

THE ETIOLOGY AND PATHOGENESIS OF LARYNGEAL CARCINOMA

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In the United States, laryngeal cancer accounts for approximately 1% of all new cancer diagnoses but fewer than 1% of all cancer deaths.⁸² Laryngeal cancer is usually diagnosed relatively early because it alters the sensitive phonatory and airway functions of the larynx; 60% of patients present with localized disease alone, 25% with local disease and regional nodal metastatic disease, and 15% with advanced disease or distant metastases or both.

Squamous cell carcinoma (SCC) is the predominant type of laryngeal cancer, accounting for over 90% of cases, and laryngeal SCC accounts for 26% of all cases of head and neck SCC.⁷ Undifferentiated carcinoma and verrucous carcinoma are major variants of SCC; lymphoepithelial carcinoma and spindle cell carcinoma ("pseudosarcoma") are less common.²⁹

Most patients who develop laryngeal SCC are men, and the overall male-to-female ratio for this disease is approximately 5:1⁸⁸; however, since the 1950s, an increasing incidence of laryngeal SCC has been observed in black male patients and in all female patients (both white and black).⁸⁸ This increase in women from 0.5 to 1.5 per 100,000 population²³ is particularly significant, and it may relate to the increased use of tobacco and ethanol by women during that period. By the mid-1980s, the overall 5-year survival rates for all patients with laryngeal SCC had improved to approximately 67%, but since then they have remained unchanged.^{13,88}

The rate of metachronous SCC disease of the upper aerodigestive

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tract, including the larynx, is reported to be between 5% and 35% of cases.^{42,64,66,89} The esophagus is the most common second site,^{66,89} and consequently, it has been postulated that gastroesophageal reflux may be a carcinogenic cofactor.⁵⁵

Changing patterns of disease with time and exposure to comparable risk factors in different countries of the world have helped elucidate a group of common carcinogenic variables that are now being associated with a broader, more multifactorial hypothesis of laryngeal carcinogenesis.^{13,55,82} This multifactorial theory is supported by observations that mucosal carcinoma is often multicentric, a phenomenon often called "field cancerization." This phenomenon is usually attributed to the panmucosal carcinogenic influences of a variety of environmental factors.^{64,66,89}

Although many of the presumed carcinogenic influences have been shown to alter the "internal (mucosal) environment," not one has been proved to cause carcinoma. Virtually all of the available data is "associative," that is, epidemiologic.⁸¹ This is even true for "well-established causes" of head and neck carcinoma, such as tobacco and ethanol.

In addition to tobacco and ethanol, it now appears that other risk factors may influence the carcinogenic process, and that identification (and correction) of specific factors in susceptible patients with laryngeal SCC may influence both management and outcome.

RISK FACTORS ASSOCIATED WITH THE DEVELOPMENT OF LARYNGEAL SQUAMOUS CELL CARCINOMA

Tobacco

Tobacco has long been implicated as an important etiologic agent in the development of laryngeal SCC, as well as for SCC in other areas of the head and neck, and for tumors elsewhere in the aerodigestive tract, such as the esophagus and the lungs. Tobacco is still considered to be the predominant risk factor in laryngeal carcinogenesis.^{11,14,22,27,62,74,82}

Jayant et al⁴⁹ have shown that smoking is a stronger risk factor for the development of laryngeal cancer than is chewing tobacco, the relative risks (compared with controls) being 11.8-fold and 7.7-fold, respectively, with the reverse being the case for SCC of the hypopharynx.⁴⁹

In two separate studies, Maier et al⁶² and DeStefani et al²² found that 96.5% and 97.2%, respectively, of patients with laryngeal SCC were smokers. Comparing smokers with nonsmokers, Wynder et al¹¹⁶ demonstrated a relative 30-fold risk of developing laryngeal SCC for men smoking at least a pack-and-a-half of cigarettes per day for more than 10 years.

Falk et al²⁷ observed a dose-dependent relationship between smoking and laryngeal SCC. The relative risk was calculated at 4.4-fold for patients who smoked up to half-a-pack per day, and at 10.4-fold for those smoking more than two packs per day.²⁷

Rothman et al⁸² also reported a linear relationship between the num-

ber of cigarettes smoked and the risk of developing laryngeal SCC; in addition, controlling for alcohol consumption, they reported the risk of developing laryngeal carcinoma to be 40-fold for those smoking more than 35 cigarettes per day, and 22-fold for cigar and pipe smokers as compared with nonsmokers.

Risk has also been delineated by the quality of the tobacco used: dark versus light. Light tobacco is flu-cured; dark tobacco is air-processed. De Stefani et al²² compared the risk of laryngeal SCC between users of light and dark tobacco in Uruguay; the relative risk for the dark tobacco users was 2.5 times higher than that for light tobacco users. In addition, the latter group was controlled for age, and the data demonstrated a doubling of the risk ratio for subjects who had started smoking prior to the age of 15 years. The larynx appears to be particularly susceptible to damage from dark blends of tobacco. In another study, the relative risk for dark versus light tobacco was 9.0-fold versus 1.6-fold.²³

The risk of cancer from tobacco usage appears to be strongest for current smokers, and it declines markedly when smoking is stopped, although Falk et al²⁷ showed a persistent threefold risk for heavy smokers who had quit at least 10 years prior to inclusion in his study. In Wynder and Stellman's series,¹¹⁷ the age-matched relative risk of laryngeal cancer in men decreased from 14.3-fold (compared with nonsmokers) to 2.5-fold after 16 years of not smoking.

Table 1 summarizes reports on the relative risk of developing laryngeal SCC, based upon epidemiology of tobacco use.

Table 1. REPORTED RELATIVE RISKS FOR DEVELOPING LARYNGEAL CARCINOMA WITH TOBACCO USE

Tobacco Type	Relative Risk	Reference
Cigarettes	5.6	(less than 50 pack-years)
	9.1	(more than 50 pack-years)
	6.0	(less than 30 cigarettes/day)
	19.2	(more than 40 cigarettes/day)
	9.6	(less than 45 pack-years)
	11.3	(more than 45 pack-years)
	14.7	(light tobacco)
	35.4	(dark tobacco)
	11.8	(less than 20 cigarettes/day)
	13.5	(less than one pack per day)
34.4	(more than two packs per day)	
Chewing tobacco	7.7	(Laryngeal and oral squamous cell carcinoma included)
	40.0	(smoking and chewing tobacco) ⁻
Cigars/pipe	22.0	
	3.9	(cigars and/or pipe alone)
	10.0	(cigars/pipe and cigarettes)

Alcohol (Ethanol)

Studies that have controlled for age, race, and smoking habits still suggest that the risk of developing laryngeal SCC is increased by the consumption of all types of alcohol (ethanol).^{22,38,74,80,87,101} Interestingly, the rate of laryngeal cancer in France dropped during the Second World War, mirroring a fall in the consumption of alcohol, despite no change in tobacco use.¹⁰⁰

The study by Jensen et al⁵⁰ of Danish brewery workers, whose consumption of beer was estimated to be four times that of the general male population, showed an increased incidence of laryngeal cancer. Although that study indicated a dose-related increase in risk of laryngeal SCC with alcohol use, in comparison with tobacco-smoking-derived data, the dose-dependent risk was not as dramatic.

In the Third National Cancer Survey (1975)¹¹² (in which most of the other carcinogenic variables were controlled), the relative risk of alcohol drinkers (compared with nondrinkers) developing laryngeal carcinoma was increased 2.2-fold.

In other studies, the relative risk of alcohol as a cause of laryngeal carcinoma (in nonsmokers) ranges up to 5.6-fold^{80,117}; this higher figure was in Wynder's series,¹¹⁷ which included heavy "hard liquor" drinkers (greater than 6 oz/day). Other studies have implicated whiskey as the most important alcoholic risk factor for head and neck cancer,^{27,118} but Williams and Horm¹¹² reported male beer drinkers to have the highest relative risk of laryngeal SCC.

In a large and carefully case-controlled study in France, Guenel et al³⁸ reported that the risk of developing supraglottic laryngeal SCC was greater for alcohol users than for tobacco users.

Finally, another source of alcohol that has been associated with aerodigestive SCC is commercially available mouthwashes,^{107,115} the ethanol content of which may range up to 28%. Weaver et al¹⁰⁷ showed that of 200 patients with head and neck cancer, 11 did not use alcohol or tobacco, but 10 of the 11 had used mouthwash daily for more than 20 years.

Table 2 summarizes reports on the relative risk of developing laryngeal SCC, based upon epidemiology of ethanol consumption.

Occupational Risk Factors

The development of laryngeal SCC related to occupational factors appears to be relatively uncommon and not well documented, as compared with other work-related head and neck cancers, such as wood-related sinonasal adenocarcinoma.^{2,13,61} Guralnick³⁹ found that all laborers except agricultural and semiskilled workers (such as factory workers) were at greater risk than professionals for developing laryngeal carcinoma. Although the study was not controlled for alcohol and tobacco, the finding was supported by the similar finding of Olsen et al.⁷³ In a study by Maier et al,⁶² 92% of laryngeal SCC patients were labeled as "blue

Table 2. REPORTED RELATIVE RISKS FOR DEVELOPING LARYNGEAL CARCINOMA WITH ETHANOL CONSUMPTION*

Variables Studied	Relative Risk	Reference
Nonsmokers	3.2	Herity et al ⁴³
Smokers	6.8	
Nonsmokers	4.6	Stevens et al ⁹⁶
Smokers	26.0	
Moderate drinkers	7.7	DeStefani et al ²²
Heavy drinkers	9.3	
Moderate drinkers	2.6	Maier et al ⁶²
Heavy drinkers	9.0	
Moderate drinkers	0.8	Olsen et al ⁷⁴
Heavy drinkers	2.2	
Moderate drinkers	1.7	Wynder et al ¹¹⁷
Heavy drinkers	5.6	
Moderate drinkers	2.0	Falk et al ²⁷
Glottic tumors	1.8	Falk et al ²⁷
Supraglottic tumors	4.6	

*All of these studies were controlled for tobacco use

collar" workers; the risk ratio for those workers having no specialist training or upper education was 3.8 as compared with those with occupational training or higher educational levels.

Of the specific occupational exposures reported, the ones most likely to be related to the development of laryngeal SCC were asbestos,^{11,30,40,70,73} wood dust,^{30,49,61,78} cement dust,^{62,73} and tar products.^{13,73} The estimated risk of laryngeal SCC due to asbestos exposure has been estimated to be from 1.5-fold to 5.1-fold.^{11,30,40} Having adjusted for smoking, alcohol, age, sex, and residence, Olsen and Sabroe⁷³ estimated the increased risk of laryngeal SCC to be 1.8-fold for workers with occupational asbestos exposure.

In a report from Spain, the exposure risk of developing laryngeal SCC was increased 5.6-fold for wood workers, and the risk appeared to increase with duration of exposure (and to decrease with elapsed time after leaving a wood-working occupation).⁷⁸

Other reported occupational risks of developing laryngeal SCC include exposure to dust and gas compounds in polluted work environments.^{17,18,63,93,105} The reported excessive risk of laryngeal SCC found in furniture workers as compared with non-wood workers may also be due to exposure to the finishing and varnishing processes.¹⁰² Pederson et al⁷⁶ found that the roasting and smelting occupations increased the risk of laryngeal carcinoma. Suspected agents include nickel,⁷³ mustard gas,⁶³ and certain textile fibers.¹⁷

Table 3 summarizes reports on the relative risk of developing laryngeal SCC, based upon epidemiology of specific occupational exposures to chemicals, dusts, fibers, and other agents.

Table 3. REPORTED RELATIVE RISKS FOR DEVELOPING LARYNGEAL CARCINOMA WITH OCCUPATIONAL EXPOSURES

Occupational Exposure	Relative Risk	Reference
Asbestos	1.8	Olsen and Sabroe ⁷³
	5.1	Burch et al ¹¹
	9.0	Morgan and Shettigara ⁷⁰
Industrial/cement dusts	1.6	Olsen and Sabroe ⁷³
	1.9	Maier et al ⁶²
Isopropyl alcohol	3.2	Lynch et al ⁵⁹
Leather working	3.0	Decoufle ¹⁷
	5.5	Decoufle et al ¹⁸
Metal processing	5.3	Burch et al ¹¹
	8.5	Flanders et al ³⁰
Mustard gas	9.3	Manning et al ⁸³
Nickel/nickel refining	1.7	Olsen and Sabroe ⁷³
	1.9	Decoufle et al ¹⁸
	10.0	Pederson et al ⁷⁶
Sulfuric acid/other acids	1.3	Olsen and Sabroe ⁷³
	13.4	Soskolne et al ⁹³
Textile fibers/processing	2.0	Decoufle ¹⁸
	5.6	Flanders ³⁰
Wood working	1.4	Olsen and Sabroe ⁷³
	3.2	Maier et al ⁶²
	5.6	Acheson et al ²
Coal and tar products	1.4	Olsen and Sabroe ⁷³
	6.1	Maier et al ⁶²

Diet and Vitamin Deficiency

It has been suggested that the consumption of fruits, vegetables, dairy products, and supplemental vitamins may confer a protective effect relating to head and neck SCC.^{36,67,111} Vitamin A has been shown to have an inhibitory effect on the development of epithelial tumors by controlling cell differentiation.⁹⁰ Retinoids, analogues of retinol (a precursor of vitamin A), have also been shown to suppress in vitro expression of malignant phenotypes caused by chemical carcinogens, radiation, or viral transforming factors, the mechanism being an unknown effect on the expression of genes involved in cell differentiation and proliferation.²⁰ Because it is the loss of cell differentiation that characterizes cancer, the lack of this control could explain the action of vitamin A and of the retinoids. In support of this theory, Bollag⁸ described regression of laryngeal leukoplakia in patients treated with retinoic acid, and Hong et al⁴⁴ showed that the incidence of subsequent second primary tumors could be reduced by prolonged daily treatment with retinoic acid.

Beta-carotene is a dimer compound that is metabolized to two molecules of retinol. Vitamin A and carotene levels have been reported to be lower in patients with head and neck SCC,⁹⁰ but relationships between pre-disease and disease levels of these vitamins are not known.⁹⁶ Like other antioxidants, beta-carotene may protect against oxidative reactions within the cell, thereby limiting damage to DNA. Graham et al³⁶ found when low levels of both vitamin A and vitamin C were ingested, the relative risk for the development of laryngeal cancer was increased 3.0-fold and 2.5-fold, respectively.

The protective effect of dietary fruits and vegetables may be related to their high content of certain vitamins, such as A and C. Mettlin et al⁶⁷ demonstrated that the greatest protective effect was in dietary fruits; they reported a 4.75 increase in risk of laryngeal SCC when fruits and vegetables were consumed less than 40 times a month as compared with more than 80 times a month. These results suggest that a person who lacks these nutrients may be at a higher risk of developing aerodigestive tract SCC.⁶⁷

A study evaluating the effect of cruciferous vegetables (such as cabbage and broccoli) containing inoles and flavinols, which are known to inhibit the development of chemically induced cancer, showed that a high intake of these vegetables did not decrease the risk of laryngeal carcinoma.³⁶

Protease inhibitors have been shown to decrease the incidence of, and to delay the onset of, some tumors in animals.⁷⁹ They affect tumor invasiveness and metastasis by inhibiting the action of numerous cellular proteases induced by tumor promoters and by cellular malignant transformation. Sources of protease inhibitors include soybeans, chick peas, and lima beans. Although epidemiologic studies on the influences of protease inhibitors are not available, the theoretic cancer protection derived from their consumption is reflected by the finding that Seventh Day Adventists, who are vegetarians, have lower rates of head and neck cancers than do other nonsmoking, nondrinking populations.⁷⁷

Irradiation Exposure

In 1902, a radiology technician developed SCC of the skin of his hand. Since that time, the relationship between irradiation and the subsequent development of SCC has been strongly suspected.^{3,32,35,41,83,86,92} The interval between irradiation and subsequent malignancy may be quite long, and tumors developing in irradiated fields have been reported to have latencies of up to 40 years.⁸³

The dosage, fractionation, rate of delivery, and distribution (field) of radiation all contribute to its carcinogenic effects.⁹² These factors appear to be important in carcinogenesis; their relationship to the host's cell cycle, and especially to DNA repair, are key factors that also account for the beneficial effects of irradiation as a therapeutic modality.^{32,35}

The long-term effects of radiation therapy are suggestive of its oncogenic potential. In adults, tumors that develop after irradiation therapy for malignancy appear to have a shorter induction period than do tumors

occurring after irradiation for benign processes.³² The total dose of radiation does not appear to affect the duration of the induction time.^{58,83,92}

The development of laryngeal SCC following irradiation for thyrotoxicosis and tuberculous adenitis has been reported.³⁵ Similarly, metachronous second laryngeal carcinomas have been associated with prior radiation therapy for an earlier malignant lesion, with the latent periods usually being longer than 4 years.⁵⁸ This occurrence seems to be more common in male patients who are ex-smokers, possibly reflecting the importance of other carcinogenic risk factors.

Viral Risk Factors

The human papillomaviruses (HPVs) are well recognized as etiologic agents for a variety of cutaneous and mucosal proliferative disorders.^{1,4,6,26,45-47,75,97-99,118} Among the papillomaviruses, more than 60 separate subtypes are now recognized. Laryngeal papillomas resemble condyloma acuminata clinically and histologically, but in one electron microscopic study, only 10% of such papillomas actually were found to contain viral particles.⁶⁰ Only recently has their true incidence been more adequately explored, with the application of modern investigative techniques such as viral nucleotide probes for hybridization, antigen immunofluorescence with permissive co-cultures, and the polymerase chain reaction for amplification of viral DNA.^{9,24,45,98} The latter has demonstrated HPV DNA in laryngeal carcinomas in up to 40% of cases studied.^{9,52,54}

Usually, laryngeal papillomas run a benign course, but recent studies suggest that the transformation to SCC occurs in approximately 2% of cases.^{60,118} Malignant transformation may take 15 to 20 years, and the role of other possible co-factors has not yet been defined. Abnormal hyperkeratotic laryngeal lesions also have been shown to contain papilloma DNA.⁵²

Cummins and Koufman¹⁶ reported that 21% (14 of 88) of patients with adult-onset laryngeal papilloma (followed an average of 10 years) developed SCC of the aerodigestive tract, either at the site of the known papillomas or at another site. In addition, the authors reported that all of the patients developing SCC were cigarette smokers and/or had documented reflux into the laryngopharynx (by double-probe pH monitoring).¹⁶

The subtypes of papillomavirus have marked differences in their predilection for different anatomic sites and in their propensity for malignant change. HPV types found in laryngeal specimens include subtypes 6, 11, 16, 18, 30, and 33.^{1,9,52} Based on studies of cervical carcinoma, different HPV types have been graded as high-risk (types 16 and 18), medium-risk (30 and 33), and low-risk (6 and 11).⁴ There appears to be a strong association with HPV subtypes 6 and 11 for laryngeal papillomatosis,^{1,4,99} and SCC arising in pre-existent benign papillomas is also associated with those same subtypes.^{24,51} HPV 16, one of the more potent subtypes associated with transformation of condylomata to invasive carcinoma, is also associated with the verrucous subtype of SCC of the aerodigestive tract.¹⁰

Although a lower prevalence of HPV has been reported in *de novo* laryngeal carcinomas, the presence of subtypes 16 and 30 has been most common.⁸³ Simon et al⁹¹ have reported the co-existence of subtypes 18 and 33 in laryngeal SCC in a child in whom no other attributable risk factors could be found, thereby suggesting a role for cooperative carcinogenic action between HPV subtypes.³¹

Recent studies have demonstrated a link between HPV and the tumor suppressor protein p53.³¹ Expression of p53 has been found with high frequency in head and neck malignancies, especially in early pre-invasive lesions. The product of the HPV gene E6 binds p53 and so sequesters or degrades it, preventing its tumor-suppressor function. A model for p53 inactivation by mutation or HPV-induced degradation could be important to the understanding of the pathogenesis of SCC.¹¹⁰

The Role of Gastroesophageal (Laryngopharyngeal) Reflux in Laryngeal Carcinogenesis

The first reports in the otolaryngology literature of the effects of gastroesophageal reflux on the larynx ("contact ulcer and granuloma of the larynx") were those of Cherry and Margulies in 1968¹⁵ and Delahunty and Cherry in 1968.¹⁹ Since that time, laryngopharyngeal reflux (LPR) has received increasing attention as a possible co-factor in laryngeal carcinogenesis.^{55,56,71,106}

In 1976, Glanz and Kleinsasser³⁴ were the first to suggest that inflammatory disease could give rise to laryngeal cancer, and a decade ago, a causal relationship among LPR, inflammation, and laryngeal carcinoma in nonsmokers was postulated by Morrison⁷¹ and by Ward and Hanson.¹⁰⁶ In each of those studies, documentation of reflux was limited to the patients' histories, the laryngeal findings, and/or barium esophagography. Ward and Hanson,¹⁰⁶ for example, in their report of 19 cases of laryngeal carcinoma in lifetime nonsmokers, relied on history and barium esophagography alone to diagnose LPR. Their studies did, however, produce the first documentation (using serial photographs of the larynx) of the progression of benign laryngeal mucosal conditions to invasive SCC of the larynx.

In 1987, Weiner et al¹⁰⁹ reported 24-hour double-probe pH monitoring results for 16 patients with laryngeal carcinoma, nine of whom had documented reflux disease. This was the first report documenting the presence of acid in the throats of human subjects with presumed LPR. The technique of "double-probe" pH monitoring they employed involved the placement of one pH probe in the distal esophagus, and the other in the hypopharynx, behind the laryngeal inlet, but above the cricopharynx.¹⁰⁹ Thus, extraesophageal reflux was documented for the first time in patients with laryngeal SCC.

In 1995, Koufman and Cummins⁵⁶ reported reflux-testing results for 50 consecutive, prospectively studied patients with early laryngeal SCC. (All of the patients underwent double-probe pH monitoring and barium esophagography.) Two thirds (33 of 50) had abnormal pH studies, and

when additional radiographic criteria were added, 72% (38 of 50) of the patients had documented reflux. The authors commented that the results seemed particularly significant, because only 44% (22 of 50) were "current smokers," 42% (21 of 50) were ex-smokers with a median duration of smoking cessation of 8 years, and 14% (7 of 50) were lifetime nonsmokers.⁵⁶

Although reflux has not yet been proven to be a carcinogenic cofactor, it is clear that reflux may cause acute and chronic laryngeal inflammation. The evidence that there may be a connection among smoking, reflux, inflammation, and malignant degeneration is beginning to accumulate.

There is an analogous model in the esophagus, in which the association among reflux, Barrett's esophagus, and esophageal malignancy is well established.⁵⁵ Barrett's esophagus occurs when there is metaplastic transformation of normal stratified squamous epithelium of the esophagus to gastric-like epithelium.⁵ Barrett's esophagus occurs as a result of the chronic inflammation caused by gastroesophageal reflux, and the incidence of malignant degeneration in Barrett's esophagus occurs in direct proportion to its duration.⁷² Conversely, prior to malignant degeneration, Barrett's esophagus will revert to normal with effective long-term anti-reflux treatment.⁵⁷

Among nonsmoking patients having an association between LPR and laryngeal SCC, there have been few other associated risk factors.^{55,56,71} Thus, in these cases, as in the Barrett's esophagus model, inflammation may cause malignant transformation. The role of reflux as a co-factor in smoking patients remains to be further investigated, but at least by association, reflux appears to be as important a factor as smoking in the development of laryngeal carcinoma.

A MULTIFACTORIAL THEORY OF AERODIGESTIVE CARCINOGENESIS

Classic carcinogenic theory relates to the dysregulation of cellular growth and differentiation.^{69,81} Discrete steps in this process permit altered cellular homeostasis with autogenous cellular function separate from the usual constraints of an orderly tissue environment and endogenous cellular control. Carcinogens cause susceptible cells to undergo this dysregulation of growth and differentiation, which is termed *malignancy*. Initiation and promotion are the two recognized stages of carcinogenesis. Initiators are those carcinogens acting in the early phase of transformation; promoters act during the more variable late phase within the latent time prior to malignant change. For example, the prolonged risk of laryngeal SCC developing after smoking cessation^{27,82,112,116} suggests that cigarette smoking may act as an initiator as well as a promoter (or other variables, such as reflux, may play a carcinogenic role).

The widespread cellular target sites for exogenous and endogenous aerodigestive carcinogens, the time course for the progression of cells toward phenotypic transformation, and the likelihood that multiple cellular

insults are necessary to induce malignant change all support a multifactorial theory of carcinogenesis.⁸¹ The theoretic model proposed by Moolgavkar and Knudson⁶⁹ is probably correct in its assertion that carcinogenesis may occur as a result of the interaction of spontaneous events, hereditary factors, and environmental agents.

In their landmark 1978 paper, Jarrett et al⁴⁸ suggested a possible relationship between viral infection and a dietary factor in causing malignancy. They observed that cattle in Scotland have a relatively high incidence of papillomas at several aerodigestive sites, but that only highland cattle develop carcinoma. In both the highland and the lowland cattle groups, the incidence of aerodigestive papillomas was 19%, yet carcinomas were diagnosed almost exclusively within the highland cattle population, 96% of which had papillomas. Jarrett et al postulated that the distinguishing difference between the two groups was that bracken fern, known for its carcinogenic potential from other animal studies, grows only in the highlands, and thus was present only in the diet of highland cattle.⁴⁸

In humans, SCC of the cervix in women is an HPV-associated malignancy.¹²⁰ In the 1960s, zur Housen et al proposed a possible link between herpes simplex virus infection and cervical SCC, but 10 years later they found that approximately 80% of cervical SCC contained identifiable subtypes of HPV.¹¹⁹ They also found that the presence of HPV was more easily detected in the dysplastic phase of cervical disease than after frank SCC had developed.¹²⁰ In the 1980s, however, with the advent of the polymerase chain reaction, their yield of HPV DNA in invasive cervical SCC increased to almost 80%. Less than a decade ago, the same authors postulated that there was a synergistic interaction between HPV infection and herpes simplex infection leading to malignant degeneration, and they reported finding that patients with HPV (condyloma acuminata) progressed to carcinoma approximately 15 years earlier than did their papilloma-free counterparts.¹¹⁹ Finally, patients with immune dysfunction or suppression (e.g., AIDS, lymphoma, renal dialysis) commonly are infected by HPV.¹² Thus, in the cervical SCC model, viral carcinogens have been shown to play a pivotal role in the pathogenesis of SCC.

Data on the role of HPV in cervical SCC^{119,120} appear to support the possibility that HPV is an important co-factor in aerodigestive, especially laryngeal, carcinogenesis.^{24,31,33,46,51,52,60,92} This concept is supported by the findings of similar HPV types at both sites. Furthermore, the finding of altered p53 in samples of papilloma-positive laryngeal SCC implies pre-existing loss of tumor cell suppression. In addition, HPV infection might theoretically provide the promoter stimuli for the induction of tumorigenesis.

Interactions Among Risk Factors for Laryngeal Squamous Cell Carcinoma

The cumulative risk of laryngeal SCC in the presence of both alcohol and tobacco does not behave in an additive fashion, but rather it follows

multiplicative ratios.^{13,22,38,74,82,117} Therefore, synergy is seen when these two risk factors are both present, and the "synergistic risk" of developing laryngeal SCC has been reported to be increased by up to 100-fold for smoker/drinkers.²² In addition, the risk of developing laryngeal SCC has been shown to follow a dose-response pattern, and the risk increases with greater alcohol consumption at every level of smoking.²⁷

Tobacco is a known reflux-inducing agent.^{19,53,55} In studying a large series of patients with reflux disease, 92% were found to be smokers.⁹⁵ Dennish and Castell²¹ reported that smoking led to a fall in resting pressure of the lower esophageal sphincter (LES) in normal nonsmokers, and Stanciu and Bennett,⁹⁵ using pH manometry in 25 chronic smokers, found that the mean basal LES pressure of 10.8 ± 3.7 cm H₂O fell to 6.4 ± 2.9 cm H₂O during smoking ($P < .01$). LES pressure began to fall within 1 to 4 minutes of the initiation of smoking, and reflux episodes occurred with 66% of all cigarettes smoked. In addition, tobacco has been shown to decrease esophageal motility and to delay gastric emptying, both of which increase the risk of developing reflux disease.^{55,68}

Alcohol also significantly increases the risk of reflux disease. Vitale et al¹⁰³ studied its effects on 17 normal volunteers using pH monitoring on two consecutive evenings. Three hours after dinner, each subject ingested 4 oz of Scotch on one night, and an equal volume of water on the other night. Reflux occurred more frequently after alcohol consumption ($P < 0.02$), and the effective alcohol peak during the third hour corresponded to the time when most of the reflux events occurred.¹⁰⁰

Alcohol also has been shown to reduce LES tone, to delay gastric emptying, to decrease esophageal motility, and to increase gastric hyperacidity, all of which are known to cause or exacerbate reflux.^{55,103} Alcohol and tobacco appear to adversely influence all of the body's natural anti-reflux barriers, and both have been shown to be refluxogenic, thus linking these three (alcohol, tobacco, reflux) as possibly interactive risk factors in laryngeal carcinogenesis.⁵⁵

As a final illustration of the logic of a multifactorial theory of carcinogenesis, chemotherapy and radiation therapy appear to sensitize anatomic "fields," which are then susceptible to activation by other carcinogens. This is exemplified by the increased rate of SCC in patients who have received doses of chemotherapy and radiation therapy.³⁵ Because such treatments are not necessarily temporally related to the development of subsequent SCC, these examples are used to illustrate the lasting tissue defects that, after the addition of a second risk factor, may lead to subsequent malignancies:

A Unified Multifactorial Theory of Squamous Cell Carcinogenesis

It is apparent that the risk factors described herein may be interconnected, and that they may play complementary, even synergistic, roles in

laryngeal carcinogenesis. The variables of induction time, latency, and field recurrence may belie the relative influence of each of the co-factors involved. It is the distinct, yet complementary, actions of these risk factors that fit a new multifactorial model of carcinogenesis in which *mucosal inflammation, injury, and/or infection* play a critical role.

Clinical observations suggest that HPV is exclusively an infection of squamous epithelium; it grows on skin, on squamous epithelial surfaces of the aerodigestive tract (such as the nose and larynx), on the cervix, and in the rectal area. When it occurs in the tracheobronchial tree and lung, squamous metaplasia of the normal respiratory epithelium is almost always found. Such metaplasia may be exacerbated by smoking, reflux, or any other cause of chronic inflammation.

As the diagnostic armamentarium has expanded, HPV has been found to be associated with SCC at all sites with increasing frequency. Whether HPV infection is a prerequisite for squamous carcinogenesis at all of its sites of occurrence remains to be seen; however, it is reasonable to postulate that HPV may be a common factor at many sites, and that a second carcinogenic co-factor may need to be present for malignant transformation to occur.

Presumably, tobacco is an important co-factor in laryngeal carcinogenesis. So too, reflux may be a very important co-factor. Ethanol appears to be a greater risk factor, compared with tobacco, for the development of supraglottic SCC.³⁸ Because ethanol is not inhaled, it probably has little direct contact with the laryngeal mucosa. One might speculate that it exerts its effects by altering the immune status of the host, by predisposing to reflux, or by both mechanisms.

Perhaps laryngeal SCC occurs in patients who drink and smoke and therefore who reflux, and perhaps some of these patients have dormant HPV infections. Could it be that most SCC, regardless of site, is related to HPV infection? As polymerase chain reaction testing has become more widely employed, the finding of HPV DNA in squamous malignancy appears to be increasing. Could it be that SCC on the skin is similar, with the carcinogenic combination being HPV and ultraviolet light? Could oral SCC be similar to cervical SCC, with the carcinogenic combination being HPV and herpes simplex?

It also seems likely that a significant proportion of SCC cases may not be involved with HPV at all, and that other combinations of carcinogens may be sufficiently potent to cause malignant degeneration. It seems likely, however, that inflammation is the most important common cause.

In conclusion, the relationship between HPV infection and environmental factors such as pollution, occupational exposures, tobacco smoke, and reflux may yet prove to be profoundly interactive, and the etiology and pathogenesis of laryngeal carcinoma may prove to be truly multifactorial. Figure 1 summarizes these possible interrelationships.

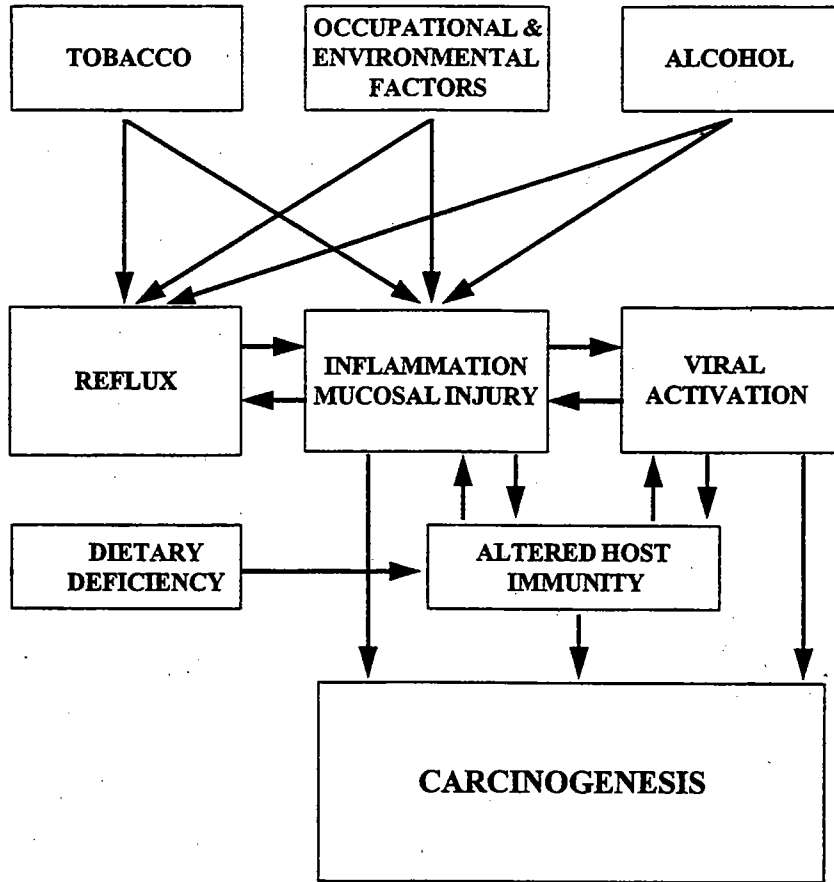


Figure 1. A multifactorial model of laryngeal carcinogenesis.

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