those now aged 12–17 years, has to be taken into account. Unfortunately, in this context the headlines of the rainbow press have to be verified: from 2004 to 2007 the total amount of ethanol consumed per week by this age group raised from 60 g to 71 g (male) and from 27 g to 29 g (female). Most alarming, such called binge-drinking (consuming more than 5 alcoholic drinks in one day) which is taken as an indicator for risky alcohol attitude has increased overall from 23% to 25%, focusing on 16–17 years old from 51% to shocking 63% [41].

Finally, the combination of both, smoking and drinking, has the highest propagating effect on head and neck cancer. This effect is over-additive and dose dependent.

Cancer of the oral cavity and the larynx had the highest consumption of nicotine, and cancer of the oropharynx and the larynx had the highest consumption of ethanol [36]. The combined consumption of >75 g ethanol per day and >30 pack years increase the odds ratio to 92 [35]. As an additional risk factor at least for cancer of the oral cavity poor oral hygiene and dental status (>20 missing teeth) has been documented (odds ratio 5.3 and 3.4 respectively) [42].

2.3 Occupational toxics

Prof. Maier and his team have detailed various occupational poisons and showed the relative risk to develop head and neck cancer associated with them: asbestos 8.7, cement 12.9, tar 6.6, and dyes/paints/solvents 2.3 (larynx) and 3.6 (oral cavity). The highest relative risk is achieved by the combination of these poisons with nicotine and ethanol [35], [43], [44], [45]. Another risk factor is the exposition to polycyclic aromates which increases the odds ratio for laryngeal cancer to 5.2 [46]. In conclusion, the group of blue-collar workers with additional high consumption of nicotine and ethanol can be identified as a high risk group for head and neck cancer. A special condition is the sinunasal adenocarcinoma. In this case, the exposition to inhalable dust of hard woods such as oak and beech carries a high risk for developing this cancer. The time delay is up to 40 years [47]. In Germany, it is a recognized occupational risk and accepted as occupational disease (BK 4203) with some 30 new cases per year (among some 70,000 employers). The odds ratio for joiners is 2.96. A concentration of 3.5 mg/m³ is definitively dangerous [48] showing a strong correlation especially for the histological subtype of intestinal type [49].

2.4 HPV-Infection

Although there was a general stagnation or even slight decrease in the overall incidence worldwide for head and neck cancer in parallel to the changing smoking habits, there was a marked increase for the incidence of oral cavity, tongue, and oropharyngeal cancer in never-smokers never-drinkers [50]. In this group, young men with tonsil cancer, young women with tongue cancer, and elderly women with cheek cancer are over-represented [51]. Several risk factors have been identified: >50% were serological positive for HPV16, 45% were passive smokers, 24% had occupational toxics in their history, and 30% had gastroesophageal reflux [51]. Together with other viral infections (HIV, HPV, HSV) there indeed is a plethora of risk factors in this group [52], [53].

The recent discussion focuses on the role of HPV16 in never-smokers never-drinkers [54]. zur Hausen is Nobel laureate for having established the concept of oncogenic viral infection [55]. First reports of HPV-positive cancer of the oropharynx and larynx date back to 1985 and 1987 [56], [57]. In 2006, in Sweden a marked percentage increase of tonsil cancer positive for HPV among all head and neck cancer cases from 1970 to 2000 was reported based on PCR on DNA-extracts from archived material [58]. Now, the presence of HPV-infection is recognized as a risk factor on its own for oropharyngeal cancer [59]. HPV-positive tonsil cancer can be rated as a clinical entity on its own [60], having a better prognosis than HPV-negative cancer [61]. A metaanalysis of 60 publications showed the prevalence for HPV-infection to be 25.9% for all head and neck cancer, highest in oropharynx (35.6%) and larynx (24.0%) and oral cavity cancer (23.5%). In oropharynx in 87% of positive cases are HPV16, whereas in hypopharynx and larynx a higher proportion of HPV18 is seen (17% vs. 38%) [62]. This is the basis for the discussion about vaccinating young males against HPV, too.

2.5 Chemoprevention

The great hopes initially put into the concept of chemoprevention have not hold true. It was just shown generally that the intake of 80 g per day of vegetables and fruit reduces the relative risk for head and neck cancer t0 0.91 and therefore can be assigned protective [63]. However, there is no single chemically identified substance yielding a preventive effect: 13-cis-retinoid acid did not influence the rate of secondary malignancies, recurrence, nor disease-free survival [64], and vitamine A and N-acetylcystein alone or in combination for 2 years had no influence on overall survival, disease-free survival, nor recurrence [65].

3 Precursor lesions

Any attempt of early detection of cancer implies a concept about how cancer arises in general. As early as 1890 Hansemann observed asymmetrical nuclear divisions in human cancer and discussed their relevance in the cancerogenesis [66]. This idea was reintroduced 25 years later by Boveri who was the first to describe chromosomes [67] and realized that a multipolare spindle apparatus yields to a misalignment and maldivision of chromosomes [68]. (For a detailed review of this historic background please refer to [69]). The development of stochiometric DNA-staining by Feulgen [70] and the construction of microscopes with quantitative densitometers [71] later on allowed to obtain the underlying quantitative data for these phenomena. As early as 1956 DNA-aneuploidy in malignant tumours was documented [72]. Casperson was then first to normalize the DNA-ploidy of cells from premalignant lesions using leukocytes as internal standard [73]. These analyses were repeated more recently in detail in breast cancer showing an inverse correlation of histological differentiation and the number of interphase nuclei with DNA-aneuploidy (>4.5c) [74].

Beginning in the 1980s a confronting alternative theory on the development of cancer has been developed. According to that theory cancer is caused by a stepwise activation of oncogenes and deactivation of cancer suppressor genes [75]. This construction is supported by the

observation of the hereditary non-polypoid colon carcinoma (HNPCC) and of familial adenomatosis polyposis (FAP); however, these cases account for not more than 5% of all colon cancers, most cases are not hereditary but sporadic. Actually, objective analyses and observations in human cancers are in contrast to this concept: about 50% of all known carcinogens are not mutagenic [76], oncogenes are not clonally expressed so that part of the cancer cells in a given malignancy lack them [77], many oncogenes are not expressed in many cancer cells, and instead of hypothesized 4–7 mutations [78] rather thousands of genes are mutated in cancer cells [79]. Spontaneous mutations, however, are extremely rare (10^{-6} per mitosis) [80] yielding to just ONE in 10^{10}–10^{28} human beings developing cancer taking the postulated 4–7 mutations as baseline! The supposed mutation of a "mutator-gene" can be found only in few cancers [81] and typically in late states [82]. Above of that, the hereditary "mutator-gene" which is postulated for Xeroderma pigmentosum does not lead to an increase of non-skin cancer in respective individuals [83].

These contradictions of this concept in itself recently have increased the interest in the prior hypothesis that aneuploidy is rather the cause but not the consequence of cancer [84], [85]. Duesberg has developed a convincing concept describing carcinogenesis as a chain reaction of aneuploidisation [86], [87].

As a matter of fact, a "precursor" lesion is defined by the clinical context. The WHO just states: "Precursor lesions are defined as altered epithelium with a high likelihood to develop into cancer". Clinically in general leukoplakia, erythroplakia, and chronic inflammation are seen, histologically represented as dysplasia and atypia. However, what exactly a dysplasia is seems to be highly variable according to Barnes and coworkers: "The terminology of these precursor lesions, however, is still evolving and no single classification has been universally accepted" [88]. Histopathologically a number of histological and cytological criteria are described leading to the diagnosis "dysplasia". In fact, also these criteria do allow neither a clear separation of hyperplasia on the one hand and early dysplasia on the other hand nor a separation of several grades of dysplasia. The only sure fact is that cancer can develop from any grade of dysplasia and can even arise from completely intact mucosa.

The corresponding theoretical concept for precursor lesions is represented by the term "intraepithelial neoplasia" (IEN) for non-invasive mucosal lesions with genetic alterations, loss of control of cellular functions, and phenotypic characteristics of invasive cancer, summing up in a lesion with high likelihood to progress into an invasive cancer [89].

In contrast, the terms leukoplakia and erythroplakia are clinically defined and have no place in pathological diagnoses [90]. Leukoplakia was first used by Schwimmer in 1877 [91] and nowadays is defined by the WHO as a "white patch or plaque that cannot be characterized clinically or pathologically as any other disease" [92]. Erythroplakia was first used by Queyrat in 1911 [93] and

in analogy is defined by the WHO as a "red patch of the mucous membrane which does not represent some specific or nonspecific inflammatory lesion" [94]. All erythroplakias presumably will show histological signs of dysplasia, in 51% invasive carcinoma has been observed and in 40% carcinoma in situ or "severe" dysplasia have been seen; as a consequence, erythroplakias even more than leukoplakias should trigger an intensive diagnostic evaluation [95]. In any case, in mixed lesions the eurythroplakia component has to be included into an incisional biopsy [90].

The problem of severe intra- and interobserver variability in histological and cytological diagnoses is increasingly realized and discussed [95], [96]. The influence of this variability on screening schemes was estimated [97]. For head and neck cancer the interobserver agreement in judging such called oral pre-malignant lesions has been evaluated. Overall only a good agreement could be achieved (κW=0.59) with further reduction in cases with accompanying inflammation (κW=–0.10) although histological instead of cytological specimens were evaluated; in addition, also the anatomical site and the way of biopsy (incision vs. punch) showed some influence on the agreement. Taking bivariate rating (carcinoma and carcinoma in situ versus less severe changes) did not improve agreement (κW=0.39). Only in non-smokers a very good agreement was achieved (κW=0.71) [98], [99]. Similar results were obtained in laryngeal lesions (κW=0.32), bivariate comparison yielding κW=0.52 [100]. In the meantime it has been realized that these problems are immanent to the histopathological analysis itself according to Lessells et al.: "Histopathological diagnosis is not carried out in an algorithmic process. Individual pathologists have a highly trained visual cortex, such that within a few seconds of looking at a slide a number of conclusions have been drawn and a diagnosis (at least provisional) made. Obviously, individual pathologists are programmed in slightly different ways, accounting for the individual variation seen in a linear spectrum of abnormality such as dysplasia. Even if strict criteria were applied, it is unlikely that any improvement would be more than marginal" [101]. This highlights the effect of screening is influenced by reproducible and correct diagnoses. Taking the variance of histology and even more cytology into account it is first of all up to the clinician to identify suspicious lesions. It is the clinician who has to decide on which lesion can be observed and which has to be biopsied as it is the case in the esophagus [102].

4 Present state of early detection

The above notes lead to the conclusion that a true screening for head and neck cancer cannot be achieved within the near future. In the following the different attempts for secondary prevention, i.e. the early detection of invasive carcinoma and its precursors, will be outlined according to the anatomical subsites.

4.1 Oral cavity and oropharynx

The oral cavity and the oropharynx are ideal for early detection due to their good accessibility for inspection. First considerations about early detection and screening were published in 1969, 1974, and 1978 [103], [104], [105]. Nevertheless, up to date there is no consensus guideline for screening of premalignant oral lesions [90]. Since the 5-year-survival correlates directly to the state at first diagnosis, early detection not only could improve the incidence but also the survival. The continuing input into patient information and the ongoing education of community based physicians and dentists are equally important [106], [107].

In a recent review by the Cochrane Collaboration [108] only one study from India where oral cavity cancer is more frequent [109] had sufficient quality. But due to methodological deficits (e.g. no data about costs and risks) also this study did not give evidence pro or con for screening for oral cavity and oropharynx cancer by inspection with or without optical tools. Nevertheless, these tools are outlined in the following chapters.

4.1.1 Chromogen-aided visual inspection

For a long time it has been recognized that altered mucosa shows a staining behavior with exogenous dyes different from that of normal mucosa. One of the early dyes is toluidine-blue which was first described in 1952 for its use as intravital dye [110]. The exact mechanism is still not understood. Several studies have evaluated its clinical use but the high rate of false-positive lesions has shown its limited use [111], [112]. Other dyes such as Bengal-Rose are little better even when intensity of the staining is compared with a standardized table [113].

Using pre-incubation of the mucosa with 1% acetic acid the ViziLite™ system tries to improve the detection of altered mucosa by blue light (490–510 nm). However, independent studies in contrary show that neither the number of detected lesions is increased nor the correlation to histology improved [114]. Some investigators rated the reflections of the blue light as disturbing [115]. This system also has a high false-positive rate leading to a number too high for routine use in screening and prompting an unacceptable number of unnecessary biopsies [116].

In conclusion, all assays based on exogenous chromogens rely on a high clinical experience of the investigator [117].

4.1.2 Autofluorescence

The phenomenon of autofluorescence is another tissue feature recognized for some time [118] and first described as a useful tool during bronchoscopy in 1962 [119]. Since then autofluorecence has been established as an integral part throughout the entire upper aerodigestive tract [120], [121], [122], [123]. Sensitivity and specificity for the discrimination of normal mucosa and cancer has been quoted to be 91% and 86%, both better than for examination with white light (75% and 43%, respectively).

Autofluorescence can be modified by applying 5-aminolevulinic acid (5-ALA) topically or systemically. While altered mucosa shows a loss of autofluorescence, it exhibits a gain of 5-ALA-induced fluorescence. The specific accumulation of 5-ALA in tumor cells was first described in 1966 [124]. The resulting protoporphyrin IX-fluorescence is used to detect cancer. Since the cells first have to metabolize the 5-ALA there has to be a time gap of 1.5–3 hours until the investigation is possible [125], [126]. For this technique, a sensitivity of 99% and a specificity of 60% are quoted.

4.1.3 Molecular markers

Cells form altered mucosa can be analyzed extensively by molecular genetic assays. Loss of heterocygocity (LOH) can be used as a marker for genetic changes: LOH at 9p and 3q has been found in dysplasias, and an additional LOH at 4q, 8p, 11q, and 17p has been associated with an increased risk for the development of cancer [127], [128].

Another genetic change is an infection with HPV. In that context HPV16-infection in non-smokers non-drinkers plays a special role. HPV16 infection is associated with a higher risk of head and neck cancer in both, smokers and non-smokers. The proportion of tumor specimens containing the genome of the virus, however, varies between neglectible and 70% [59], [61], [129]. A study comparing HPV-status in biopsies and exfoliative cytology from the same patient showed no correlation between the both: 90% of patients HPV-positive in the biopsy where negative in the exfoliative cytology [130].

The analysis of cytological parameters is given an increasing importance in the context of early detection of oral cavity and oropharyngeal cancer. Their value will be further increase by including the analysis of quantitative parameters [131] (see 5.1).

4.2 Larynx

Classical screening for laryngeal cancer is based on the subjective analysis of conventional indirect laryngoscopy with or without magnification [16]. Initially "optical amplifiers" were used such as the topical application of toluidine blue [132]. This dye, however, was last mentioned for topical application in the larynx in 1982; in that study, the comparison with histology in 272 cases showed a sensitivity of 91% and a specificity of 52% [133].

4.2.1 Autofluorescence

The application of autofluorescence in the larynx was reported first in 1995 [134]. Autofluorescence has been shown to improve both, sensitivity and specificity, in rigid and flexible endoscopy for the delineation of the borders of altered mucosa (to 97% and 84–92%, respectively). The presence of inflammation, scaring, and hyperkera-

tosis has been identified as limitations of this technique leading to false-positive and false-negative results [121], [135], [136], [137], [138].

The use of 5-ALA has also been evaluated for laryngoscopy. Again, a gain in 5-ALA induced fluorescence has been observed in laryngeal cancer [125], [139]. A first trial of topical 5-ALA for photodynamic therapy in a pilot study, however, has been disappointing [140].

4.3 Hypopharynx

Since early symptoms in hypopharyngeal carcinoma are vague or absent and since prognosis depends on size at first presentation as in any other region, early detection of cancer of the hypopharynx would be especially effective [141], [142], [143]. In 2006, 9,296 patients have been treated as in-patients in Germany with the diagnoses C12 or C13 (cancer of the pririform recessus or cancer of the hypopharynx; for comparison: C32 – cancer of the larynx: 14,656 cases) according to the DRG-statistics of the Statistisches Bundesamt [2].

The odds to detect hypopharyngeal cancer at an early stage are best for patients who already had a carcinoma of the upper aerodigestive tract or of the esophagus in their history. This risk group has a substantial benefit from routine endoscopies even if no symptoms are present. The proportion of second primaries of the hypopharynx in stage I + II versus stage III + IV can be dramatically increased: from 22:78 without routine endoscopy to 90:10 with routine endoscopy. As a consequence, radical therapy with larynx preservation was possible in 79.4% with routine endoscopy as compared to 45.5% without routine endoscopy [144].

4.4 Salivary glands

Tumors of the salivary glands have an especially broad histological spectrum. Accordingly, the complex classification has been revised several times [88]. For this reason the data for some entities are insufficient. The overall incidence for malignant salivary gland tumors is 0.4–2.6/100,000. The most common malignant salivary gland tumor seems to be the mucoepidermoid carcinoma [~50% of cases], adenoidcystic carcinoma raging second. The incidence increases with age culminating around the age of 70. Males have a slight preponderance [145].

A known risk factor for malignant salivary gland tumors is ionizing radiation (by the atom bomb [146] or by radiotherapy [147] or by J131 e.g. as part of treatment for thyroid disease [148]). Electromagnetic radiation of mobile phones has no demonstrable negative effect [149]. Known occupational risk factors are nickel, chrome, asbestos, and cement dust, and ingredients used in rubber production [150], [151], [152]. Also hair dressers were shown to be at increased risk [153]. Exceptionally there is no increased risk for malignant salivary gland tumors associated with smoking and drinking [154].

As a consequence there is only a small population at risk that can be defined as a target group for screening. In 2006, there have been 2,457 patients treated as in-patients in Germany with the diagnosis C07 or C08 (cancer of the parotid gland or of other salivary glands of the head) according to the DRG-statistics of the Statistisches Bundesamt [2].

4.4.1 Fine-needle aspiration biopsies

Fine-needle aspiration biopsy (FNAB) has been long established as part of the diagnostic work-up for salivary gland tumors and can in principle be used as a tool for screening schemes [155], [156], [157], [158], [159]. These studies report false-positive and false-negative results in 1–14% of cases. The correct diagnosis was made in 81–98% of cases. Schröder et al. have evaluated the use of FNABs in the work-up of parotid gland tumors in 2000 [160]. They did not see seeding of tumor cells along the biopsy canal leading to satellite metastases or any other relevant complication. Retrospectively, only 284 specimens out of 336 had been sufficient for pathology due to the presence of tissue fragments; with these 284 specimens, sensitivity was 93.1% and specificity was 99.2%. Those cases where only single cells but no tissue fragments were obtained by FNAB were scored "inadequate". Obviously, conventional "cyto"pathology is rather some kind of "microhisto"pathology. For true cytopathology, i.e. the analysis of single cells, quantitative and objective assays are available (see 5.1).

4.4.2 Ploidy

Up to recently the DNA-ploidy could only be determined from freshly resected tumor tissue. Using flow cytometry DNA-aneuploidy could be found in 24–28% of the malignant tumors, and all histologically benign tumors were DNA-diploid [161], [162]. The drawback of this method is that the tumor first has to be resected before tissue can be taken in order to obtain single cells for flow cytometry. Consequently, this test cannot be used for screening or preoperative analysis. As a matter of fact, however, the analysis of DNA-ploidy has been shown to be beneficial as part of the diagnostic work-up of salivary gland tumors. For example, this test was able to identify a carcinoma ex pleomorphic adenoma that was undetected by routine histology [163].

4.5 Second primaries and recurrences

The concept of field cancerisation was first published by Slaughter et al. in 1953 following their observation that head and neck carcinomas in general have a rather lateral spread as compared to their vertical dimension and that there is always surrounding mucosa which also shows alterations to some extent [164]. This is also highlighted by anecdotic reports of synchronous multiple carcinomas of the same anatomical sublocalization [165]. Recently, the respective molecular changes have been shown [166]. The common criteria for a "second primary" have been defined in 1932 [167]: tumors have to have a clear,

but different malignant histopathological signature, and a metastasis has to be excluded. Rather arbitrary a lateral gap between the two lesions of 2.5 cm and a time interval of 3 years has been defined. In any case, patients with a newly diagnosed head and neck cancer or a positive history are at high risk to develop a second primary and should be thoroughly investigated as well initially at first presentation and during follow-up in order to detect a second primary in the entire upper aerodigestive tract. The rate of second primaries ranges from 9 to 19%; 41–46% are synchronous and 54–59% are metachronous [168], [169], [170], [171]. Using panendoscopy up to 85% of synchronous and 67% of metachronous secondary primaries can be detected [172].

Whereas recurrence occurs within the first 3 years, second primaries show an unchanged incidence beyond the 5th year [168]. For laryngeal cancer, a population based study on 20,074 patients showed a second primary in 3,533 cases (=17.6%); the cumulative risk was 26% at 10 years and 47% at 20 years. Treatment with radiation therapy increased the risk to develop a second primary especially in those patients who survived the 5th year [173]. In general, a second primary is correlated with a poor survival: the median survival is approximately 25 months [174].

The paradoxical late adverse effect of radiation therapy in terms of inducting a second primary has been first described by H. Glanz as early as 1976 [175]. Since then, the cancerogenic effect of low ionizing radiation doses has been well documented [147], [173], [176]. On that background, there is an additional risk to develop a second primary for patients with a head and neck cancer: the cancerogenic effect of radiation therapy. Especially patients with a low stage first primary should benefit from intensive follow-up since they have good chances for curative treatment of the second primary, too. Extensive surgery with reconstruction by microvascular reanastomised flaps had no influence on the risk to develop a second primary [177].

4.5.1 Endoscopy

Repeated endoscopy both, as rigid hypopharyngo-laryngo-esophagoscopy or as flexible videoesophagoscopy, are a standard that can be offered to patients with head and neck cancer and with esophageal cancer [178], [179], [180]. The highest benefit is seen for patients with a small first primary: in T1-T2 carcinoma of the oral cavity, the time gap from the end of the initial therapy to the detection of a second primary was beyond 60 months in 23% of cases [181]. The survival time was significantly increased in patients who underwent routine endoscopies as compared to patients with disease-triggered endoscopies from 32 months to 58 months [182].

4.5.2 "Tumormarker"

In a review on circulating tumor makers of head and neck cancer in 1994, not a single marker or a combination of several markers had a level of sensitivity or specificity high enough to justify its use as a "tumor marker". If at all some use for monitoring of therapy seemed helpful [183]. This judgment was unchanged by two later reports which included molecular parameters [184], [185]. Out of the proteins Cyfra21-1, SCC- and TPS-antigen (squamous cell carcinoma antigen and tissue-polypeptide specific antigen, resp.) Cyfra21-1 seems to be adequate to detect a recurrence or a second primary (sensitivity 96%, specificity 87%) [186], [187], [188]. Indeed, the failure to define a single parameter or a combination of markers predictive for head and neck cancer is due to the fact that this group of malignancies has a wide spectrum of molecular genetic changes represented by the diverse histopathologic findings [189].
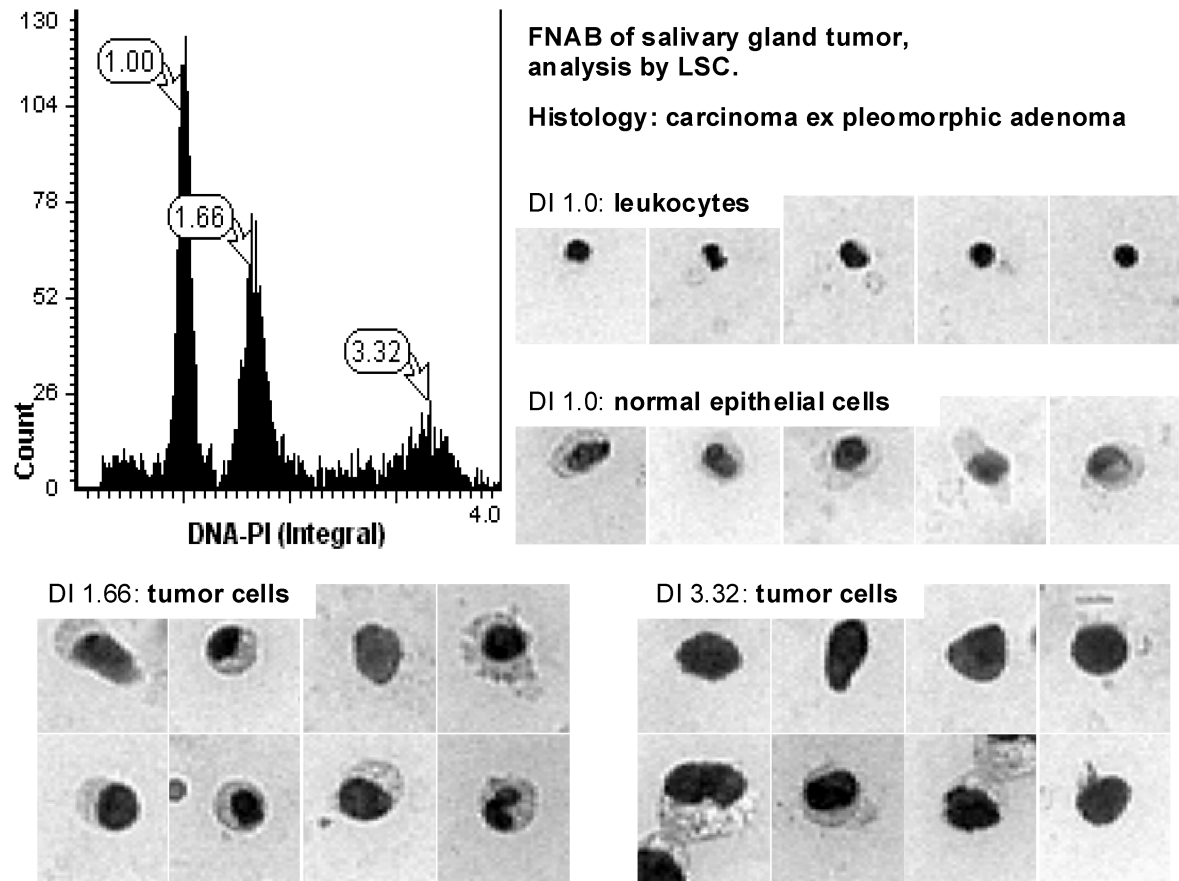
4.5.3 PET-CT

Since its introduction as fused PET-CT into oncology in general [190] there have been several studies for head and neck cancer as part of the initial staging [191], [192] and during follow-up for the detection of recurrence and second primaries [193], [194], [195]. In general, these studies just compare PET-CT with other imaging modalities. Only rarely the result of PET or PET-CT is compared to the histopathological diagnosis or the clinical course of untreated PET-CT-positive lesions is evaluated, e.g. if these lesions lead to locoregional recurrence. For example, there was a consensus of PET-CT and histopathology in only 9 out of 16 neck dissections [196]. Another study compared PET, PET-CT, CT, and MR and showed that PET-CT had a better accuracy than CT/MR in detecting primaries and metastases (98% vs. 86% and 92% vs. 85%, respectively) [197] but still there were false-positive and false-negative results [198].

5 Perspectives

In summary there are three areas that have the potential to yield an improvement of the early detection of head and neck cancer in the near future: 1. improved analysis of cytological specimens, 2. novel approaches to identify tumor markers, and 3. improved investigation techniques for the detection of altered mucosa.

5.1 Cytodiagnostic

There is a renaissance of cytodiagnostic assays in the head and neck region recently [131]. This is due to the novel insights into the development of cancer [86], [87], [199], to better minimal-invasive approaches to obtain specimens, and to improved optical (detection) systems. In general cytopathology can be improved by the use of cytometric techniques as this has been advocated as "intensified cytodiagnostic" by Hanson et al. in 1989 [200] and lined out by Böcking et al. in 2004 [201]. Since the specimen in general is analyzed on a slide as a solid

FNAB of salivary gland tumor, analysis by LSC.

Histology: carcinoma ex pleomorphic adenoma

DI 1.0: **leukocytes**

DI 1.0: **normal epithelial cells**

DI 1.66: **tumor cells**

DI 3.32: **tumor cells**

Cells from a FNAB taken from a solid salivary gland tumor were prepared on a slide and stained stoichiometrically with the DNA-dye propidium-iodie (PI). The fuorescence intensity of single cells was analyzed using a LSC (see [202]). The DNA-index (DI) of leukocytes was taken as internal standard for epithelial cells. Tumor cells show clear DNA-aneuploidy (DI=1.66 and DI=3.32) typical for a malignancy. Conventional HE-staining and re-localisation of cells proves altered morphology of respective single cells. Routine histopathology showed a carcinoma ex pleomorphoc adenoma.

Figure 2: Slide-based quantitative cytometry

carrier these techniques are summarized as slide-based cytometry (SBC) [202].

A major focus is set on the determination of the DNA-ploidy of the tumor cells. Shortly after patenting the world's first flow cytometer by Partec in Münster, Germany (Impulscytophotometer IPC 11) [203] the analysis of DNA-content of single cells was established [204]. The first application for head and neck cancer was published in 1972 [205]. In parallel, the analysis of DNA-ploidy by image cytometry was developed and applied to head and neck cancer, too [206]. This lead to the identification of DNA-ploidy as a prognostic parameter on its own for head and neck cancer: most tumors are DNA-aneuploid, and patients with a DNA-diploid tumor have a better prognosis for both, locoregional metastases and 5-year-survival rate [207], [208], [209], [210], [211], [212], [213].

Unlike flow cytometry which needs larger amounts of cells obtained by tissue specimens, for SBC minimal (hypocel-lular) specimens suffice (such as FNABs or exfoliative cytologies). The DNA-ploidy can be determined by image cytometry after Feulgen staining [214], [215] or by laser scanning cytometry after fluorescent staining [216], [217], [218] (Figure 2), both methods being adequate [219]. The sensitivity and specificity for detecting a head and neck cancer are 79–96% and 93–100%, respectively. In addition to the DNA-ploidy, other markers can be analyzed [220]. The analysis of DNA-ploidy allows to detect a carcinoma months before it can be identified by histopathology [221]. SBC allows to reduce the proportion of "inadequate" specimens for cytological analysis. This technology can be applied throughout the entire upper aerodigestive tract even in areas not accessible for conventional swabs: esophagus and hypopharynx can be screened in a cursorical manner by using sponge that is swallowed by the patient [222].

In other areas the cytological work-up has been already updated for its clinical use to a much better extent than in ENT. Screening for cervical cancer and its precursors for example in many schemes includes a variant of SBC, such called liquid-based cytology: the specimen is obtained as a suspension and dispersed on a microscopic glass slide in a special centrifuge and analyzed automat-

ically [223]. This approach has been shown to be superior to conventional swabs where cells tend to lay in clusters of several layers and show substantial variation in fixation and staining [224], [225]. In the follow-up of urological tumors, a quantitative cytological analysis of specimens obtained from voided urine using fluorescence-in-situ-hybridization has been established [226].

SBC is leading to an objective and quantitative histopathological analysis even avoiding staining with exogenous dyes at all (see 5.3.4) [227], [228], [229], [230].

5.2 Proteomics

The transition of a cell from normal to cancer includes significant changes in the expression of cellular proteins. This includes the cancerous cell itself, but affects also the surrounding normal cells of blood vessels, of the connective tissue and infiltrating inflammatory cells. This yields to a change in the proteins circulating in the serum throughout the body. Proteomics intends to identify single biomarkers or clusters of protein changes (signatures) which allow to detect the presence or recurrence of a cancer. It aims to help in screening, initial staging, and follow-up, too. Proteomic based biomarkers are thought to open the door to personalized medicine in near future [231]. Using a plethora of different assays biomarkers are targeted from the DNA via RNA to protein expression on cell, tissue, and plasma level (cDNA array, oligonucleotide arrays, reverse-phase proteomic arrays, and tissue arrays) [231]. For head and neck cancer, there have been applications developed on protein level using surface enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS) [232] and evaluated [142], [233], [234]. A SELDI-TOF-MS-based classification had a sensitivity of 82–92% and a specificity of 76–90%. As long as biomarkers are proteins they can be bound by antibodies. Novel techniques allow to bind a set of different antibodies to a glass slide [235]; this approach has already been applied for the identification of novel biomarkers of breast cancer analyzing synchronously 387 proteins on a single array [236]. Flow cytometry can also be used for the simultaneous analysis of several blood-bound proteins, e.g. cytokines [237], [238].

A fusion of different novel detection assays on the one hand and of minimal specimen requirements on the other hand is targeted by the "lab-on-the-chip"-technology. These applications could finally be less expensive than present detection systems and are reviewed excellently by Ziober et al. [239]. Taking saliva as the specimen, applications cover the entire spectrum of oncology, from screening via initial staging to detection if minimal-residual disease or recurrence.

The major problem of proteomics actually is to extract that part of information out of the vast amount of data which is relevant for the respective application. Therefore, following Virchow's concept of Cellularpathologie putting the cell as the smallest unit of life [240] the Human Cytome Project was initiated [241]. So far, the bottom-up concept of integrating single bits of information into one working concept has not been successful: looking at protein chemistry, it took more than 30 years of research but still there is no way to predict the 3D-structure of proteins based solely on their amino acid sequence. Instead, proteins are synthesized and then their 3D-structure is analyzed. One has to keep in mind that this is a problem of just 23 variables (i.e., amino acids). Obviously it will take some time until one can construct the cellular phenotype after analyzing the up to 20,000 genes and their products as it is tried in the Human Genome Project. Besides the fact that there are novel members detected continuously, not all "rules" of interaction between the 20,000 variables are understood and even novel "regulatory boards" are discovered (e.g., siRNA). Respecting these frustrating attempts, the Human Cytome Project is working top-down: taking the known phenotype represented by the cell (normal vs. ill) the obtained cell-based information is classified in a learning set and then applied to a test set [242], [243]. This allows to predict the clinical course (i.e. survival) of colon cancer with almost 100% accuracy [244] and to identify those patients with acute myeloic leukemia (AML) who need maximal therapy (i.e. stem cell transplantation) even before any therapy has been initiated [245]. This approach has also great potential in drug discovery [246], [247].

For screening, at present those assays have the biggest potential that allow to globally determine the individual risk of cancer with a simple test. First, there are analyses of saliva detecting its genotoxic potential (AMES-test) [248] or the (hyper)methylation of specific sequences within the genome [249]. Second, there are cytological analyses of cells contained in the saliva in order to detect global changes of the genome such as micronuclei or DNA-ploidy [250], [251]. In addition, peripheral blood leukocytes can be analyzed for their susceptibility to cancerogenes (using the bleomycin-test [252] or the COMET-assays [253], [254], [255], [256], [257]). These tests could be used for a pre-selection in a population-based screening.

5.3 Optimized visualization

The assays discussed in 5.1 and 5.2 yield to identify individuals with a (so far undetected) head and neck cancer. Most assays have the major drawback that they are not able to pinpoint the exact location of the cancer in the wide field of the upper aerodigestive tract. In the following some endoscopical approaches will be discussed that deal exactly with this problem: to highlight areas within the mucosa with altered architecture or even single cancer cells.

5.3.1 Chromoendoscopy

For a long time it has been recognized that mucosa with an altered structure shows a different behavior when stained with exogenous dyes as compared to normal mucosa. In 1835 the French physician Jean Guillame Lugol described the iodine-kaliumiodine-solution named
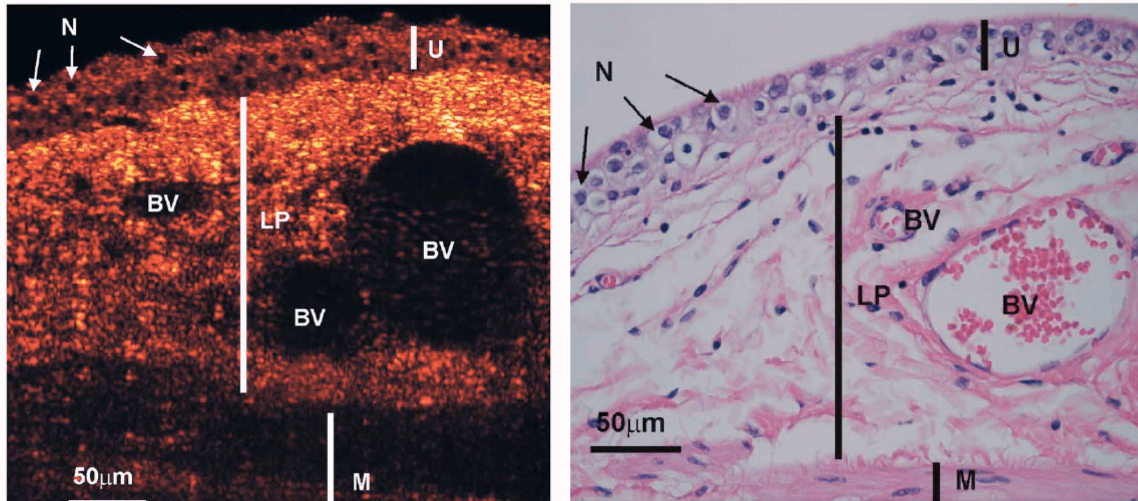
Comparison of subcellular µOCT (left) with the corresponding histopathologic section (right) of rat bladder. U: irothel; LP: lamina propria, M: muscularis; BV: blodd vessel; N: nuclei. From [268] with permission.

Figure 3: Optical coherence tomography

after him. A first report about its use during an esophagoscopy dates as its latest back to 1966 [258] but it almost certainly was widely used earlier than that. Recently it is re-discovered by colleagues from surgical and medical departments for endoscopy [178], [180], [259] although it was widely practiced in many ENT-departments as standard procedure according to personal communication of the author with senior members of the society. Lugol's staining has a sensitivity of 96% and a specificity of 63% in identifying highly dysplastic lesions of the esophagus [260]. Due to its minimal technical requirements, its safe use, and the low prize justify its use for the investigation of the esophagus by ENT-doctors, too. However, its application in the oral cavity, the oro- and hypopharynx and the larynx leads to dysesthesia and even pain, and there have been warning comments about aspiration [261], so that it should not be applied for the investigation of these anatomical regions.

The potential of autofluorescence alone or in combination with 5-ALA-induced PPIX-fluorescence have already been discussed in 4.1.2 and 4.2.1. Sensitivity and specificity for the discrimination of normal mucosa and cancer by autofluorescence are quoted as 91% and 86%, respectively, by 5-ALA-induced PPIX fluorescence as 99% and 60%, respectively, and therefore are better than with white light (75% and 43%).

5.3.2 Optical coherence tomography

In optical coherence tomography (OCT) light with long wavelength (>1,300 nm) is used similar to ultrasound in order to obtain sectional views or 3D-reconstructions of the mucosa. Similar to applications at the retina [262] the reflected light is used to imagine thickness and structure of the mucosa with a lateral resolution of 10 µm and a depth of penetration of 2 mm. So far applications for laryngeal mucosa have been published; especially in non-exophytic lesions with smooth surface the mucosal

thickness and the basal membrane can be analyzed [263], [264], [265]. Further technical modification could allow to integrate this technology into conventional endoscopes [266], [267]. Recently there has been the development of a µOCT that has a resolution down to the subcellular level showing a very good correlation with conventional histological sections [268] (Figure 3). OCT still has to be evaluated for its clinical use.

This also applies to narrow band imaging (NBI). This technology also makes use of the reflected light which is passed through adequate filters limiting the wavelengths to "narrow bands" (i.e. 400–430 nm, 430–460 nm, and 485–515 nm). This allows to better visualize the microvasculature within the mucosa and in deeper layers [261]. This technique has already been integrated into conventional endoscopes and can be used for the detection of superficial mucosal changes in the oral cavity and the oro- and hypopharynx [269].
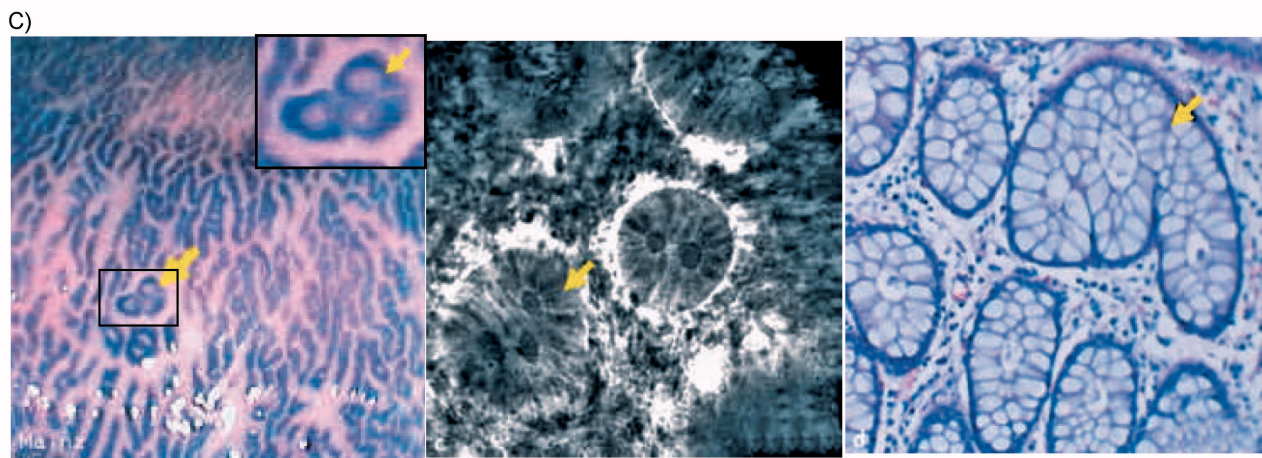
5.3.3 Confocal endomicroscopy

Another step to in-vivo-histology is offered by confocal laser scanning microscopy (cLSM). This technology yields a lateral resolution of 0.5–1 µm, an axial resolution of 3–5 µm, and a maximal depth of 200–500 µm [270]. This allows to gain most of the information that is obtained by conventional routine histopathology in order to analyze tissue and to discriminate benign from malignant lesions: size and configuration of nuclei, morphology of the chromatin, prominent nucleoli, mitoses, and the ratio nucleus:plasma. Based on microfabrication [271] the tip of conventional endoscopes can be fitted with a miniaturized cLSM-objective and to mount it to 50–100,000 optical fibers; this allows confocal endomicroscopy [272]. First applications have been described for colon cancer highlighting the optical properties impressively [273], [274] (Figure 4). Shortly after that first applications for laryngeal endoscopy have been published [275]: the re-

A)

Design of a confocal endomicroscopy: a confocal laser microscope protrudes 2 mm out of the distal tip of the endoscope and is manipulated by two additional buttons (left). Blue laser light penetrates the tissue and is reflected. Focusing on different depths yields optical sections at different levels (right).

B)
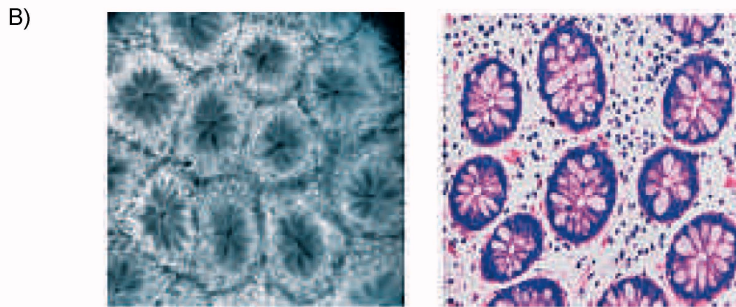
Comparison of confocal endomicroscopy (left) and histology (right) exemplified in healthy colonic mucosa. Goblet cells, crypts, and stromal cells can be clearly cifferentiated. Fluorescein was injected i.v. for better contrast.

C)

Aberrant crypts in a colorectal carcinoma. Left: chromoendoscopy (overview and *en detail*); middle: confocal endomicroscopy showing an aberrant crpyt; right: targeted biopsy yields histologic proof. From [274] with permission.

Figure 4: Confocal endomicroscopy

sected mucosa in this paper actually has been studied using conventional upright confocal laser scanning microscopy. However, in principle the step to laryngeal confocal endoscopy has been made.

In conclusion, these techniques (optical coherence tomography, confocal endomicroscopy) will yield bloodless, optical in-vivo sections. However, as long as there are no automatic algorithms analyzing the images there will be a reduced information obtained by these sections just as it is the case with routine histopathology: the sections have to be assessed by the observer's eye and his or her brain behind them. At least they are looking at living cells in action which is not the case in pathology.

5.3.4 Hyperspectral imaging

The cellular function can be better assessed by analyzing the electromagnetic spectrum. This approach has been used by geoscienticts when analyzing and classifying satellite images. "Spectral imaging" is defined as analyzing the entire spectrum from infrared to ultraviolet for every single pixel of an image. This generates an "image
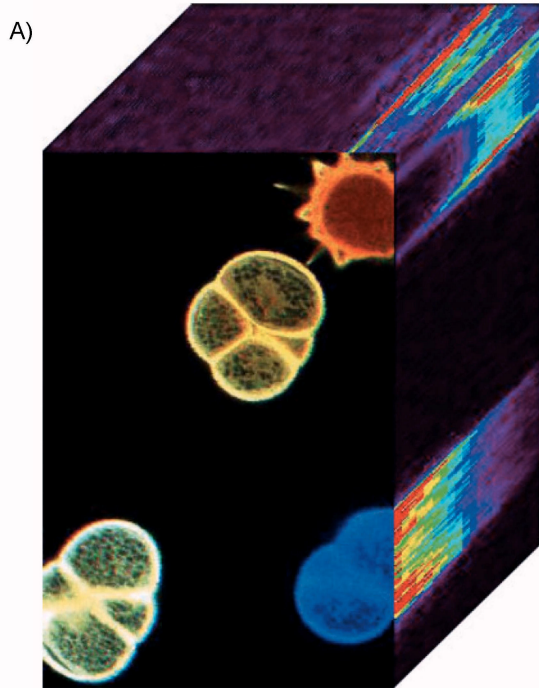
A)

Image cube exemplified with a pollen: a stack of 2-dimensional intensity profiles each specific for a single wave lenght band from 600 to 700 nm (visible only a the edges of the cube). The *en face* image is a reconstruction mixed from these profiles. From [276] Copyright 2006 Wiley & Sons. Reprint with permission from Wiley-Liss Inc., a subsidiary of John Wiley & Sons, Inc.
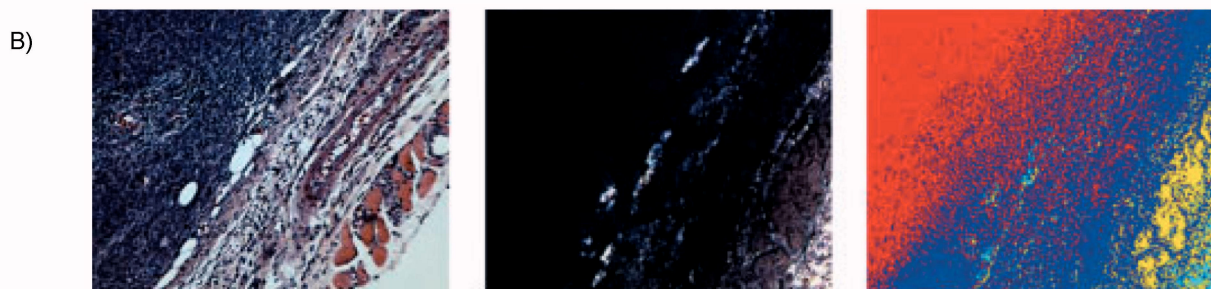
B)

Hyperspectral analysis of breast cancer. Left: HE-staining; middle: spectral reflectance of a unstained parallel section; right: color-coded classification by spectral signatures (red: cancer; blue: stroma; yellow: muscle). From [284] Copyright 2006 Wiley & Sons. Reprint with permission from Wiley-Liss Inc., a subsidiary of John Wiley & Sons, Inc.

Figure 5: Hyperspectral imaging

cube”, i.e. a stack of 2-dimensional intensity maps for single wavelengths [276] (Figure 5). This allows to identify optical signatures specific for malignant cells. The term “spectral histochemistry” was coined 1998 [277] and points out the possibility to analyze tissue non-invasively: any altered cellular function yields to a change of the contents of the cellular and extracellular constituents. This in turn changes the spectrum of the reflected light. The potential of this technology has been described first for the diagnosis of malignant melanoma [278], [279]. Another very practical application is the determination of the Hb-content non-invasively spectroscopically by analyzing the conjunctiva within 1 second [280]. On tissue sections hyperspectral imaging allows to quantitatively analyze the expression of a receptor [281].

This can be achieved even without the use of any exogenous dyes [282]. A pilot study of the use of hyperspectral imaging in mucosal lesions of the oral cavity showed a sensitivity and a specificity of 96% for the in-vivo detection of normal versus dys- or neoplastic mucosa [283]. The group of Dan Farkas showed that the combination of different optical techniques [reflectance, fluorescence, scattering, 2-photon-excitation) yield even better sensitivity and specificity [284]. They have developed a suitable hyperspectral endoscopy, too [285].

5.3.5 In vivo-cytometry & molecular imaging

The ultimate analytical tool is a technology that does not take a specimen at all and avoids invasive means. Such a technology is in vivo-cytometry. Models so far have been

the analysis of leukocytes in peripheral blood [286] where several modifications for murine models have been developed [287], [288], [289], [290]. In animal there have been further assays analyzing circulating tumor cells [291] and tissue analyses [292].

Novel concepts go even further in the animal model making use of the entire plethora of available technologies in order to analyze cancer cells at their different characteristics (proliferation, infiltration, metastasis) non-invasively in the living organism [293]. Genetically encoded reporter systems leading to triggered fluorescence allow to track a single cells or a cluster of cells through the body of the test animal. The number of available dyes is steadily increasing and is ever better fitting the special needs of the various applications [294], [295]. At present the aim is to tag single genome sequences and to make them work visibly in the living animal in order to better understand processes such as metastasis [296]. Another application is the fluorescence based detection of sentinel lymph nodes [297]. Instead of turning the cell to express the fluorochrome and hence light up the cells can alternatively be tagged with a fluorochrome directly; for this purpose quantum dots have proven beneficial [298]. However, some obstacles have to be taken until this can be applied to human patients; for example, quantum dots set heavy metal ions free yielding toxic effects so that they cannot applied in medicine yet.

6 Concluding remarks

We have to anticipate that most patients at risk (smokers) also in future will not attend screening programs as long as they have no symptoms. Global test that help to identify asymptomatic cancer patients using saliva specimens could however easily be used in occupational health screening of risk population ("blue collar workers").

The most powerful tools in early detection of head and neck cancer still will be taking a thorough history concerning signs and symptoms and risk factors including previous head and neck cancer, and a close look during examination. The clinician's eye already has some support by optical tools that guide to altered mucosal areas such as chromoendoscopy and autofluorescence. Up to now there is a lack of a tool that can be used throughout the entire upper aerodigestive tract. Hyperspectral imaging seems to be very promising in order to highlight suspicious lesions which then can be investigated by optical sectioning using optical coherence tomography or confocal endomicroscopy. These applications yield a lateral resolution in the subcellular dimension.

Pilot studies have shown that patients with detected areas of altered mucosa as well as solid tumors of salivary glands benefit from an intensified cytodiagnostic work-up: computer-based, quantitative, and objective techniques allow to analyze especially hypocellular specimens better than the naked eye. On the horizon already techniques for quantitative histology can be seen which will rapidly gain importance due to the increasing diversity of

therapy strategies for a growing number of tumors (antibody-based, "targeted" therapy).

Novel technologies might in future allow to perform in vivo-cytometry and molecular imaging with revolutionizing applications for both, therapy and diagnostics, of malignancies. However, these approaches are at the test animal level at present.

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