HEAD AND NECK PARAGANGLIOMAS — A. G. L. VAN DER MEY

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A CLINICAL, GENETIC AND PATHOLOGICAL STUDY OF GLOMUS TUMORS

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CONTENTS

CHAPTER 1

GENERAL INTRODUCTION AND OUTLINE OF THE STUDY

HISTORY

Identification of paragangliomas was reported in the German literature more than 200 years ago. The carotid body at the carotid bifurcation (ganglion minutum), discovered by Haller in 1742, received the most of attention up to 1941, when Guild first described the *'hitherto unrecognized structure, glomus jugularis in man'*. In 88 temporal bones obtained from asymptomatic patients, Guild identified 248 paraganglionic formations, also called glomus bodies. Four years later, Rosenwasser removed a middle ear tumor that histologically resembled the carotid body, but was considered to be a glomus jugulare tumor. In 1950 Lattes re-presented Stout's (1935) original case of a vagal body tumor arising from the nodose ganglion of the vagus nerve.

The earliest mention of the familial occurrence of carotid body tumors was made by Chase (1933) when he described two sisters, one of whom had bilateral carotid body tumors and the other a unilateral tumor. In the same year Goekoop described three 'fibro-hemangiomas' of the temporal bone and middle ear in three sisters, and Lubbers (1937) described two concurrent and histologically identical tumors in the neck and ear. The ear tumor was thought to be a metastasis of a carotid body tumor. Bartels (1949) reviewed these vascular lesions and was the first to appreciate the heredo-familial tendency of glomus jugulare tumors. He concluded that the trait was conveyed by autosomal dominant inheritance and stressed their association with carotid body tumors. In the van Baars' thesis, until 1979, reference is made to 32 articles in the literature, covering 160 patients with 225 hereditary tumors (van Baars,1982).

Table 1. gives an update of the data in the papers on familial appearances of non-chromaffin paragangliomas. It also illustrates the substantial Dutch contribution to early reports on familial glomus tumors (see * in table 1).

The first excision of a carotid body tumor was performed by Riegner in 1880; the patient did not survive. In 1886, Maydl removed a carotid tumor, his patient survived but with hemiplegia and aphasia. Three years later, Albert performed the first successful excision of a carotid body tumor without ligating the carotid vessels. Matthews observed in 1915 that *'this rare tumor presents unusual difficulties to the surgeon and should he encounter it without having suspected the diagnosis, the experience will not soon be forgotten'*. Another example of the difficulties encountered with the surgical treatment of these tumors, was stated by Hayes Martin in 1957: *'there are tumors both malignant and benign which the judicious surgeon will conclude to be touch-me-nots..., although I have successfully removed several carotid body tumors, I deeply regret not having backed out in some others...'*. These cautious words from the doyen of head and neck surgery initially promoted the conservative approach to this problem, which has been reversed completely in the last two decades. With the advances in vascular surgery, the chance of successful surgical treatment of carotid body tumors has greatly increased.

The treatment of glomus tumors originating from the jugular bulb has both challenged and frustrated an entire generation of otolaryngologists, as well as neurosurgeons and radiologists. Since the early 1940's, a continuing effort has been made to accomplish total removal of this uniquely difficult tumor. Otolaryngologists need hardly be reminded of the difficulties presented by a glomus tumor originating from the dome of the jugular bulb and involving the base of the skull. The problems of inaccessibility to conventional surgical approaches, the close proximity to vital structures, and the vascular nature of this tumor are all well known.

Table 1. Publications on Familial Occurrence of Glomus Tumors (* = Dutch)

	Author	Year	Patients	Localisation
1.	Chase	1933	2	CBT
2.	Goekoop*	1933	3	GJT
3.	McNeally	1939	2	CBT
4.	Bartels*	1949	7	CBT/GJT
5.	Wassink/Elders*	1949	4	CBT/GJT
6.	Sprong	1949	9	CBT
7.	Lewison	1950	6	CBT
8.	Lahey	1951	5	CBT
9.	James	1953	2	CBT
10.	Linn	1956	2	CBT/VBT
11.	Fabre	1958	2	CBT
12.	Lyk	1959	2	CBT
13.	Hardy	1960	2	CBT
14.	Ladenheim	1961	2	GJT
15.	Desai	1961	5	CBT
16.	Gastpar	1961	2	CBT
17.	Elders/Wassink*	1962	7	CBT/GJT
18.	Soraluce	1963	2	CBT
19.	Conley	1963	2	CBT/GJT
20.	Dibble	1963	2	-
21.	Rush	1963	6	CBT
22.	Katz	1964	6	CBT
23.	Kroll	1964	12	CBT
24.	Leden	1965	4	CBT
25.	Gimino	1965	2	GJT
26.	Staats	1966	2	CBT
27.	Farr	1967	2	CBT
28.	Del Fante	1967	3	CBT
29.	Lewis	1967	2	CBT/GJT
30.	van der Borden*	1967	2	GJT
31.	Brown	1967	2	GJT
32.	Farrel	1967	2	GJT
33.	Piaget	1968	2	CBT/GJT
34.	Ribet	1969	7	CBT/VBT
35.	Rosenwasser	1969	2	CBT/GJT
36.	Wilson	1970	9	CBT
37.	Sugarbaker	1971	5	CBT
38.	Gerlings*	1970	2	GJT/CBT
39.	Palacios	1970	2	GJT/CBT
40.	Pollack	1973	2	GJT/CBT
41.	Pratt	1973	8	CBT
42.	Chedid	1974	6	CBT/VBT
43.	Frisch	1974	2	CBT
44.	McGuirt	1975	2	CBT
45.	van Baars*	1975	5	CBT/GJT
46.	Boon*	1976	4	VBT/GJT
47.	Boon*	1976	4	CBT/VBT
48.	Kahn	1976	6	VBT/GJT
49.	Dent	1976	7	CBT/Pheo
50.	Lack	1977	9	CBT/GJT
51.	Conley	1977	5	CBT/VBT
52.	Hageman*	1978	4	CBT
53.	Schmidt	1978	2	CBT/GJT
54.	Ruys*	1978	10	CBT/GJT
55.	Rose	1979	4	CBT/VBT
56.	van Baars*	1980	44	CBT/VBT/GJT
57.	Grufferman	1980	5	CBT
58.	Parry	1982	22	CBT
59.	Santini	1989	7	CBT/GJT
60.	van der Mey*	1989	64	CBT/VBT/GJT
61.	Shedd	1990	2	CBT
62.	Sobol	1990	4	CBT/VBT
	Total	1933-1990	371	

ANATOMY AND TOPOGRAPHY

Paragangliomas, which are predominantly benign hypervascular tumors originating from the tiny glomus bodies are rarely seen. Each glomus body is a flattened, ovoid structure measuring about 0.5 x 0.5 x 0.25 mm. More than one glomus body may be present at the same anatomic location.

The most common site of origin is the carotid body located at the carotid bifurcation from which the carotid body tumors (CBT) are derived (fig 1.). Glomus tumors have also been found associated with the glomus bodies from the jugular bulb, the so-called glomus jugulare tumor (GJT). The glomus tympanicum tumor (GTT) originates from the glomus bodies of the tympanicum plexus of Jacobson's nerve, which is found in the mucosa of the cochlear promontory. Sometimes the glomus jugulare and tympanicum tumors are grouped together and called glomus jugulo-tympanicum tumor (GJTT). Vagal body tumors (VBT) typically arise from nests of the paraganglionic tissue within the perineurium of the vagal nerve near it's ganglion nodosum.

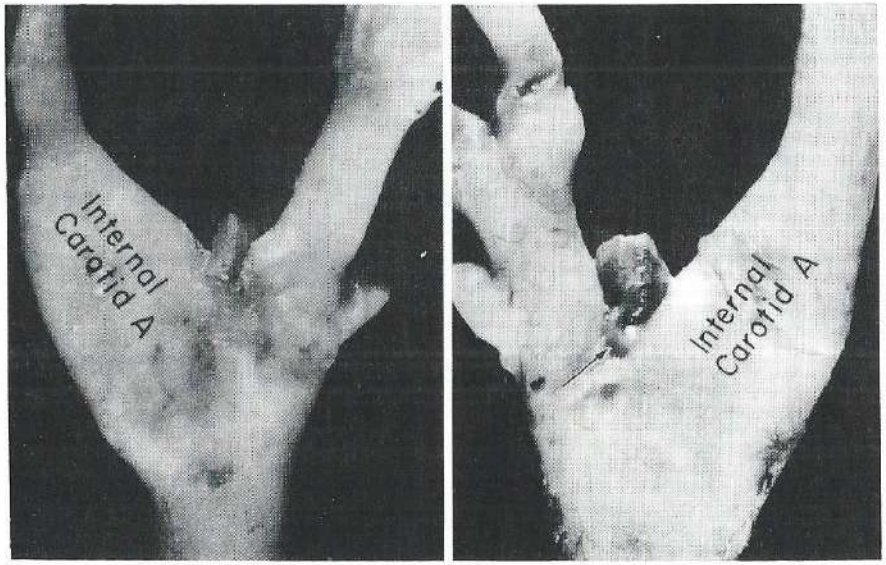

Figure 1. Biffurcation of the carotid artery with a normal carotid body (left) and a still very small carotid body tumor (right). (Courtesy of AFIP, Washington, 1973)

Glomus tumors rarely present in the larynx (Hordijk,1981; Konowitz,1988), nasal cavity and nasopharynx (Lack,1977), orbit (Lack,1977), trachea (Zeman,1956), or in the thyroid gland (de Vries,1989).

In animals too, paraganglioma can occur (Mulligan,1950) but in contrast with man, the paraganglioma aorticum (the so called heartbase tumor) in dogs appears to occur more frequently than the paraganglioma caroticum does. The combination of paraganglioma with pheochromocytoma has been described in dogs as well as in man (Hayes,1988). The Boston-terrier and Boxer seem to be overpresented by a familial risk, and among bull-dog related breeds an elevated risk was found of developing aortic body tumors (ABT) for male dogs. It has been suggested that this finding was related to a genetic predisposition in combination with chronic hypoxia due to brachycephaly (Hayes,1988).

INCIDENCE

The rarity of these tumors is illustrated by the finding that the literature offers hardly any valid figures representing the incidence of these tumors. It is difficult to establish the exact number of CBTs reported in the literature because republication of cases is a frequent occurrence. As an example of the latter, material from the Mayo Clinic has been reported consecutively by Rankin (1931), Harrington (1941), Pemberton (1951), Brown (1967), Shamblin (1971), and Hallet (1988). Furthermore, some of these patients also sought consultation or treatment elsewhere and were consequently included in series of other authors as well. In the decade between 1960 and 1970, Zak and Lawson (1982) found over 200 CBT reported in the American literature alone. Despite the large number of published cases, these tumors are still relatively uncommon. Lack et al.(1977) found 69 paragangliomas among 600,000 surgical cases seen at the Sloan-Kettering Memorial Cancer Centre from 1937 to 1975, this represents an incidence of 0.0012%.

Neoplasms of the temporal bone (GTT and GJT) too, are relatively rare. Although malignant tumors predominate, the glomus jugulare tumor is the most commonly benign tumor of the middle ear. Review of the literature up to 1975 revealed a cumulative experience with glomus jugulare tumors in excess of 1000 reported cases (Zak and Lawson, 1982). According to information accumulated by the Dutch National Medical Registration over the last ten years (1980 - 1989), 90 CBT's were registered but no reliable data were available as to the exact number of GJT's and VBT's (source Stichting Informatiecentrum Gezondheidszorg, Utrecht, 1990).

The exact incidence of familial disease is difficult to determine. Elders (1962) found 7 instances of familial occurrences of CBT and GJT's on review of 92 Dutch patients with head and neck paragangliomas, but, as in the case of several other authors (Grufferman,1980; Parry,1982), the exact frequency of familial disease may have been underestimated, because initially these tumors hardly cause symptoms and may not be noticed.

A striking characteristic of heredo-familial tumors is that they are much more likely to be multicentric (30%) than sporadic tumors are (10%), which holds for both synchronous and metachronous lesions (Sugarbeker,1971; Grufferman,1980). The most common type of multicentric glomus tumor is the bilateral CBT. This increased incidence of multicentric tumors in familial tumors has prompted to recommend bilateral carotid angiography as a part of the evaluation of patients with a unilateral glomus tumor and a positive family history.

THE EXTRA-ADRENAL PARAGANGLION CONCEPT

The concept of a unitary system of paraganglia linking the adrenal medulla with extra-adrenal chromaffin tissues was put forward by Kohn in 1903 (fig 2.).

PARAGANGLION SYSTEM

EXTRA-ADRENAL — ADRENAL MEDULLA

parasympathetic — orthosympathetic

- branchiomeric paraganglia/bodies
  - carotid
  - jugulotympanic
  - laryngeal
  - subclavian
  - coronary
  - aortico-pulmonary
  - orbital
- intravagal paraganglia
  - vagal body
- aortico-sympathetic paraganglia
  - Zuckerkandl

orthosympathetic
- para-aortic
- thoracic
- abdominal

Figure 2. Diagrammatic representation of the paraganglion system according to Kohn (1903).

The paraganglion system can be divided into (1) the adrenal medulla, an innervated neuroendocrine organ of primary importance in the orthosympathetic system (fig 3.), and (2) the extra-adrenal paraganglion system. This second system is more dispersed and two components can be distinguished: (A) one associated with the orthosympathetic system in the para-aortic, thoracic, and abdominal regions, which is functionally related to the adrenal medulla, and (B) the other, related to the parasympathetic system, which functions as a series of afferent receptor organs. The extra-adrenal portion of the paraganglion system will be considered as comprising several interrelated 'families' which can be grouped logically on the basis of anatomic distribution, innervation, and microscopic structure (fig 4.). These subdivisions are termed as follows (I) branchiomeric, which includes the carotid, jugulotympanic, subclavian, laryngeal, coronary, aorticopulmonary, and orbitalparaganglia; (II) intravagal paraganglia; and (III) aorticosympathetic paraganglia, associated with the orthosympathetic system. The latter group is usually axial, extends from the aortic arch to the urinary bladder, and includes the organs of Zuckerkandl. Tumors arising from the branchiomeric and intravagal paraganglia are generally considered to be chromaffin negative; whereas those from the aortosympathetic paraganglia are often chromaffin positive and secrete catecholamines similar to the pheochromocytomas of the adrenal medulla. However, the chromaffin reaction is not always reliable and may

not correspond with the functional activity. Conversely, some tumors store catecholamines but lack the ability to secrete them, and such non-functioning paragangliomas are chromaffin positive. Paraganglia of the head and neck tend to be distributed symmetrically, closely resemble the carotid bodies and are typically associated with arterial vessels and cranial nerves of the onto-genetic gill arches.

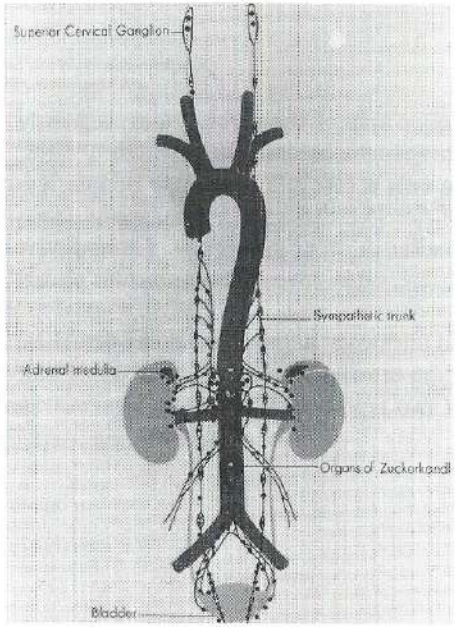

Figure 3. Distribution of aorticosympathetic paraganglia in a new born child. (Courtesy of AFIP, Washington, 1973)

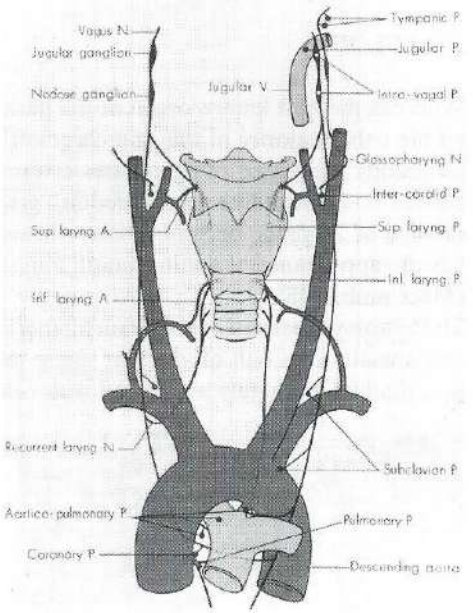

Figure 4. Sites and anatomic relationship of branciomeric and intravagal paraganglia.

EMBRYOGENESIS

Of all the non-chromaffin paraganglia, the embryologic development of the carotid body has been studied the most extensively in both mammals and man. Theories claiming origin from all three germ layers, either singly or in various combinations, have been proposed by various authors during the last century. The parenchymal cells of paraganglia appear to arise from the neural crest (neuroectoderm) along with other elements of the autonomic nervous system (Kohn,1903). A prominent mesodermal contribution to some of the branchiomeric paraganglia presumably represents the origin of stromal tissue and vessels, but the possibility that some of the parenchymal cells could arise from the mesoderm or branchial ectoderm as well, has not been excluded (Kjaergaard,1973). It is now generally accepted that the neuroectodermal cells

are the forerunners of the glomus tissue and that the pre-existing mesenchymal cells give rise to the fibrous stroma of the carotid body (Heath,1991).
The distribution of paraganglion cells in the human fetus and newborn infant is considerably wider than in the adult (Kohn,1903; Becker,1966). This observation probably accounts for the occurrence of well differentiated paragangliomas at sites where no conspicuous or constant paraganglion has been described as a normal occurrence in the adult.

HISTOLOGY

Whereas the best known organ of the paraganglion system, the carotid body, is grossly visible , the majority of the non-chromaffin paraganglia are microscopical structures, frequently composed of aggregates comprising only a few cells. In young people, the carotid body looks like a small reddish grain of rice (5x5x2.5 mm) and is largest about the age of 20 years, becoming sclerotic and smaller in older individuals. Generally, it has the appearance of a thin smooth tumor capsule, often covered by a vascular plexus (MacComb,1948).
The histologic pattern of the branchiomeric and intravagal paraganglia is characteristic and consists of a ball of vascular tissue including arteries, capillaries, and veins; that is, a glomus intimately associated with cells of two types (fig 5.).

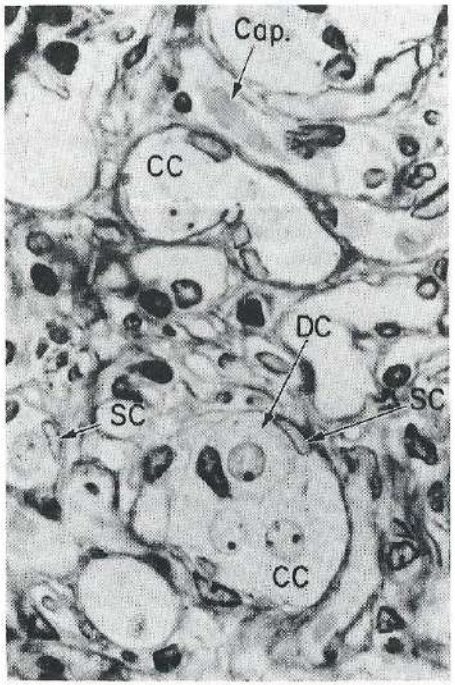

Figure 5. Detail of a human carotid body. **CC**: chief cell; **SC**: sustentacular cell; **DC**: dark variety of chief cell; and **CAP**: capillary. (Courtesy of AFIP 1973, Washington)

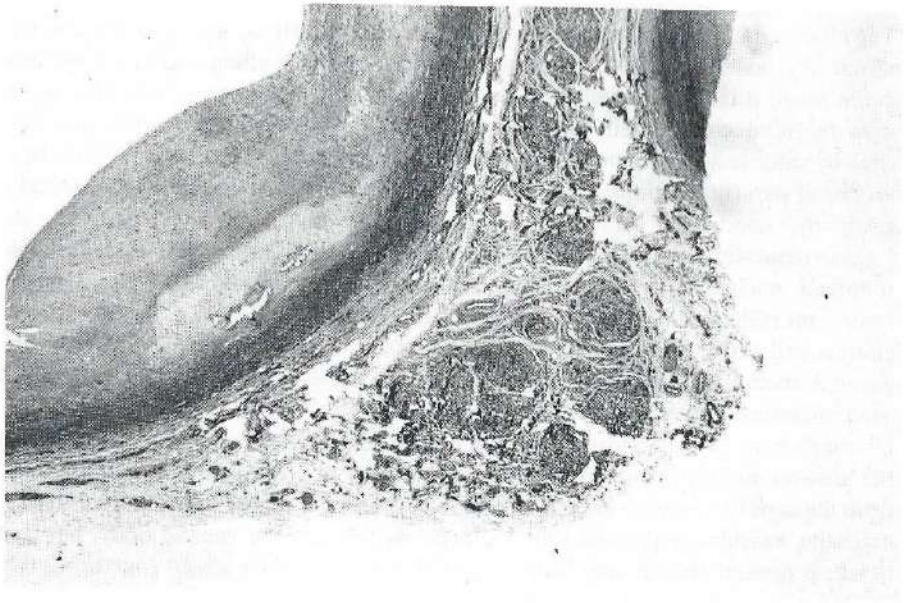

Figure 6. Normal glomus bodies at the bifurcation of the carotid artery.

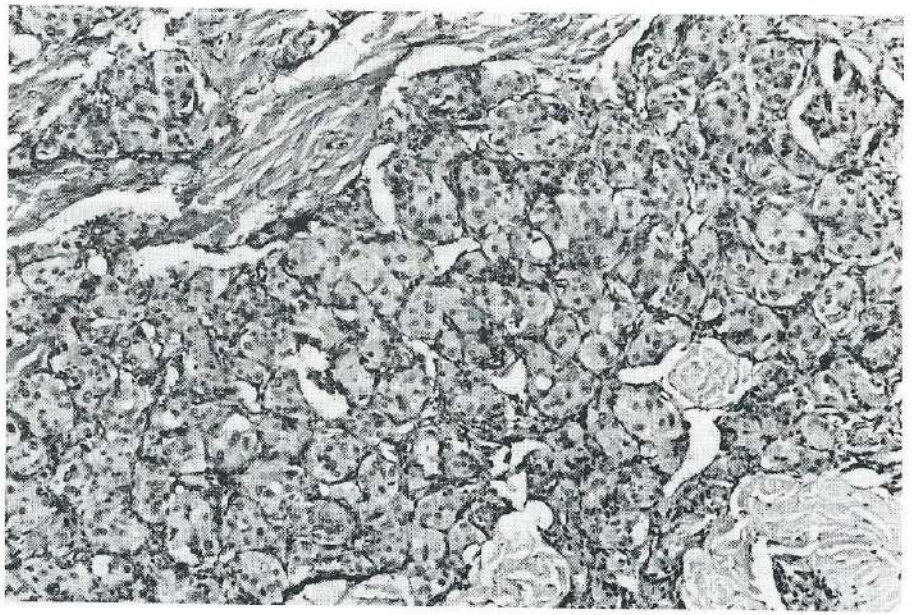

Figure 7. Microscopically a glomus tumor imitates the normal glomus body structure with the presence of clusters of tumor cells ('Zellballen') interspersed among an extensive capillary network.

The chief (type I) cell has a copious cytoplasm with poorly-defined margins and a large round or oval nucleus. The sustentacular (type II) cell is elongated and ensheathes a nerve axon; it closely resembles a Schwann cell and the two types of cell may merge with one another. The cells are arranged in clusters (Zellballen) formed by a central core of chief cells surrounded by a shell of sustentacular cells (Heath,1991). In well-prepared paraffin sections, the nuclei of sustentacular cells can be distinguished by a relatively condensed chromatin pattern, and an oval or lenticular shape. Ultrastructurally the chief cell is a round or polygonal cell, with a central, ovoid or spherical nucleus and abundant, finely granular or vacuolated, pale eosinophilic cytoplasm (Glenner and Grimley, 1974). The distinctive ultrastructural feature of the glomus cell is the presence of dense-cored granules identical to those found in the adrenal medulla and other chromaffin cells. These have been shown to contain catecholamines and are exclusively found in the type I cells.

Glomera have an extensive innervation by the nerve fibres from the carotid branch of the glossopharyngeal and vagus nerves but also by non-myelinated sympathetic fibres from the superior cervical ganglion (de Castro,1926). Another important feature is their extensive vascularity (Boscia,1990). Bloodflow through the carotid body, per unit of tissue, is greater than in any other organ of the body, being about four times that of the thyroid gland (Batsakis, 1982).

According to Lattes and Waltner (1949), there is no clear histologic difference between a CBT and GJT or VBT; in fact, they are microscopically identical with other non-chromaffin paragangliomas. It should be kept in mind however, that uniformity of histological structures of components of this system does not imply uniformity of function. Furthermore, a satisfactory separation into benign, locally invasive and malignant metastasizing tumors cannot readily be made solely on histological grounds.

Paragangliomas resemble the tissue from which they arise (paraganglia) and are in fact neoplastic caricatures of that tissue (fig 6. and 7.). Hyperplastic carotid bodies are unequivocally enlarged and their combined weight exceeds 30 mg, although their normal weight ranges between 6 and 29 mg (Heath,1991).

Lack (1979) believed that a glomus tumor does not represent hyperplasia but is a true neoplasm, because residual compressed normal paraganglionic tissue was found outside the tumor capsule.

MALIGNANCY

The non-chromaffin paragangliomas may be clinically aggressive, producing disabling symptoms and even death by slow, progressive enlargement with encroachment on vital organs, or by infiltrative growth into surrounding structures. Incidentally, they are clinically malignant, owing to the development of regional and distant metastases. Difference in the reported percentage of 'malignant' CBT's and other paragangliomas are undoubtedly attributable to the differences in the criteria of malignancy accepted by different authors. According to Batsakis (1979), there is no correlation between the histological appearance of these tumors and their biological behavior, because follow-up studies have shown that survival rates of histologically malignant tumors are almost the same as those of histologically benign tumors. If we accept -as the sine qua non of malignancy- histologically proven metastases, we must conclude that less than 10% of all paragangliomas are malignant (Lack,1977; Batsakis,1979). Even in such cases, the course of the disease may be protracted. Several authors have reported metastasis to the regional lymph nodes (Pryse Davies,1964; Staats,1966; Oberman,1968; Kahn,1976) to the dorsal spine, and to the ribs (Rosenwasser,1958). The incidence of malignant transformation varies with the site of origin of the tumor (Batsakis,1979). The malignant potential of VBT's is relatively high, according to Kahn (1976), i.e., 16%, compared with 4% in GJT's, and 6% in CBT's (Staats,1966) (see table 2.). According to Brown (1967), metastases are rare but may occur after a protracted latent interval (7-15 years), therefore suggesting the need for life-long follow-up. Lack (1979), reported on six cases of malignant paraganglioma, there are some features that are not shown by benign tumors. These include focal (within Zellballen) or confluent necrosis, vascular and lymphatic invasion, and the invariable presence of mitotic figures.

In view of the increasing number of reported glomus tumors, it is surprising that metastatic spread to regional lymph nodes, lung, liver, and bone is not seen more frequently. The extended series (175 glomus tumors) of Leiden University Hospital contained one malignant paraganglioma of the larynx (Hordijk,1981) and one uncertain malignant CBT. It should also be kept in mind that the tendency of non-chromaffin paragangliomas to have a multicentric origin must be considered before claiming metastases, even at sites where the glomera have not been discovered.

Table 2. **Malignant paragangliomas according to site of origin**
(Courtesy of Batsakis, Tumors of the Head and Neck, 1979)

Location	Nº of cases with metastases	Nº of patients	
Carotid body	± 500	32	(6%)
Jugular body	316	6	(2%)
Vagal body	25	4	(20%)
Mediastinum	20	0	(-)
Lung	25	0	(-)
Duodenum	9	0	(-)
Retroperitoneum	21	6	(28%)

NOMENCLATURE

Similarly, many descriptive terms have been applied to glomus tumors, reflecting differences in interpretation of their basic nature by analogy with the normal organ. Many terms have persisted through continued usage based on individual preferences rather than on scientific grounds, but none of the designations can be considered entirely satisfactory (Rauch,1969). Use of the term glomus was extended from the body to the tumor. The main objection to this term is, that it implies a vascular nature and invites confusion with the glomangioma (glomus tumor of Masson). The term chemodectoma (chemeia = infusion; dechesthai = to receive; -oma = tumor) was introduced by Mulligan (1950) for heartbase (ABT) tumors in dogs to denote a neoplasm of chemoreceptor cells in contradistinction to neoplasms of true paraganglionic cells of the sympathetic nervous system. The term chemodectoma has been accepted in the international histologic classification of tumors adopted by the World Health Organization (Enzinger,1969). One serious objection to this term is that only the carotid and aortic bodies have been shown to be receptor organs, and none of these neoplasms has been found to be functionally active as a chemoreceptor. The term paraganglioma, modified by combination with the term non-chromaffin, has been widely applied to these tumors. Its usage has been criticized, because it implies a common histogenesis with endocrine neoplasms of the adrenal medulla and sympathetic nervous system. To avoid controversy concerning histogenesis, some authors favor the noncommittal term, tumor of the ---- body, (specifying the anatomic site), thereby implying homology with the carotid body or other non-chromaffin paraganglia. In the present report, however, we have retained the alternative names glomus tumor, chemodectoma, and non-chromaffin paraganglioma, because of the frequency of their clinical application and general acceptance among clinicians.

PHYSIOLOGY AND RELATION TO HIGH ALTITUDES

Experimental physiologic studies in mammals have shown that the carotid and aortic bodies are sensitive to fluctuations in arterial oxygen tension and pH (Comroe,1964). Reflexogenic regulation of the cardiorespiratory system mediated through these paraganglion chemoreceptors may be of particular importance, i.e., in hypoxic conditions, pulmonary insufficiency at high altitudes or during sleep (Biscoe,1971).
The discovery that the carotid bodies may become enlarged in generalized disease was made as late as 1969 in the high Andes of Peru and, was subsequently confirmed by Arias-Stella and Valcarcel. The carotid bodies of Quechua Indians from the Peruvian mining town of Cerro de Pasco (4330m) were found to be heavier and larger than those of mestizos from Lima on the coast, and these differences became greater with increasing age (fig 8.). On histological examination they showed hyperplasia of the chief cells with loss of formalin-induced immuno-fluorescence indicative of loss of biogenic amines (Arias-Stella,1969). The hyperplasia was said to be so dense and diffuse as to give the section a homogeneous appearance and the intervening bands of fibrous

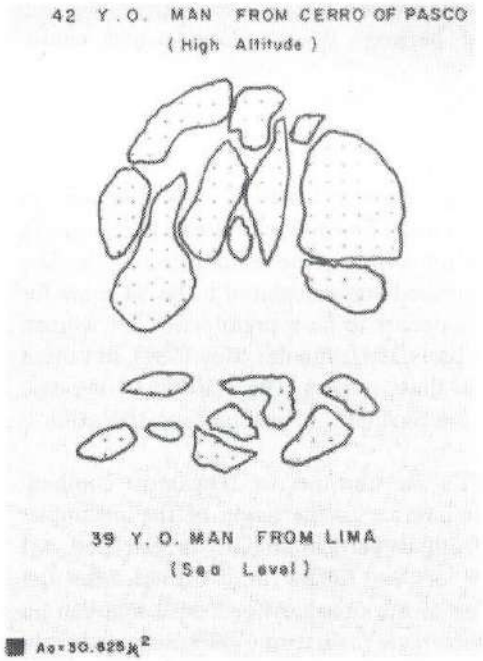

Figure 8. Drawing of the relative amount of parenchymal tissue in the carotid body of high-altitude sea-level dwellers. (Courtesy of Arias-Stella and Valcarcel, 1976)

stroma were thinner. Such hyperplastic changes were thought to be brought about by sustained stimulation of the chemoreceptor tissue by the hypobaric hypoxia of high altitude (Heath,1991). It remains to be established whether there is a significant risk of occurrence of a carotid body paraganglioma under normobaric conditions in patients with chronic hypoxia (Lack,1985). Heath (1970) found no solid evidence of a relationship between the severity of a pulmonary emphysema and the carotid body enlargement. Saldana (1973) has reported that in Peruvians living at high altitudes, chemodectomas occur about ten times more frequently than in those living at sea level. This finding was confirmed by a review of the incidence of tumors in two hospitals situated 11,000 feet above sea level (reference Saldana,1973). This again raises a fundamental problem, i.e., the distinction between carotid body neoplasia (paraganglioma) and carotid body hyperplasia.

HEREDITARY ASPECTS AND RELATION WITH OTHER DISEASES

With reference to remarks and discussions in this thesis, especially in chapters 1, 3, and 7, the familial tendency of these tumors will be extensively outlined. The remarkable finding that familial glomus tumors are exclusively inherited via the paternal line, can be explained in terms of genomic imprinting (van der Mey,1989). This new genetic theory has considerable implications for genetic counselling with respect to glomus tumors and the understanding of other hereditary diseases.
The association of head and neck paragangliomas with pheochromocytoma (Parkin,1981; Dunn,1986; van Gils,1990) as well as several other tumors, i.e., carcinoid (Farrior,1980), pituitary adenoma (Berg,1976) and thyroid-gland carcinoma (White,1979) derived from the neural crest have been reported, but these combinations are considered to be very rare. The occurrence of a glomus tumor in MEN II or Sipple's syndrome (the association of pheochromocytoma, medullary carcinoma of the thyroid, and parathyroid hyperplasia) was first reported by Kennedy (1986). Hereditary deficiencies of clotting factors VII and X associated with carotid body tumors were

mentioned by Kroll (1964), and the association between multiple paragangliomas and neurofibromatosis suggested a relationship between neuroendocrine and neuro-cutaneous disease (DeAngelis,1987).

CLINICAL PRESENTATION

Carotid body tumors have been recorded for all stages of life from early childhood to old age (Choa,1987). Helpap (1966) described a CBT with lymph-node metastases in a 10-month-old child with a paraganglionic tumor arising in the temporalis muscle. The average age at which the diagnosis was established was calculated to be 34 years for 174 cases reviewed by Monro (1950). There appears to be a predilection for women in the non-familial group (Grufferman,1980; Parry,1982; van der Mey,1989), but there is no evidence of preferential lateralization of these tumors. The majority of the case reports concern Caucasians, but no studies are available to substantiate this clinical impression of racial prevalence.

In general, the signs and symptoms of CBT's are non-specific. The most common complaint or finding is a cervical mass just inferior to the angle of the mandible. Because the tumor adheres to the carotid sheath, its vertical mobility is restricted, but it can be movable laterally. Five to 10% of the CBT's may extend into the parapharyngeal region and produce a bulging of the oropharyngeal wall that can be observed at intraoral examination. One characteristic feature of CBT's is slow growth, which is reflected clinically in generally long histories before the correct diagnosis was established. Histories spanning between 20 and 25 years are not uncommon (Elders, 1962; Conley, 1963; Farr, 1967). Review of large numbers of cases yields an average interval of 4 to 7 years from the time of onset to diagnosis (Monro, 1950; Shamblin, 1971; van Asperen, 1981).

In a survey of the literature done in 1988, Green collected 140 vagal body tumors. An upper-cervical mass, situated behind and relative deep to the angle of the mandible, was the most common finding on presentation. The incidence of preoperative vocal-cord palsy was relatively low 30% (Biller,1989), but tumors extending into the jugular foramen (fig 9.) were associated with a higher incidence of preoperative cranial nerve palsy (Kleinsasser,1963; Green,1988). Clinical features of VBT's, such as, sex, age at onset, and duration of symptoms did not differ essentially from those of CBT and GJT's.

The clinical presentation of GTT and/or GJT's may range from the classic picture of a vascular middle ear tumor to cases in which differentiation from primary neurologic disorders or brain tumors is virtually impossible without further diagnostic procedures. The key to understanding of the presenting symptoms is knowledge of the distribution of the temporal bone glomera. The site of origin of the tumor influences the direction of its growth (fig 10.). Classically, patients usually complain of pulsatile tinnitus and hearing loss. As the lesion expands, involvement of the lower cranial nerves (VII-XII) will significantly influence morbidity. Facial-nerve paralysis was listed in the series (n=277) reported by Alford and Guilford (1962) as the third most commonly produced

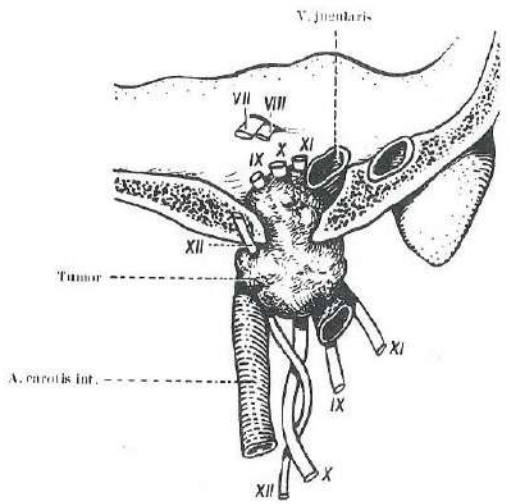

Figure 9. Dumbell-shaped tumor with extension into the jugular foramen having intracranial and cervical components. (Courtesy of Kleinsasser, 1963)

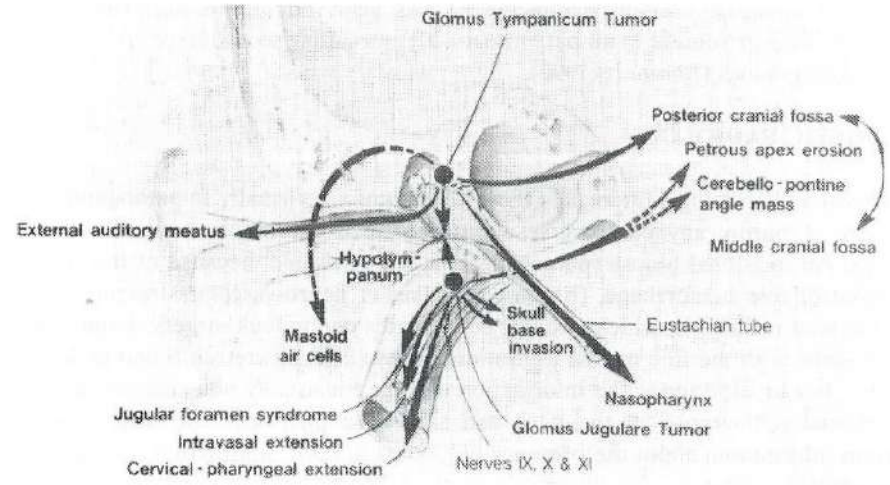

Figure 10. Diagram of the direction of spread of glomus jugulare and glomus tympanicum tumors. (Courtesy of Zak and Lawson, 1982)

symptom (33%), whereas the series (n=57) of Spector (1979) cited an incidence of 21%. The incidence of neurologic findings produced by temporal-bone glomus tumors ranges from 30 to 60% in different studies (Bickerstaff, 1953; Spector, 1976; Brown, 1985). The differences in incidence are illustrated by several case reports from the neurosurgical literature, which show greater central nervous system involvement with cranial nerve palsies, whereas those published in otolaryngologic journals stress aural findings with deficits primarily of nerves VII and VIII.

FUNCTIONAL ACTIVITY

Glomus tumors and pheochromocytomas form a class of neuro-endocrine tumors with as a common feature, the presence of numerous neuro-secretory granules containing catecholamine. The few reports on functional activity in the literature describe a pheochromocytoma-like syndrome resulting from excessive tumor catecholamine production. The patients may present symptoms that are indistinguishable from those of pheochromocytomas, with headaches, palpitation, labile hypertension, and flushing. The first description of a functioning norepinephrine-secreting CBT was made by Glenner et al.(1962). Although these cases are extremely rare, all patients with non-chromaffin paragangliomas should be evaluated with respect to elevated catecholamine production, because of the catastrophic results of surgery in unidentified cases. Schwaber (1984) discussed the indications for selective venous catheterization and pharmacologic blockage of catecholamine-secreting glomus tumors. The incidence of hormonally active head and neck glomus tumors, in contradistinction to pheochromocytomas and sympathetically derived paragangliomas, has been estimated to be about 1% (Lawson,1980). Almost all reported vasoactive cranio-cervical glomus tumors secreted nor-epinephrine. Epinephrine or dopamine secretion has only been reported once (Schwaber,1984). Since (functional) head and neck paragangliomas and pheochromocytomas may arise in association with each other, appropriate screening should be performed (van Gils,1990). Further details on imaging of these vasoactive tumors can be found in Chapter 6.
Recently, imaging of somatostatin-receptors was achieved after administration of ^{123}I labeled Tyr3-octreotide in all patients (n=20) amounting to a total of 29 head and neck paragangliomas (Lamberts,1990).

DIAGNOSTIC RADIOLOGY

The clinical and radiologic findings in these neoplasms are virtually so pathognomonic that there is hardly any controversy about the need for biopsy to establish the diagnosis. An incisional biopsy (paracentesis) is not advisable, because of the risk of (a) uncontrollable hemorrhage, (b)injury of adjacent neuro-vascular structures, and (c) it will lead to fibrosis, which strongly complicates continuous surgery. Experience at some scale, with the fine needle aspiration biopsy (FNAB) are not found in recent literature. In our experience the information obtained is usually non-conclusive.
Conventional radiography of the neck and skull-base provides little diagnostically important information about the presence of glomus tumors. Subtraction angiography of the carotid system is the diagnostic method of choice for these lesions. The angiogram made in the lateral position shows a characteristic highly vascular CBT and provides insight into the size, patency, and course of the displayed carotid arteries. Angiography is especially useful in differentiating a cervical paraganglioma (CBT;VBT) from an aneurysm of the carotid system or a coiled internal carotid artery (Tsai,1977; de Jong,1989). Bilateral (so-called 'four-vessel') angiography is essential for the visualization of occult (multifocal) uni-or bilateral head and neck glomus tumors.
The blood supply of temporal-bone glomus tumors is derived from the external carotid system, principally the ascending pharyngeal artery but may also have supplying vessels from the internal carotid artery. With intracranial extension, vertebral angiography is imperative because the blood supply to this portion of the tumor can also derive from the vertebral-basilar system. The venous 'run-off' phase of the angiogram often outlines the jugular drainage system, making retrograde venography unnecessary.
Less invasive techniques such as digital vascular imaging (DVI) with intravenous enhancement have also been reported (Kinney,1982; Carmody,1983; Tange,1983) and ultrasound has been recommended in case reports to be used in the neck for screening or follow-up (Gooding,1979; Baatenburg de Jong,1989). These modalities need further evaluation together with the Color-Duplex Sonography.
Interpretation of the radiologic findings produced by GJT's is based on the understanding of their site of origin and direction of spread. The high-resolution CT-scan is the most valuable method for assessment of the extent of lesions at the skull base. This modality allows determination of the presence of intracranial intradural disease, medial extension, and the relationship between the tumor and the carotid artery, and the inferior extent of the lesion can be determined as well (Mafee,1983; Chakeres,1984). On the basis of contrast-enhanced, with thin section, multiplanar (axial and coronal) high resolution CT, virtually all temporal-bone glomus tumors could be classified according to a surgical classification system (Valavanis,1983).
Magnetic resonance imaging (MR) has added a new dimension, particularly for assessment of intracranial extension and involvement of the internal carotid artery (Vogl,1989). Phelps (1990) examined 20 GJT's by CT and MR with gadolineum enhancement, and concluded that these two imaging modalities, combined with clinical assessment, can provide all of the necessary information with respect to the diagnosis, localization, and extent of the majority of skull-base glomus tumors. 3-D reconstruction of lesions in the head and neck will become a valuable diagnostic method to demonstrate space-occupying lesions, particularly with respect to surgical planning (Vogl,1990). This means, especially for skull-base tumors that angiography is no longer a first-line imaging method but rather a preoperative evaluation. It can also be used in those tumors in which embolization is required. The use of MIBG scintigraphy can add an extra physiological dimension to functioning glomus tumor imaging (Rockall,1990). Further details and results will be found in Chapter 6 (van Gils,1990).

SYSTEM OF CLASSIFICATION

The three stage classification of Shamblin et al.,1971 was used to grade the difficulty of resection in CBT's (fig 11.). Type I tumors were defined as localized and relatively easily resected.

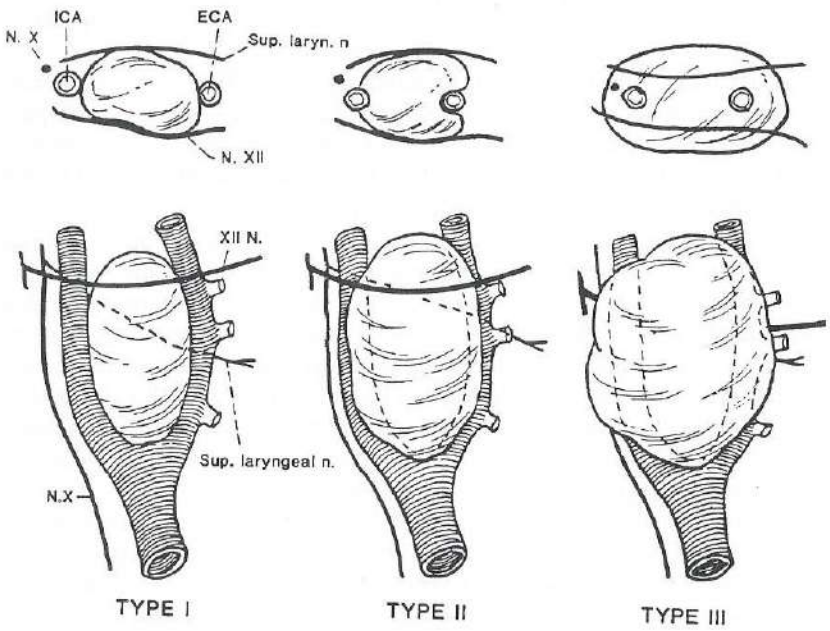

Figure 11. The classification of Shamblin (1971) of the difficulty of surgical resection. (ICA = internal carotid artery; ECA = external carotid artery).

Type II covers tumors adherent to or partially surrounding the vessels. Type III paragangliomas completely surround the vessels. Most of the CBT's (70%) of in the Mayo Clinic series (n=153) were designated as Types II and III, and considered to increase the risk of injury to the carotid vessels and cranial nerves during resection (Hallet,1988). However, most of the reports on the diagnosis and surgical treatment of CBT's published so far make no mention of the use of a specific tumor classification. For GJT's, many classifications (Alford and Guilford,1962; Glasscock-Jackson,1982) have been proposed, but the one devised (1979) and modified (1982) by Fisch is most successful in addressing the surgeon's need for anatomic landmarks (see table 3 and fig 12.). This classification refers to four classes.

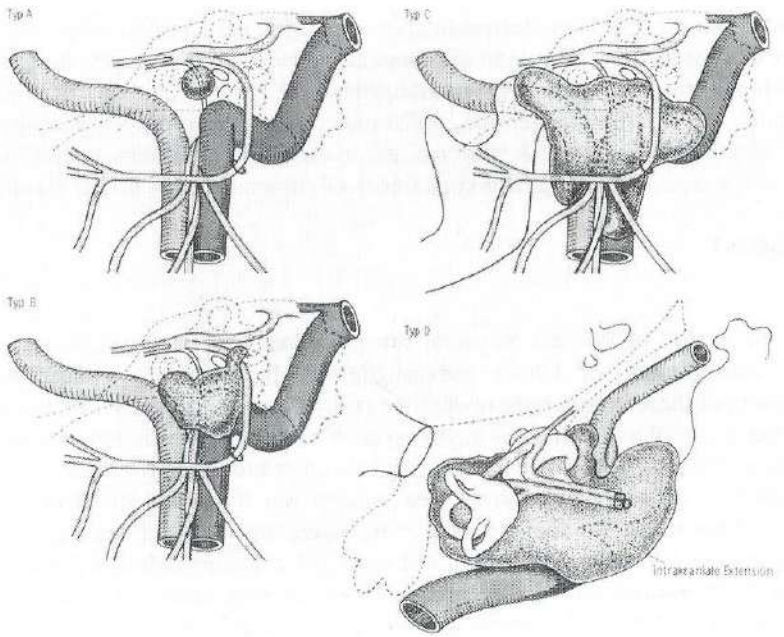

Figure 12. Representation of Fisch's four different types of glomus jugulo-tympanic tumors (GJTT).

Table 3. Classification of glomus temporale tumors (Fisch, 1982)

Class A	:	Tumor confined only to the tympanum and arising from the promontory without evidence of bone erosion.
Class B	:	Tumors involving the tympanum with or without mastoid involvement but always arising from the hypotympanic region. The cortical bone over the jugular bulb must be intact.
Class C	:	Tumors eroding the bone over the jugular bulb. The tumor may extend into and destroy the bone of the infralabyrinthine, retrolabyrinthine, and apical compartments of the temporal bone.

Subclassification depends on internal carotid artery involvement:

C1	:	Tumors involving the foramen caroticum.
C2	:	Tumors involving the vertical segment of the carotid canal.
C3	:	Tumors involving the horizontal segment of the carotid canal.
C4	:	Tumors extending to the ipsilateral foramen lacerum and cavernous sinus.

Class D	:	Classification used in conjunction with class C and denotes intracranial extension.

Extradural and intradural extension are distinguished by the suffix 'e' and 'i' respectively:

De1	:	Tumors with intracranial extradural extension up to 2 cm.
De2	:	Tumors with intracranial extradural extension greater than 2 cm.
Di1	:	Tumors with intracranial intradural extension up to 2 cm.
Di2	:	Tumors with intracranial intradural extension greater than 2 cm.
Di3	:	Tumors with inoperable intracranial extension.

As stated above, the use of modern radiographic imaging has contributed greatly to the accuracy of classification. This kind of preoperative evaluation provides the necessary information for the skull-base surgeon to determine the presence and degree of carotid, intracranial, and intradural extension, which means that the proper surgical approach can be selected (Fisch,1982). A uniform tumor classification is also needed for the purposes of analysis of results, and consistency of communication in the literature.

TREATMENT

Surgery

During the period of the last 50 years, several trends can be distinguished in the surgical management of CBT's paragangliomas, but opinions concerning the management of these lesions have undergone many changes over the years. LeCompte (1951) was doubtful as to whether anything more than a diagnostic biopsy should be done. He stated that more patients died from the operation than from the tumor and concluded that *'the greatest danger to these patients was the treatment rather than the disease'*. Others based on the use of a more aggressive surgical approach on the invasiveness and unpredictable natural history of these tumors (Monro,1950). During the years most authors agreed that small, actively growing tumors in young patients should be removed before the arterial wall became extensively involved, thus increasing the risk of surgical morbidity, whereas asymptomatic neoplasms in the elderly should not be removed surgically, since they represent little threat to the patients survival (Conley,1965; Javid,1967). At present however, instead of biopsy alone or combined with partial excision, complete surgical resection of CBT's is generally achieved in almost all patients (Hallet,1988; van Asperen,1981).

Use of the subadventitial plane dissection is largely responsible for the decreased morbidity and mortality associated with the surgical removal of these tumors (Conley,1965). The embryologic development of the carotid body in the adventitia of the carotid artery, coupled with the clinical observation that CBT's do not invade the media, offered a basis for the subadventitial dissection of these tumors originally outlined by Gordon-Taylor (1940). Hallet (1988) reported that arterial repair (33% of cases) has become common practice to replace ligation. Complicated arterial reconstruction (patch, graft, or end-end anastomosis) had to be undertaken in 25%, a carotid artery shunt was used in only 9% of cases, and the external carotid artery was ligated or resected with the tumor in 33%. In his series of 153 paragangliomas, Hallet et al. concluded that almost all CBT and VBT's can be completely resected without minimal risk of stroke or death.

Most surgical morbidity in VBT's is the result of neurologic rather than vascular damage. The vagus nerve is almost always sacrificed, because of its intimate connection with the tumor capsule (Green,1988). A small number of VBT's extend into the the skull-base (jugular foramen) to involve the 9th - 11th cranial nerves and embed the internal jugular vein and carotid arteries. Ligation of the vessels at this level can become difficult or impossible, and sometimes does not allow radical resection of a VBT.

At present, surgery is also considered the preferred method for the treatment of patients with GTT or GJT's (Fisch,1982; Jackson,1989). Contemporary diagnostic and (micro) surgical techniques in the area of the skullbase have greatly improved the results of complete removal of extensive vascular tumors.

The atlas *'The surgeon's view of the skull base from the lateral approach'* by Goldenberg (1984) and *'Microsurgery of the skull-base'* by Fisch (1988) will contribute to a better understanding and increased knowledge of the surgical anatomy and their relationship in this area.

The development of the infratemporal fossa approach with permanent anterior transposition of the facial nerve by Fisch in 1978 has opened the way for radical removal of lesions situated in the infralabyrinthine and apical compartments of the temporal bone and require exposure of the infratemporal course of the carotid artery. Radical removal of types C 1-3 and D 1 (70% of cases) was possible in a one-stage procedure via the infratemporal fossa approach. D 2 tumors require a two-stage, combined otological and neurosurgical removal, while type A and B tumors were removed with conventional tympanoplastic techniques and preservation of middle ear function.

Jackson and Glasscock (1990) recently introduced their concept and reported their results obtained with conservation surgery at the lateral cranial base. The goal of conservation surgery is to preserve as much as possible of the normal ear anatomy and cranial nerve function which also depends on accurate and early diagnosis. Rates of cranial nerve preservation amounting to 90% were possible with small tumors, but with increasing tumor size the chance of cranial nerve preservation decreased sharply (Fisch,1982; Jackson,1990).

A few other approaches (case-reports) to the lateral cranial base have been described; the transcervical transmastoid approach to the jugular bulb, which permits access to the jugular bulb between the undersurface of the skull and C 1, thus avoiding transposition of the facial nerve (Donald,1984). Al-Mefty (1987) combined the infratemporal and posterior fossa approaches for the removal of giant glomus tumors and chondrosarcomas in one stage.

Preoperative embolization has been recommended by several authors for the management of GJT's and advocated for the reduction of per-operative blood loss (Hekster,1973; Cece,1987; Young,1988; Murphy,1989). Opponents of embolization understate its risk and minimize its effect on the tumor. The most significant potential complications are stroke and death, but several other neurological deficits and vascular injuries can occur. Murphy and Brackmann (1989) concluded from a study of 35 patients that pre-operative embolization helps to reduce per-operative blood loss and operation time; however, it does not appear to reduce the risk of injury to the cranial nerves. The experience accumulated by the surgeon and the surgical team also plays a significant role in the reduction of blood loss and operation time.

Management of the internal carotid artery in skull base surgery can be a difficult problem when disease involves this vessel or resection is anatomically limited by it. The recent development of the detachable balloon catheter has permitted occlusion of the internal carotid artery in a controlled setting (1-3 days) prior to any surgical procedure (Andrews and Valavanis,1989). The use of this technique enables to create compensation for the alteration in intracranial hemodynamics through a well-developed collateral circulation. Essential to this is certainty as to adequate collateral blood flow to the brain as shown by arteriography and balloon occlusion under monitoring of physical signs and the use of electroencephalography (EEG).
Despite these new techniques it must be said that cranial-base operations still remain an extraordinary undertaking associated with a substantial chance of invalidating morbidity and mortality.

Radiotherapy
Several authors have claimed a high efficacy of radiation therapy in the control of these slow-growing tumors (Spector,1975; Cummings,1984; Konefal,1987; Springate,1990). The optimum dose is still under discussion, but many radiotherapists have reported excellent response and minimal side effects with doses of 40 to 50 Gy using conventional fractionation (Lybeert,1984; Dawes,1987; Boyle,1990). Others have stated, that the response of these vascular tumors to radiation is limited to microvascular damage, leading to sclerosis and fibrosis, since a direct cell-killing effect cannot be demonstrated (Spector,1975).
In a histopathological study, Hawthorne (1988) showed that tumor behavior after irradiation is unpredictable and concluded that it should be reserved for the elderly and those in poor health with the aim of slowing down local tumor growth. Morfit (1967) doubted whether any patient was ever cured by irradiation and believed that claims of control or regression were related to the natural history of the tumor,i.e., which tends to remain stationary for years. Even today the position of irradiation as primary treatment for, temporal-bone paragangliomas is heavily debated (Brackmann,1987).
In the management of CBT's or VBT's radiotherapy is not considered - a primary modality of treatment because the majority of tumors at these sites remain more accessible to complete surgical resection tham do GJT's. In our experience surgical removal of CBT or VBT's after radiotherapy is, even for the experienced surgeon, extremely more difficult, it severely increases morbidity as well as the chance on non-radical surgery.

AIM OF THE STUDY

Controversy concerning the management of head and neck glomus tumors persists. Although these tumors are extremely slow-growing and rarely malignant, they have a critical localization. Surgical therapy of these benign lesions especially in the temporal bone is criticized because of the accompaning perioperative complications, cranial nerve palsy, and decreased quality of life. Radiation therapists have focused on the surgical complications to continue arguing for non-operative management of these tumors, in spite of the question as to whether these tumors are truly radiosensitive. Representatives of both modalities probably make good use of the unknown and usually indolent natural course of disease in favor of their results.
For the clinician it remains difficult to decide whether and when to perform such an extensive tumor resection. This dilemma increases when dealing with bilateral tumors.

The main purpose of the present study was to obtain more information about the 'natural course' of the disease in glomus tumor patients. In familial and hereditary disease, information can be obtained on the probable course of disease of affected family members, i.e., ancestors that in the past were sometimes not even diagnosed or treated.
Chapter 2, discusses a comparison between the (reconstructed) 'natural course' of disease and the outcome of different treatment modalities. It also gives an overview of the clinical data of 108 patients accounting for a total of 175 head and neck paragangliomas treated in Leiden University Hospital in the last 32 years (1956 - 1988).
With respect to the study on the familial aspects of the disease, **Chapter 3** describes a genetic analysis of thirty pedigrees (including fifteen from the literature). The finding that the pattern of inheritance in familial glomus tumors deviates from that of the classic Mendelian autosomal dominant trait is discussed and support for a new genetic theory is described, and the implications for genetic counselling are dealt with.

With respect to both sporadic and familial disease, there is an urgent need for a tumor-biological marker that gives information on the clinical behavior of patients with glomus tumors. Our aim was to find out whether tumor ploidy or immunohistochemical findings could help to discriminate between clinically indolent and aggressive tumors.
Chapter 4 deals with a study on the predictive value of flow-cytometric DNA ploidy analysis of a series of 99 head and neck paragangliomas. The question of whether there were differences in DNA ploidy between familial and non-familial tumors on the one hand and the ploidy distribution of multicentric tumors within the same patient or family on the other is also discussed.
Chapter 5 presents the pattern of immunohistochemical staining of 114 glomus tumors with the use chief and sustentacular cell markers. Correlation with the clinical outcome in hereditary and sporadic disease is described. Expression of these markers and the DNA-ploidy status of the tumor were compared to obtain insight into the relation between the presence of these antigens and DNA-ploidy.
Chapter 6 reports a study on hormonally active paragangliomas diagnosed with the uptake of radioactive (I-123) labelled metaiodobenzylguanidine (MIBG) in 14 patients with a total of 24 head and neck paragangliomas. The yields of MIBG screening are compared with urinary catecholamine measurements, computed tomography (CT), and magnetic resonance (MR) studies.
Finally, in **Chapter 7**, the results of the studies reported in this thesis are summarized and an outline for further tumor biologic and genetic studies is given.

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CHAPTER 2

DOES INTERVENTION IMPROVE THE NATURAL COURSE OF GLOMUS TUMORS?

(a series of 108 patients seen in a 32-year period)

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ABSTRACT

To acquire more insight into the results of treatment versus the 'natural' course of glomus tumors, we studied the clinical data of 108 patients, in 58 of whom the disease was hereditary. During a period of 32 years (1956-1988), 175 tumors were diagnosed, viz. 52 glomus jugulo-tympanic tumors, 32 vagal body tumors, and 91 carotid body tumors. The results of radical surgical treatment were disappointing for tumors located at the skull base i.e., non-radical in 59% (n=23) of the cases, but very good for the carotid body tumors, for of which 96% (n=68) radical excision was achieved. Moreover, surgery at the level of the skull-base dramatically increased morbidity since it frequently induced cranial nerve palsy.

During the follow-up period (maximum observation time 32 years, mean 13.5 years) none of the patients died from residual or recurrent tumor or developed distant metastases irrespective the mode and outcome of treatment. When combined with the results of pedigree analysis a realistic approximation of the 'natural' course of the disease for both hereditary and non-familial tumors is made. The results make it questionable whether this natural behavior is really improved by intervention.

It is concluded that the option to remove carotid body tumors and solitary vagal body tumors should be considered in order to prevent future morbidity. However, for skull-base and bilateral glomus tumors a more conservative monitored 'wait and see' policy can be sensible and should be considered in any proposal to treatment concerning head and neck paragangliomas. When there is serious progression of cranial nerve palsy or when intracranial growth becomes life-threatening surgical intervention cannot be avoided. The main goal of glomus tumor treatment should be to reduce morbidity rather than trying to increase survival rates.

INTRODUCTION

In the middle of the eighteenth century Haller was the first to describe the carotid body situated at the medial wall of the common carotid artery at its bifurcation. In 1941, Guild reported *'a hitherto unrecognized structure, the glomus jugularis in man'* located in the adventitia of the jugular bulb[1]. Rosenwasser recognized the relationship between an unusual vascular tumor of the middle ear and the normally occurring glomus jugulare body[2].

One of the main characteristics of glomus tumors (syn.: non-chromaffin paragangliomas, chemodectomas) is the presence of clusters of tumor cells ('Zellballen') interspersed among an extensive capillary network. This 'Zellballen' system is demonstrated best by silver impregnation of the reticulin fibers[3].

As Bartels mentioned as early as 1949, glomus tumors can also occur as an autosomal dominant hereditary disease[4]. Pedigree analysis showed that tumor expression was restricted to inheritance via the paternal line. This finding is inconsistent with the autosomal dominant transmission assumed before but it can be explained in terms of genomic imprinting, i.e. the maternally derived gene is inactivated during female oogenesis and can only be reactivated during spermatogenesis. This new genetic theory has considerable implications for genetic counselling in hereditary glomus tumors[5]. Further genetic studies are currently being performed to localize and identify the predisposing gene defect. Multicentricity whether uni-or bilateral is an important feature of hereditary glomus tumors. It occurs in at least 30% of cases.

The clinically most relevant tumor sites in the head and neck are the carotid body (CBT), the glomus tympanicum (GTT), the glomus jugulare (often grouped together as glomus jugulo-tympanicum tumor GJTT), and the vagal body (VBT).

These benign tumors grow extremely slowly and are associated with a generally low mortality rate, but they can enlarge relentlessly to encompass the adjacent neuro-vascular structures, and even extend intracranially. When surgery is performed on such a large tumor there is an increased risk of neurovascular complications as well as of incomplete tumor resection. Almost 75 years ago this was already recognized by Balfour and Wildner when they wrote[6]: *'a cure without permanent disability is rare'*. Consequently, at that time the complications of surgical treatment were worse than the natural course of the disease itself.

Especially for lesions at the base of the skull, tumor resection frequently leads to considerable and permanent cranial nerve palsy. It is even more difficult to decide whether and when to perform such extensive tumor resection, when dealing with bilateral tumors.

Therefore we felt the need to compare the natural course of disease with the outcome of different treatment modalities and studied a series of 108 patients with a total of 175 glomus tumors located in the head and neck region treated in our hospital during the last 32 years.

MATERIALS & METHODS

Patients

During the past 32 years (1956-1988) the diagnosis glomus tumor was made in 108 patients seen in the Departments of Otolaryngology and Vascular Surgery of the Leiden University Hospital. In 75% of the cases the diagnosis was confirmed histologically. The clinical data in this retrospective study were retrieved from the status reports which in most cases supplied information on the following items: family history of glomus tumor, age at first diagnosis, presenting signs and symptoms, duration of symptoms, catecholamine secretion, tumor size and localization, multicentricity, mode of treatment, radicalness of surgery, morbidity and follow-up data. A questionnaire was sent to all 108 patients or relatives (in case of death) requesting additional data on the medical and family histories. Only three patients could not be traced or were not informative otherwise. Patients whose response was positive or equivocal with regard to hereditary disease were visited at home to obtain more information about family members who might have had a glomus tumor. In this way 15 families with hereditary glomus tumors were retrieved. Sometimes a family conference or photograph of an ancestor helped to complete the pedigree. The results

of this study on the hereditary aspects have been published elsewhere[5]. Functioning glomus tumors or extra-adrenal paragangliomas were detected both clinically and on the basis of elevated urinary catecholamine levels. Recently, our group established scintigraphy with labeled metaiodobenzylguanidine (MIBG) as a valuable diagnostic technique for vasoactive paragangliomas[7]. It is now routinely used in preoperative analysis. Since many patients in our study had multicentric glomus tumors, we prefer to report the follow-up data for individual tumors rather than that for patients.

Tumors

- Localization, size, and histology

The 108 patients accounted for 52 GJTT's, 32 VBT's, and 91 CBT's, giving a total of 175 glomus tumors. Tumor size had been recorded for 124 glomus tumors. The size of 38 temporal bone glomus tumors (GJTT) was estimated according to the following semiquantitative categories defined by Rosenwasser[2]: *small* tumors are confined to the middle ear space without extension in the hypotympanum. These are usually GTT's arising from the promontory or floor of the tympanum (n=12). Tumors of *intermediate* size have penetrated beyond the middle ear in the direction of the mastoid and with involvement of the hypotympanum. This indicates that the process originated in the jugular bulb (n=6). Finally, *large* tumors show considerable extension into the base of the skull or intracranially (n=20).

The volume of the VBT's and CBT's (n=86) was calculated from the dimensions given in the histo-pathological reports[6]. Tumor volume ranged from 1 to 224 cm^3. Since no established or uniform classification was available before the introduction of the CAT-scan, we arbitrarily subdivided tumor volume into three size classes, each of which covered approximately one-third of the size range. Tumors measuring between 1 and 18 cm^3 (n=37) were called *small*; those between 18 and 60 cm^3 *intermediate* (n=39), and those of 60 cm^3 or more *large* (n=10). In table 1 this subdivision is shown for the three tumor localizations studied.

As described earlier[8], all histological slides of 132 excised tumors were reviewed using the histological criteria for glomus tumors as established nowadays[3]. No histological signs of malignancy or clinical evidence of distant metastases were found in this series.

Table 1. Distribution of tumors by size class and localization.

			GJTT	VBT	CBT
Small	n=49	(39%)	12	3	34
Intermediate	n=45	(36%)	6	9	30
Large	n=30	(24%)	20	2	8
All sizes	n=124	(100%)	38 (31%)	14 (11%)	72 (58%)

- The selection of the time periods.

The results of treatment during the past ten years were compared with those of two earlier ten year periods. This method for the assessment of trends with respect to tumor resectability and neuro-vascular complications of surgical management was also used by Hallet et al.[9].

Period 1: (1956-1966; 14 patients, 20 tumors) refers to 'the early days' of microscopical ear surgery, when the otologic surgeons obtained their initial experience with glomus tumor surgery and the vascular surgeon had to perform ligation of the carotid artery in some difficult cases.

Period 2: (1967-1977; 44 patients, 59 tumors) Size and extension of a skull base tumor often had to be estimated clinically with little help from polytomography and angiography. Frequently, surgical management failed to control the tumor in several areas including the jugular foramen. Repair or grafting of the extra-cranial carotid artery was possible.

During the past ten years (*Period 3*, 1978-1988; 50 patients, 96 tumors) CT and MR imaging techniques became widely available, allowing for new and safer surgical approaches to the skull base. In our hospital systematical scanning in axial and coronal planes became routine from 1984. Furthermore, better pre-operative evaluation of cerebral blood flow was introduced[10]. Improved and less invasive imaging techniques are also used more and more to support the traditionally clinical follow-up.

Reconstruction of the 'natural' course of disease of glomus tumors

One of the purposes of this study was to find the most likely approximation of the 'natural' course of disease of head and neck paragangliomas. A contribution to this could not only come from the history of disease of patients treated and followed in our hospital (n=25). Especially informative are those patients that lived with such a tumor without seeking medical help. We traced 16 such patients from the material used in our previous study on hereditary glomus tumors[5] and observed that many of them had become old with the disease and did not die from it. These persons or 'patients' (n=16) were not initially diagnosed, treated or followed in our own hospital and information about them was sometimes gathered from family members. For patients with non-hereditary disease these data could be retrieved from the status report and follow-up. Now, the course of disease for both hereditary and non-familial tumors could be reconstructed from (a) patients that were left untreated, (b) those with residual tumor after surgery or (c) had undergone irradiation or (d) a combination of surgery and irradiation.

RESULTS

Epidemiologic data

In table 2 some epidemiologic data for familial and non-familial glomus tumors are summarized. In the non-familial group (47% of cases) there was a marked female preponderance (68%) in contrast to the familial group in which multicentricity

(55%) was a distinctive feature. At this point it must be noted that the results of this study are gathered retrospectively and that pedigree analysis has influenced the number of familial cases.

Table 2. Epidemiological data on the incidence of familial and non-familial glomus tumors.

	number of patients	♂	♀	multiple tumors (patients)	age at diagnosis (years)
Familial	58 (53%)	28	30	32 (55%)	36 (16-66)
Non Familial	50 (47%)	16	34	11 (22%)	45 (12-84)

For both groups most glomus tumors were diagnosed in the fourth decade. At the time of diagnosis the symptoms had been present for three years on the average. Note however, that in 17% of the patients the duration of symptoms had amounted to even more than ten years.

Presenting signs and symptoms

Patients with a GJTT were mainly suffering from hearing loss (88% of cases) and/or pulsating tinnitus (86%). Less frequently (3%) the initial sign was a brisk bleeding from the external auditory canal. Voice change and a facial nerve palsy were both recorded once as a presenting symptom. The classical otologic finding of a mass behind the tympanic membrane, usually with a positive Brown's pulsation sign, was often recorded indeed (32 patients = 64%). Initial cranial nerve deficit was relatively frequent and included the facial (n=9; 18%), the cochlear (n=9; 18%), the glossopharyngeal (n=5; 10%), the vagal (n=7; 14%), the spinal accessory (n=2; 4%), and the hypoglossal nerves (n=4; 8%).

Table 3. Results of surgical treatment in relation to tumor localization and time period. Five tumors were either irradiated (1 VBT, 2 CBT) or embolized (1 VBT, 1 CBT).

	number of patients	number of tumors	GJTT (n=52)				
			radical surgery		non radical surgery		no treatment
1956-1966	14	20	2	(28%)	5	(71%)	1
1967-1977	44	59	4	(40%)	6	(60%)	3
1978-1988	50	96	10	(45%)	12	(55%)	7
1956-1988	108	175	16	(41%)	23	(59%)	13

VBT's almost invariably presented with a cervical neck mass (25 patients; 89%), which in 18% of cases extended as a pulsating mass into the oropharynx. Cranial nerve dysfunction was less prominent than in GJTT. The facial and cochlear nerves were never affected, whereas the glossopharyngeal, vagal, spinal accessory, and hypoglossal nerves were involved in 10, 17, 3, and 4% respectively.

Similarly, a cervical mass at the level of the carotid bifurcation (97%) was the leading sign at the onset of CBT's. Cranial nerve deficit was restricted to the vagal and hypoglossal nerves, in 11 and 4% of the patients, respectively.

Thirty glomus tumors (2 GJTT's, 4 VBT's and 24 CBT's) were detected in a subclinical stage when searching for multicentric tumors. This occurred mainly in *period 3* due to the availability of imaging techniques and our special interest in pedigree analysis.

Treatment

In 25% (13/52) of GJTT's, 34% (11/32) of VBT's and 18% (17/91) of CBT's we refrained from treatment because of the extent of the tumor, the age or the condition of the patient. Also in many cases of bilateral disease no treatment was installed avoiding the risk of bilateral cranial nerve palsy. In recent years, we were more and more inclined to prefer such a conservative approach initially, as demonstrated in table 3, especially for (bilateral) localizations at the skull base.

Furthermore this Table shows that 75% of GJTT's, 59% of VBT's and 78% of CBT's were treated surgically. Surgery was radical in 41%, 58% and 96% of cases respectively. Recorded data allowed determining the relation between tumor size and the results of surgical excision for 116 tumors. As could be expected beforehand, large GJTT's are more prone to non-radical surgery than smaller ones, irrespective the time period. However, for tumors originating from the neck, tumor localization rather than tumor size was predictive for the outcome of surgery, which generally appears to have been better than in GJTT's in all three time periods (table 4.).

One VBT and one CBT were embolized as a sole form of treatment.

Table 3. (continued)

VBT (n=32)					CBT (n=91)				
radical surgery		non radical surgery		no treatment	radical surgery		non radical surgery		no treatment
0		1		0	8	(89%)	1		0
4	(50%)	4	(50%)	2	25	(96%)	1		8
7	(70%)	3	(30%)	9	35	(97%)	1		9
11	(58%)	8	(42%)	11	68	(96%)	3	(4%)	17

Only in period 1 radiotherapy (2000 - 4500 rad) was a treatment modality, used in four patients (GJTT). Three of them required surgical intervention for the same lesion within ten years.

Table 4. Results of treatment in relation to tumor size and tumor localization.

	GJTT (n=36)		VBT (n=14)		CBT (n=66)		Total (n=116)	
	radical surgery	non-radical surgery	radical surgery	non-radical surgery	radical surgery	non-radical surgery	radical surgery	non-radical surgery
Small (n=44)	6	5	2	1	29	1	37	7
Intermediate (n=43)	2	3	7	2	28	1	37	6
Large (n=29)	6	14	0	2	6	1	12	17

Complications of surgery

Surgical removal of GJTT's (n=39) increased the prevalence of cranial nerve palsy (7th-12th) more than two-fold. E.g., facial nerve palsy increased from 20% to 43% and the prevalence of deafness increased from 18% to 64%. Similar observations were made for the 9^{th}-12^{th} cranial nerve function.

90% of VBT excisions resulted in loss of function of the 10th cranial nerve, whereas in CBT's deficit of the 10th and 12th cranial nerve occurred just four times resp. once in 71 excisions.

The carotid artery was ligated four times (CBT), i.e., in two times in *Period 1* and in the other Period's each one time. One of these patients suffered from a transient hemiplegia. Reconstruction of the internal carotid artery was performed four times after CBT removal.

One fatal case occurred in a patient referred to our hospital in 1976, one day after the excision of a CBT elsewhere. Revision surgery did not reveal macroscopic damage to the vessels, but the patient remained hemiplegic and died soon afterwards.

Follow-up

33 GJTT's were available for an average follow-up period of 13.5 years (range 1-32). 13 of these were monitored without treatment, while 20 tumors had previously undergone non-radical surgery and three patients were lost to follow-up. All patients in this group are still alive and without serious complaints. Clinically their disease is not progressive with the exception of one case. This patient had to undergo further surgery within five years after incomplete surgical removal. The usual rather indolent clinical course can be illustrated by four cases whose radical cavities show relatively unchanged residual and recurrent glomus tumor tissue thirty years after incomplete removal of the GJTT via a mastoidectomy approach. Unfortunately, follow-up imaging with CT for confirmation of the patient's clinical status was not performed routinely until 1984.

Similar observations can be made on the 20 VBT's that had a mean follow-up of 7.3 years. Virtually no long term follow-up data were available for solitary CBT's due to the usually radical excision. Consequently, the untreated CBT's mentioned in Table 3 are mainly accompanied by another paraganglioma at another location. With the help of our Medical Registration we obtained additional information about patients that had radical surgery in the past and were discharged from further follow-up. From those previously treated for a CBT (n=68), 8 'patients' had died of unrelated causes with an average time of 17 years after their initial diagnosis and operation. For those with a GJTT (n=16), 3 'patients' had died with an average time of 6 years after surgery of unrelated causes.

Reconstruction of the 'natural' course of disease of glomus tumors

Especially for hereditary disease a remarkable observation on the reconstructed course of disease could be made: Including the 'patients' (n=16) from the pedigrees we collected a group of 50 tumors (41 patients) which we subdivided into four groups: patients which were left untreated, patients after non radical surgery, those after irradiation, and those who had undergone both surgery and irradiation.

Table 5. Reconstruction of the 'natural' course of disease of 50 hereditary glomus tumors found in 41 patients in relation to tumor site (a.o. = average age at onset of disease).

	no treatment (17 patients) a.o.=38 yrs		non-radical surgery (12 patients) a.o.=31 yrs		irradiation (7 patients) a.o.=37 yrs		combination (5 patients) a.o.=35 yrs		total (41 patients) a.o.=35 yrs
GJTT (n=21)	31 yrs	(n=4)	17 yrs	(n=9)	14 yrs	(n=4)	23 yrs	(n=4)	20 yrs
VBT (n=9)	7 yrs	(n=7)	16 yrs	(n=1)	23 yrs	(n=1)	–		10 yrs
CBT (n=20)	18 yrs	(n=14)	40 yrs	(n=2)	25 yrs	(n=3)	20 yrs	(n=1)	21 yrs
duration of disease (50 tumors)	17 yrs	(n=25)	21 yrs	(n=12)	19 yrs	(n=8)	22 yrs	(n=5)	19 yrs

The average duration of disease was about 19 years irrespective of tumor site as is illustrated by table 5. Also for this group (i.e., affected family members or 'patients') a marked increase in cranial nerve palsy was most clearly observed in patients who underwent surgery. For example, in 4 out of 9 patients with GJTT's the operation induced an increased deficit of the 7th, 8th and 10th cranial nerve, and three of them also revealed new deficit of the 9th, 11th, and 12th cranial nerve. Three patients showed no adverse effects of surgery and in the other two no reliable information was available.

As demonstrated by table 6, similar observations were made for non familial tumors for which the average course of disease accounted for about 13 years.

In our study we included 12 patients with a familial and 4 patients with a non-familial glomus tumor who had an age of onset of disease under 30 years (average follow-up 17 resp. 19 years). With the exception of one patient who died of it we did not observe any significant difference in the course of disease between these patients and the ones with a later age of onset.

Table 6. Reconstruction of the 'natural' course of disease of 35 non-hereditary glomus tumors found in 31 patients in relation to tumor site (a.o. = average age at onset of disease).

	no treatment	non-radical surgery	irradiation	combination	total
	(9 patients) a.o. = 52 yrs	(20 patients) a.o. = 43 yrs	--	(2 patients) a.o. = 44 yrs	(31 patients) a.o. = 46 yrs
GJTT (n=22)	7 yrs (n=7)	16 yrs (n=14)	--	16 yrs (n=1)	13 yrs
VBT (n=9)	2 yrs (n=2)	13 yrs (n=6)	--	25 yrs (n=1)	12 yrs
CBT (n=4)	9 yrs (n=2)	19 yrs (n=2)	--	--	14 yrs
duration of disease (35 tumors)	6 yrs (n=11)	15 yrs (n=22)	--	21 yrs (n=2)	13 yrs

DISCUSSION

The current study is comparable to the five largest studies in the literature with regard to the number of cases reported (range 74-231) and the duration of the follow-up (range 1 to 27 years)[9,11-14]. Also, the number and nature of signs and symptoms are consistent with these earlier reports. The mean age at onset of disease was 33 years for the familial group and 44 years for the non-familial group, which is accordance with the literature [5,15]. It seems likely that familial cases are diagnosed earlier, as the family is aware of the possibility of the disease.

However, the observed incidence of familial glomus tumors (50%) is much higher than in most other series[15-17] where it is about 10%. Partly this may be attributed to the relatively close-knit communities in which families tend to dwell in the region nearby our institution. Probably it is also due to our screening procedure, which focussed on the familial aspects of glomus tumors. We demonstrated that genomic imprinting may account for the finding that inheritance is almost exclusively via the paternal line[5].

Familial occurrence of glomus tumors is often accompanied by multicentricity[15,16]. Our zeal to detect additional tumors will have contributed to an unusually high percentage (55%) of multicentric tumors.

The possibility that the multicentric occurrence of glomus tumors should be explained as metastatic disease has been invalidated by the results of our recent DNA ploidy study[8]. With flow cytometry we could not find any arguments for a common cellular origin of different tumor sites in patients with multifocal, hereditary disease.

The biological behavior of this tumor is highly intriguing. Although roughly 40% of the cases show frank DNA-aneuploidy, this is not associated with the development of either metastatic disease or an aggressive growth pattern[8]. Thus it remains the question which factors prevent these tumors from progressing to overt malignancy. As a matter of fact, the incidence of malignancy in glomus tumors as indicated by lymphogenic and hematogenic metastases is probably not greater than 10%[3]. These tumors may penetrate into nerves and bone tissue but local infiltration is not correlated with the occurrence of distant metastases[18]. We observed just one malignant laryngeal paraganglioma which caused massive metastatic disease 2.5 years after the initial diagnosis[19].

In the present series, angiography was used in almost all patients as the main diagnostic technique since 1962. It was accompanied by CT scanning for lesions at the skull base since 1977. From 1984 we scanned more and more routinely in two perpendicular planes. In 1987 we implemented magnetic resonance imaging (MRI) because it provides superb soft-tissue contrast if Gadolinium DTPA enhancement is used and offers the possibility of scanning in the sagittal plane. It is particularly useful for the assessment of the intracranial extent of the tumor[20]. We are currently using it as a non-invasive screening technique in a study on familial glomus tumors.

In the past surgeons often had to remove GJTT's piece by piece, inducing tremendous bleeding and damage to adjacent neurovascular structures[21]. Complete surgical removal of GJTT's was usually extremely difficult due to their location in the petrous bone and the impossibility to know the true extent of the lesion in advance. Therefore, these neoplasms were known for their high recurrence rate. We share the opinion of Glasscock[22], that *'the early literature must be regarded with a certain amount of skepticism'*.

The development of the infratemporal fossa approach by Fisch greatly improved the completeness of GJTT resection at the skull base[23]. Techniques for safe resection of CBT's are based on excision in the subadventitial plane from which the blood supply of the tumor is derived[24].

In 1969 McCabe advocated that the size and extent of the tumor is decisive for the selection of the most appropriate form of therapy[25]. Unfortunately, this information could not be obtained before the introduction of CT-scanning in 1977. Since many surgical classifications for GJTT's are based on CT-scanning data[12-13], it is not possible to apply any of these classifications in the current review over the past 32 years.

Our surgical results for GJTT's (59% non-radical) are comparable with the literature[2,14,17,26] with the exception of a few series[12,13,22] which claim better results. Our preliminary results with the infratemporal fossa approach (7 tumors) were more favorable, but conclusions with regard to the surgical morbidity are not yet permitted. It is conceivable that even large GJTT's with involvement of the internal carotid artery at the skull base can be resected completely with this technique[10].

With 96% radical excision of CBT's our results were comparable to those reported by other authors[6,9] as well as the amount of vascular and neurological post-operative complications of CBT and VBT excision.

The position of irradiation in the management of glomus tumors is still heavily debated[27]. Several authors have claimed a high efficacy of radiation therapy in the control of these slow-growing tumors[28-30]. Others use it in elderly patients or in individuals in poor health to slow down local tumor growth. The response of these vascular tumors to irradiation is limited to microvascular damage, leading to sclerosis and fibrosis since a direct cell-killing effect cannot be demonstrated[28]. The histopathological study done by Hawthorne showed that tumor behavior after irradiation is unpredictable[31]: He investigated 18 tumors requiring surgery after irradiation and detected vital tumor tissue without signs of necrosis in twelve ones. This observation clearly supports the vast experience of Rosenwasser[2]: *'the longer an irradiated patient is followed clinically, the more likely the recurrence'.*
The relatively large number of tumors in our series that had not or had not yet been treated is explained by the high number of multifocal bilateral tumors in our series. This is due to the high percentage (50) of familial glomus tumors, which are often multicentric (see introduction). In order to avoid bilateral cranial nerve palsy we only decided to operate on the second side if the first resection had not caused essential cranial nerve palsy. When we refrained from surgery in recent years, we relied on a 'wait and see' policy under the control of CT and MR imaging in preference to irradiation for reasons mentioned below.

In this retrospective semiquantitative study covering many years a comparison is made between the results of the different therapeutical modalities and the usually indolent natural course of the disease rather than to compare the follow-up of radically and non-radically treated patients. From table 5 and 6, it follows that survival cannot be improved by any treatment since the vast majority of patients with hereditary and non hereditary disease have a close to normal life expectancy. The surgically treated group showed most complications. Although it may not be concluded from these data that treatment increased morbidity, one may ask whether the patients did benefit from (non-radical) surgery or other treatment. In view of this, results reported in the literature for treatment and follow-up of glomus tumors (both of surgery and irradiation) may well have been influenced by the course of the disease. Certainly, in our series there are exceptions too, in which tumor progression is far more rapid. This makes the decision to refrain from intervention even more difficult especially when they are found in young people. However, from our material it cannot be concluded that patients with an early onset of disease do have a more severe outcome. Unfortunately, at present we haven't any tumor-biological marker at our disposal that can predict the growth pattern.

CONCLUSION

In general, our series demonstrates that surgical removal of CBT's and solitary VBT's is quite possible without major complications. Usually it prevents future morbidity since the resection is almost always radical. Moreover, the smaller a CBT, the more easy the resection.
Contrarily, we feel that for GJTT's a monitored 'wait and see' policy can be sensible and is justified by the morbidity frequently induced by surgery and the observation that survival is not improved by any treatment. Once a skull-base tumor is causing progressively invalidating cranial nerve palsy or it becomes life-threatening due to the intracranial extension, the decision to perform surgical intervention should be made. At this point both the surgeon and the patient will be convinced that this decision and its consequences are inevitable. With multifocal bilateral tumors we propose a similar strategy.

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CHAPTER 3

GENOMIC IMPRINTING IN HEREDITARY GLOMUS TUMOURS: EVIDENCE FOR NEW GENETIC THEORY

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ABSTRACT

A study based on fifteen pedigrees showed that familial glomus tumours are inherited almost exclusively via the paternal line, a finding inconsistent with autosomal dominant transmission. The results can be explained in terms of the genomic imprinting hypothesis - the maternally derived gene is inactivated during female oogenesis and can be reactivated only during spermatogenesis. Genomic imprinting may have considerable implications for genetic counselling with respect to glomus tumours and also for the understanding of other hereditary diseases.

INTRODUCTION

Glomus tumours, known as chemodectomas or non-chromaffin paragangliomas, derive from glomus body tissue. Their usual sites are in the head and neck. The most common types, in the order of frequency, are the carotid body tumour, in the adventitia of the carotid bifurcation, the glomus jugulare tumour, and the vagal body tumour. In general, these slow-growing tumours are benign and usually present as an enlarging cervical mass or cranial nerve palsy.

Familial occurrence of carotid body tumours was first noted by Chase[1] in 1933 in two sisters, one of whom had bilateral carotid body tumours and the other a unilateral tumour. Bartels[2] (1949) appreciated the familial tendency of glomus jugulare tumours and drew attention to their association with carotid body tumours. The study by van Baars[3] was consistent with autosomal dominant transmission. Familial glomus tumours are much more likely to be multicentric than sporadic tumours.[4] Patients with a positive family history have an incidence of bilateral carotid body tumours of at least 30%, as opposed to 5% in non-familial cases,[5] and thorough investigation including bilateral carotid angiography and m-iodobenzylguanidine (MIBG) scintigraphy is advisable.

Total surgical excision is the treatment of choice.[6,7] Irradiation may be more appropriate for advanced lesions involving the skull base or extending intracranially. Radiotherapy is preferable for the elderly and those in poor health.[8] Surgical treatment of bilateral glomus tumours carries a risk of permanent bilateral cranial nerve palsy. The purpose of the present study was to elucidate the natural course of the disease and investigate familial and hereditary aspects of these tumours.

MATERIALS AND METHODS

During the past 32 years (1956-88) the diagnosis of glomus tumour was made in 69 patients seen in the department of otolaryngology of Leiden University Hospital, and in most cases was confirmed by histology. A questionnaire was sent to the patients requesting data on the past medical and family histories as such information had not always been recorded. Patients who gave a positive or equivocal response were visited at home to obtain more information about family members who might have had a glomus tumour. Sometimes a family conference or a photograph of an ancestor helped to complete the pedigree. Occasionally, a first-degree relative was examined but in general no other screening techniques were used. 35 (50%) patients, comprising fifteen kindreds, had a positive family history and their pedigrees were suitable for analysis.

Photograph of kindred 'J' made in 1923 when grandfather (having at least bilateral cervical paragangliomas) was 60 years old. He died at the age of 84 of unrelated causes. According to his grandchildren, he had had these large cervical masses for over 43 years, which illustrates the usually indolent growth pattern of glomus tumours.

RESULTS

The 69 patients had a total of 115 glomus tumours - 50 glomus jugulotympanic tumours, 21 vagal body tumours, and 44 carotid body tumours.

Patients Without a Family History

In the group of 34 patients (50%) without a family history there was a female preponderance: 9 male, 25 female. Multicentricity was found in 8 patients (23%). The mean age at the time of diagnosis was 44 years (range 12-84) and the average duration of symptoms was 4.7 years (0.5-1.8). 12 patients had had symptoms for more than 12 years. No deaths due to either the disease or the treatment occurred in this group.

Patients With a Family History

The group with a positive family history comprised 35 index cases and 82 patients (40 M, 42 F) after completion of the pedigrees. The diagnosis was well established in 64 patients (33 M, 31 F) and for the other 18 patients seemed likely from the available information. Of the 64 patients, 31 (48%) had multicentric tumours. The median age at the time of diagnosis was 33 years (17-72) and the duration of symptoms was 3-9 years (0.5-15). The median age at the time of death was 67 years for 23 patients in fifteen kindreds. One patient died from intracranial extension of the glomus tumour. The average age at the time of diagnosis differed between the generations. In the first generation the average age was 51 years, in the second generation 38 years and in the third and fourth generations 28 and 21 years.

No clinical or histological evidence of malignancy was found in any of the patients with a positive family history.

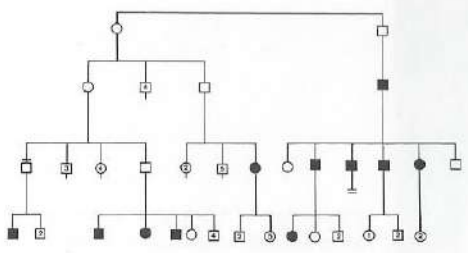

Fig 1—Pedigree O.

Pedigree symbols:
■ ● = Affected (male, female respectively)—confirmed in most cases, by histology.
▨ ◍ = Affected—history only.
③ ② = No of (male, female) siblings.
□ ○ = Personally examined, not affected.

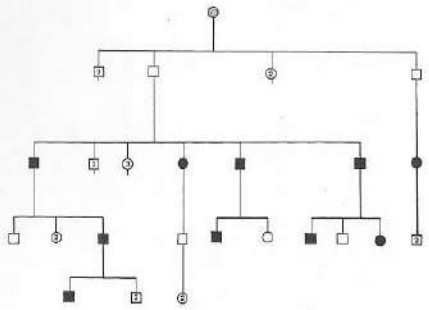

Fig 2—Pedigree B.

Pedigree Analysis

Analysis of the fifteen pedigrees in our study shows that the disease is transmitted almost exclusively by males, both affected and unaffected. In families where the father had a glomus tumour, 23 descendants were affected (11 M, 12 F) and 59 were not affected (37 M, 26 F). When the mother was affected, no descendants had tumours except one in whom the diagnosis of glomus tumour was not confirmed, either clinically or histologically. When the father was unaffected by the disease 41 descendants were affected, however when the mother was unaffected no descendants were affected. Whenever the disease was transmitted by the maternal line, this always happened via unaffected males (figs 1. and 2.).

DISCUSSION

Clinical Findings

The familial occurrence of glomus tumours is well known. We found a positive family history in 50% of our patients, in agreement with the percentage found by van Baars.[3] Grufferman et al,[4] who reviewed 923 cases in an epidemiological study, found only 88 (9.5%) familial cases but, as he pointed out, this percentage may have been inaccurate because several reports gave incomplete or no family histories. We too may have underestimated the frequency of familial disease and the number of affected individuals within a family, because initially these tumours, which are often small and slow-growing, hardly cause symptoms and may not be noticed.

In our study, fifteen extensive pedigrees were analysed. Earlier family studies were based on small pedigrees covering only few generations or just one large family.[3]

One of the most distinctive features of the familial versus the non-familial disease is the tendency for the former group of patients to get multiple tumours.[5] We found multicentricity in 31 (48%) of our familial cases as against 8 (23%) of the non-familial group.

The mean age at the time of diagnosis was 33 years for the familial group and 44 years for the non-familial group, which is in accordance with the findings of Parry.[9] It seems likely that familial cases are diagnosed earlier, as the family is aware of the possibility of the disease. The median age at the time of death was similar for treated and untreated patients.

In our series there was a big difference in sex ratio between the familial and non-familial groups; like other authors,[3,4,9] we found a female preponderance (9 M, 25 F) in the non familial group.

Pedigree Data

In familial cases the inheritance pattern was autosomal dominant but there were some atypical findings. There were several instances of skipped generations, where the disease was found in sibs with normal parents (fig 2.). Inheritance from father to son, which was often seen, excludes X-linked inheritance. Remarkably, inheritance from

Table 1 - Author's data on pedigree analysis

PEDIGREE	No Gen	♂ +	offspring +	offspring -	♀ +	offspring +	offspring -
A	4	1	1	4	2	0	11
	4	4	5	7	3	0	9
B	3	2	0	5	na	na	na
C	2	1	2	1	na	na	na
D	2	1	1	3	na	na	na
E	3	3	2	8	na	na	na
F	2	1	1	3	na	na	na
G	2	na	na	na	1	0	5
H	4	1	2	1	3	0	6
I	4	1	0	5	1	0	2
J	3	na	na	na	2	0	8
K	3	1	0	1	2	0	6
L	2	2	2	8	na	na	na
M	2	1	2	5	3	1*	10
N	4	3	5	8	2	0	9
O							

LEGEND: No Gen = number of generations reported
+ = affected
- = not affected
na = not available
nr = not registered
* = glomus tumor uncertain or inherited from both family lines
§ = non-affected males with affected offspring not reported

Table 2 - Data from the literature on pedigree analysis.

AUTHOR	No Gen	♂ +	offspring +	offspring -	♂ -	offspring +	offspring -	♀ +	offspring +	offspring -
Bartels 1949	3	2	5	7	1	nm	na	2	0	8
Sprong 1949	2	1	9	2	1	nm	na	5	nm	nm
Lewison 1950	3	4	nm	nm	3	5	2	1	0	10
Desai 1961	3	2	3	11	2	2	3	9	nm	nm
Kroll 1964	3	1	3	6	na	na	na	2	1*	3
Ribet 1969	3	3	6	5	na	na	na	3	nm	nm
Wilson 1969	3	3	7	1	1	1	nm	1	nm	nm
Sugarbaker 1971	4	3	1	4	1	1	2	1	1*	3
Pratt 1973	3	1	nm	nm	2	6	8	1	0	4
Chedid 1974	3	2	1	3	2	2	4	1	1*	nm
Kahn 1976	3	2	4	1	na	na	na	1	nm	nm
Hageman 1978	2	1	2	6	na	na	na	2	nm	13
Van Baars 1980	3	20	19	51	§	§	§	5	0	nm
Gruffermann 1980	2	1	2	3	2	nm	nm	2	nm	21
Parry 1982	3	9	8	23	3	6	4	5	0	

LEGEND: No Gen = number of generations reported
+ = affected
- = not affected
na = not available
nm = not mentioned
* = glomus tumor uncertain or inherited from both family lines
§ = non-affected males with affected offspring not reported

mother to offspring was not observed except in one case where the diagnosis was unconfirmed (table 1).

Review of published reports[2-5,9-19] confirmed our conclusion that inheritance along the maternal line never occurred, but for three questionable exceptions (table 2). Ribet[14] described a pedigree with an affected female having a possible affected descendant with minimal tumour near the carotid bifurcation. Pratt[16] reports a family where the disease was present in both paternal and maternal family lines, but, unfortunately the pedigree given is not informative. Kahn[18] mentioned another example, of a possibly affected descendant in the maternal line but the history was of attacks of vertigo only.

Genetic Implications

A high frequency of multifocal and bilateral disease, an equal sex ratio, and evidence of vertical transmission are all characteristic of hereditary tumours. It is increasingly recognised that many tumours may occur both sporadically and as a heritable disorder. Retinoblastoma and Wilms tumour are examples. Several hypotheses have been put forward to explain these findings. Knudson et al[20] put forward a two-step-mutation theory to explain the inheritance of retinoblastoma and other embryonic tumours. According to this hypothesis, in hereditary cases the first mutation is prezygotic and may be inherited in a dominant way; subsequently tumours develop only in somatic cells that sustain a postzygotic second mutation. In non-hereditary cases, Knudson suggested, tumours develop only when both mutations occur post-zygotically in a single somatic cell line. This hypothesis is consistent with the predominance of bilateral and multifocal tumours within affected families as well as with the dominant transmission. Hermann[21] postulated delayed mutation of a highly penetrant autosomal dominant gene and the transmission of a premutant allele through unaffected carriers. Both of these theories explain the difference in age at the time of diagnosis between the familial and non-familial group, but not the female preponderance among patients with non-familial tumours or the apparent absence of transmission via the maternal line.

Genomic Imprinting

The inheritance of glomus tumours and, in particular, the finding that offspring of female patients are not affected, might be explained by genomic imprinting. There is evidence, mainly from studies done in mice, that the expression of certain genes differs according to whether they are inherited from the mother or the father. Furthermore, there are reports suggesting that methylation of the regulatory regions of genes is associated with gene inactivation by transcriptional inhibition.[22] Parent-specific gene expression has been demonstrated in transgenic mice[23,24] and might explain the paternal influences found in some inherited disorders such as juvenile Huntingon's chorea and congenital myotonic dystrophy.[23] Wilkins[25] suggested a modification of Knudson's two-mutational-event theories involving genomic imprinting which would explain the non-random retention of the paternal allele of chromosome 11 in Wilms tumour.

Genomic imprinting satisfactorily accounts for the transmission of glomus tumours in the pedigrees in our study and those previously reported. We suggest that the

autosomal dominant gene, which is inactivated during female oogenesis, with the result that the first generation is not affected, can be reactivated after demethylation during spermatogenesis and lead to affected offspring in the second generation. This hypothesis would explain the pedigrees where unaffected males, with affected mothers or maternal grandmothers, had several affected children. There were a few instances of transmission of the gene through apparently unaffected males in two generations (fig 3.) which might have occurred because the gene escaped activation during male spermatogenesis or the tumour was present but undetected. Only once a "carrier" female was said to be unaffected (fig 4.).

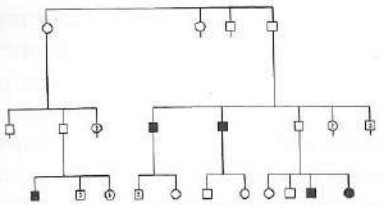

Fig 3—Pedigree C.

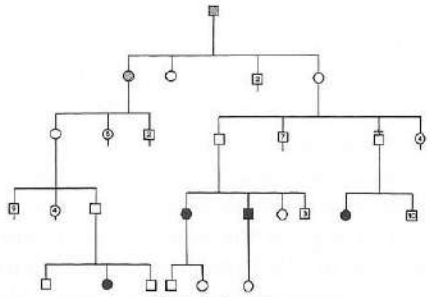

Fig 4—Pedigree A.

Genomic imprinting also offers an explanation for the female preponderance in non-familial patients, which is very pronounced for such a heterogeneous group. Very early post-zygotic mutations can produce germline mosaicism.[26] Some females are thought to have sporadic glomus tumour because the heritable character is not apparent until the mutation, passed through a paternal germline, is expressed in the second or later generation (fig 1.). In contrast, males are more often identified as having an heritable tumour as the disease becomes manifest in the next generation (figs 1. and 2.). In addition some patients represent true sporadic post-zygotic mutations.

Genomic imprinting is consistent with the extensive pedigree data which we present. Similar explanations have been proposed for the non-random retention of alleles in retinoblastoma and Wilms tumour.[25,27,28] Genomic imprinting may be a more general phenomenon in genetic tumour susceptibility than previously recognised. The involvement of genomic imprinting, derived from allele retention in carcinogenesis is still a matter of much discussion[29] - our pedigree studies provide strong and independent evidence for a role in the control of cell growth.

These findings are highly relevant for genetic counselling. The risk for the offspring of affected males remains 50%, for those of affected females the risk is very low, and for the children of sons of women with glomus tumours the risk is 25%. The grandchildren or greatgrandchildren of female patients who are thought to have sporadic tumours may also be at risk. DNA studies are needed to confirm the genomic imprinting hypothesis in relation to glomus tumours and other hereditary diseases.

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CHAPTER 4

DNA FLOW CYTOMETRY OF HEREDITARY AND SPORADIC PARAGANGLIOMAS (GLOMUS TUMORS)

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ABSTRACT

Paragangliomas (glomus tumours) are benign, hypervascular tumours which in general are treated by surgical excision. The indication for treatment of these often slow-growing tumours needs additional criteria for predicting tumour progressiveness.
For this reason the nuclear DNA content of 99 paragangliomas, 65 of them originating from patients with a positive family history, was analysed by flow cytometry. Unequivocal evidence of DNA aneuploidy was found in 37% of these clinically and histologically benign tumours, the average duration of follow up amounting to at least 10 years. The DNA index of the aneuploid tumours ranged from 0.90 to 2.03. No correlation was found between DNA ploidy and familiality or between DNA content and clinical criteria indicative of tumour progression, which means that DNA ploidy of these tumours cannot serve as a predictor for an expected growth pattern or familiality. DNA aneuploidy in hereditary and sporadic paragangliomas is not clinically related to malignancy but indicates that these tumours are true neoplasias cytogenetically.

INTRODUCTION

Paragangliomas (syn.: glomus tumours, chemodectomas) are rare benign hypervascular tumours originating from the tiny glomus bodies which are present throughout the body. In the head and neck area these tumours are mostly found at specific locations. The most common types, in order of frequency, are the carotid body tumour, the glomus jugulare tumour, and the vagal body tumour.
Paragangliomas can also present as an autosomal dominant hereditary disease for which, as we showed recently, genomic imprinting may account for the finding that the inheritance is almost exclusively via the paternal line[1]. Familial paragangliomas are often multicentric, whether uni-or bilateral[2].
The neoplastic nature of paragangliomas has been a subject of debate[3,4]. According to some authors, carotid body tumours are due to hyperplasia. In Peruvians living at high altitudes the glomus bodies are larger and heavier than in those dwelling at sea level, possibly due to hyperplasia of parynchymal tissue in response to lower PO_2 levels[5,6]. Others believe that the transformation of a carotid body into a carotid body tumour is due to neoplasia, because they found residual normal paraganglionic tissue outside the tumour capsule[7,8].
The morbidity caused by paragangliomas is not related solely to the highly variable growth pattern, but also to the mode of treatment applied. Surgical excision, where feasible, is considered to be the treatment of choice[9,10]. For the glomus jugulare tumours invading the skull base and sometimes the posterior and middle fossa such surgical resection is a major intervention, frequently resulting in considerable and permanent cranial nerve palsy.
In general the growth pattern of these neuro-endocrine tumours is characterized by extremely slow progression and in such cases it is difficult to decide whether to perform

extensive tumour resection. Sometimes, however, the tumour develops rapidly, threatening a number of functions or even the life of the patient.
With respect to both sporadic and familial paragangliomas, there is an urgent need of information about the growth kinetics, for definition of the indication, but also for the timing of surgical treatment. Such information is not provided by the histological features of the tumour[8].
Flow cytometric analysis of the DNA content has revealed the widespread occurrence of DNA ploidy changes in human malignancies[11,12]. Evidence pointing to an association between DNA aneuploidy and clinical aggressiveness in various types of solid tumour has been accumulating[13].
The aim of the present study was to find out whether DNA aneuploidy could be detected in paragangliomas as support for a neoplastic origin and, if so, whether this DNA aneuploidy is associated with the clinical extension of the tumour and can be used as a predictor of the growth rate. Furthermore, we were interested in finding out whether there were differences in DNA ploidy distribution between familial and sporadic tumours on the one hand and the ploidy distribution of multicentric tumours within the same patient or family on the other. The results indicate that DNA aneuploidy occurs relatively frequently in both familial and sporadic paragangliomas but no correlations with clinical extension were found.

MATERIALS AND METHODS

Patients
During the past 32 years (1956-1988), the diagnosis paraganglioma was made in 108 patients (male : female = 44 : 64), referred to the departments of Otolaryngology and Surgery of the Leiden University Hospital. A positive family history was found in 58 cases (53%). These 108 patients accounted for a total of 173 paragangliomas, i.e., 50 glomus jugulotympanic tumours (GJTT), 32 vagal body tumours (VBT), and 91 carotid body tumours (CBT). The diagnosis was histologically confirmed in almost all of the 132 excised tumours.
Tissue blocks cut from 132 paragangliomas were available for DNA flow cytometry (FCM). This study was limited to 99 tumours providing sufficient material to permit conclusive interpretation of the DNA profile (obtained from 77 patients, 47 of whom had a positive family history). Prior to surgical removal none of the tumours had been irradiated.
All histological slides were reviewed by two of the authors (AGM, GJF) according to the established histological criteria for paragangliomas. One of the main characteristics of paragangliomas is the presence of clusters of tumour cells (Zellballen) interspersed among an extensive capillary network. This Zellballen pattern is demonstrated best by silver impregnation of the reticulin fibres[8,14]. No histological or clinical evidence of distant metastases was found in any of these 99 tumours (77 patients).
The clinical data were retrieved from the status reports and in most cases supplied information on the following items: family history of paragangliomas; tumour

localization (GJTT, VBT, CBT), and data providing an indication about the extension of the tumour and the rate of growth, e.g. the age at first diagnosis, duration of symptoms, and the size of the tumour.
For 20 skull-base tumours (GJTT), the tumour size and FCM were available. According to Rosenwasser[9], three size classes can be recognized:
Small tumours: tumour confined to the middle ear space without extension or tumour in the hypotympanum, usually a glomus tympanic tumour that arises on the promontory or the floor of the tympanum (n=6); tumours of *intermediate* size: the tumour has penetrated beyond the middle ear in the direction of the mastoid and with involvement of the hypotympanum, indicating that the process originated in the jugular bulb. (n=3); and *large* tumours: the tumour shows wide spread extension into the base of the skull or intracranially (n=11).
The volume of the vagal and carotid body tumours (n=74) was calculated from the dimensions given in the histopathological report. Tumour volume ranged from 1 cm^3 to 224 cm^3. Since no established or uniform size classification was available (before and after introduction of the CT scan) for paragangliomas, we arbitrarily subdivided tumour volume into another three size classes each of which covered approximately one-third of the cases. Tumours measuring between 1 and 18 cm^3 (n=33) were called small; those between 18 to 60 cm^3 intermediate (n=32), and those of 60 cm^3 or larger (n=9). In all, 39 tumours were classified as *small*, 35 as *intermediate*, and 20 as large.
For 5 tumours no size had been recorded.

Flow cytometry
The procedures used for cell preparation and the staining of fresh and paraffin-embedded tissue have been described elsewhere[15]. Briefly, suspensions of isolated nuclei were prepared from fresh or frozen tissue specimens according the detergent-trypsin procedure and stained with propidium iodide (PI)[16]. Rainbow trout red blood cells (TRBC) were added to the suspensions of isolated nuclei prepared from fresh or frozen samples as an internal ploidy standard. Frozen and paraffin sections of each tissue block were examined to see whether there was an adequate proportion (>10%) of tumour cells. The pepsin-digestion technique was used to release nuclei from 40-50 μm sections of paraffin-embedded tumour specimens according to Hedly et al[17]. with some minor modifications[18]. Deparaffinized samples were stained with DAPI (4',6,-diamidino-2-phenylindol ; ICP-22 flow cytometer) or PI (FACSCAN flow cytometer). Measurements were made initially with an ICP 22 flow cytometer and later with an FACSCAN flow cytometer (Becton and Dickinson, Mountain View, CA, U.S.A.) with use of the appropriate filter combinations for the excitation of DAPI and PI fluorescence, respectively. DNA profiles produced by the two instruments had a similar resolution and did not show systematic differences. DNA profiles showing only a single G_0/G_1 peak were classified as DNA diploid. The position of the diploid peak in DNA profiles from fresh or frozen samples was verified with aid of the TRBC standard. For DNA profiles from deparaffined samples the most left G_0/G_1 peak was considered to represent the diploid population. Single G_0/G_1 peaks with a coefficient

of variation (CV) of > 5.5, were classified as peridiploid, and DNA profiles showing two or more G_0/G_1 peaks as aneuploid. The peridiploid group may thus contain both tumours from which the high CV has obscured the presence of a near-diploid, aneuploid DNA stemline as well as 'true' DNA-diploid tumours which yield broad G_0/G_1 peaks because of e.g. suboptimal fixation of the paraffin embedded tissue[18]. The overall median CV was 5.7, whereas the median CV for the diploid G_0/G_1 population in aneuploid tumours was 4.5. We consider the latter the better estimate for the quality of the measurements since these consist of normal cell populations. There were 8 fresh and 91 paraffin embedded samples. In several cases, DNA profiles showing a single G_0/G_1 peak with an enlarged G_2M peak were observed. However, no second G_2M fraction was seen at twice the modal channel number of the first (enlarged) G_2M fraction. Because of the uncertainty as to whether these profiles indicated the presence of a true tetraploid DNA stemline, they were classified as peridiploid with enlarged G_2M fraction, and due to the variable quality of the DNA profiles derived from deparaffinized tumour samples, no attempt was made to calculate S-phase fractions.

Statistics
Differences between tumour groups in frequency tables and cross tables were evaluated by the t-test, analysis of variance, and the chi-square test.

RESULTS

Of the 99 tumours, 14 were DNA diploid, 33 peridiploid, and 37 aneuploid. Among the DNA aneuploid tumours, only two had multiple aneuploid DNA stemlines. Fifteen tumours showed a peridiploid DNA profile with an enlarged G_2M fraction. For the statistical analysis used to detect correlations between DNA ploidy and clinical features, DNA diploid and peridiploid cases were grouped together into one near-diploid class.
The distribution of the ploidy classes in the familial and non-familial groups is shown in table 1a. The frequency distribution of DNA indices shows scattering between the diploid and tetraploid ranges (fig 1.). Apart from the fact that more familial tumours were measured, there was no significant difference in DNA index distribution between the familial and non- familial cases.
Individual patients belonging to the same family, showed no tendency to similarity in DNA ploidy pattern. Multicentricity was found predominantly in the familial group of 47 patients, 16 of whom had a double tumour (uni- or bilateral) and one even had three tumours. In the non-familial group (n=30) only 4 patients had a double tumour. The DNA ploidy distribution in double tumours (table 2.) showed that both tumours were DNA diploid or peridiploid in 10 patients and that in three out of five patients with DNA aneuploid tumours, both tumours had almost identical DNA indices. For case no. 48, the second stemline of tumour T1, which DNA index of 1.85 is nearly similar to that of the contralateral tumour T2 (DNA index = 1.79) probably arose via polyploidization of the first, hypodiploid stemline (DNA index = 0.90).

Table 1a.

	ND (Di)	PD+G2↑	AN	Total
Fam	33 (10)	10	22	65
Non Fam	14 (4)	5	15	34
Total	47 (14)	15	37	99

Table 1b.

	ND (Di)	PD+G2↑	AN	Total
GJTT	16 (2)	1	3	20
VBT	5 (3)	2	7	14
CBT	26 (9)	12	27	65
Total	47 (14)	15	37	99

Table 1c.

		ND	PD+G2↑	AN
Age	mean	36	42	39
	s.d.	13	8	14
Duration of symptoms	median	3	4.5	2
	(min;max)	(1;35)	(1;20)	(1;10)

Results of DNA flow cytometry.
(ND = Near-Diploid; Di = Diploid;
AN = Aneuploid; PD + G_2 = Peridiploid with elevated G_2M fraction).

1a) Distribution of 99 tumors with respect to familiality of disease.
1b) With respect to tumor localization (99 tumors).
1c) With respect to age at onset of disease (77 patients) and duration of symptoms (73 patients), both given in

However, this hypodiploid stemline is not present in tumour T2 (fig 2.). In the remaining patients the DNA stemlines of double tumours differed significantly.

DNA ploidy and clinical characteristics

After subdivision according to tumour localization in the head and neck (i.e., GJTT, VBT, CBT), no correlation was found between DNA ploidy and these localisations (table 1b.).

The average age at first diagnosis was available for 77 patients and was somewhat lower for the familial group, but this difference was not statistically significant.

A correlation between DNA ploidy and the age at first diagnosis was not found. The mean age of the patients with DNA diploid tumours was 36 years and that of the peridiploid with elevated G_2M phase and DNA aneuploid stemlines was 42 and 39 years, respectively (table 1c.).

The average duration of symptoms was known for 73 patients. For the duration of symptoms there was a remarkable great difference between the minimum and maximum number of years recorded (table 1c.). Irrespective of ploidy class, the average duration of symptoms amounted to about three years.

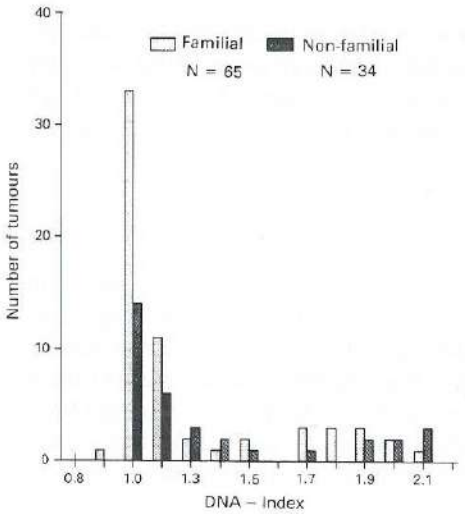

Figure 1. DNA index frequency for familial and non familial paragangliomas (n=99).

Table 2. DNA indices of tumors in patients with multiple tumors. Note that the cases are grouped by ploidy class. DI=DNA index; T1, T2, T3=number of tumors.

	Case No	DI (T1)	DI (T2)	DI (T3)
Diploid	085	1.00	1.00	
	095	1.00	1.00	
	099	1.00	1.00	
	106	1.00	1.00	
Peridiploid	015	± 1.0	± 1.0	
	023	± 1.0	± 1.0	
	028	± 1.0	± 1.0	
	029	± 1.0	± 1.0	
	097	± 1.0	± 1.0	
	100	± 1.0	± 1.0	± 1.0
Aneuploid	017*	1.80	1.95	
	048*	0.90/1.85	1.79	
	107*	1.18	1.19	
	055	1.61	1.10	
	098	1.69	1.16	
Mixed	004	1.82	± 1.0	
	016	1.00	1.58	
	038	1.42	1.00	
	044	1.00	1.66	
	075	± 1.0	1.65	
	096	± 1.0	1.75	

DI = DNA index; T1, T2, T3 = Number of tumours.

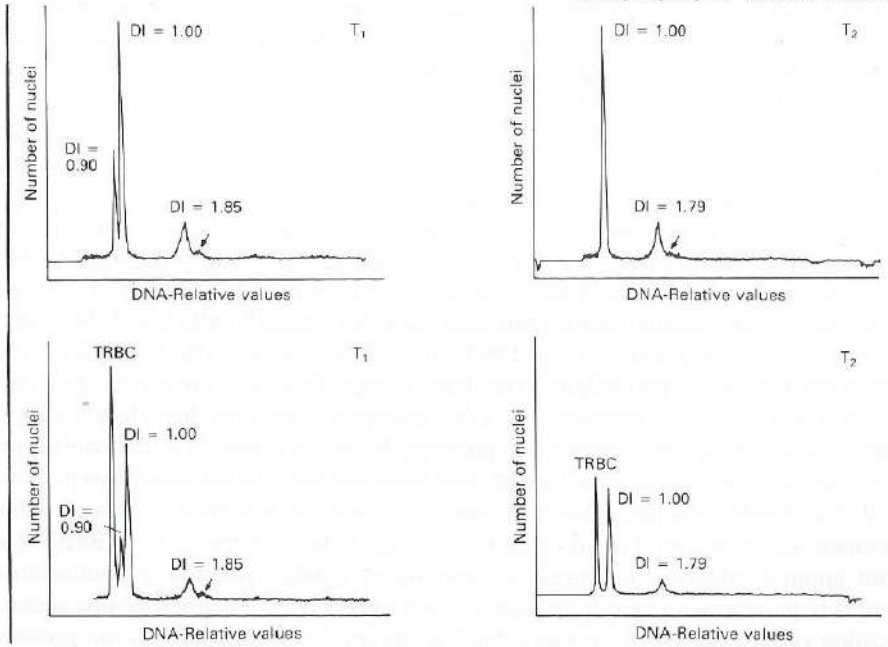

Figure 2. DNA profiles of two carotid body tumors (CBT's) in a single patient (viz. case no. 048).
CBT right-sided T_1 without TRBC-standard; T_1* with TRBC-standard.
CBT left-sided T_2 without TRBC-standard; T_2* with TRBC-standard.

For 94 paragangliomas, DNA ploidy was analysed in relation to tumour size. However, the DNA ploidy distribution for small, intermediate, and large tumours did not differ significantly. When familiality was taken into account with respect to the average age at diagnosis, the duration of symptoms, and the size of the tumour, no significant differences were found.

DISCUSSION

The results of this study show that aneuploid stemlines occur relatively frequently in clinically and histologically benign paragangliomas (37%), indicating that cytogenetically, these tumours represent true clonal proliferations. Although no definite proof, this strongly supports the neoplastic rather then the hyperplastic nature of these lesions as argued by some authors[5,6]. Similar results have recently been reported for a series of 13 carotid body tumours analyzed with DNA-image cytometry by Barnes and Taylor[19] who found abnormal DNA-histograms in 69% of the cases of which 15% were true aneuploid.

DNA aneuploidy has also been described for other benign tumours of neuro-endocrine orgin. In a flow-cytometric study of pituitary adenomas done by Anniko et al[20], aneuploidy was found in 49% of the cases, and Joensuu[21] reported aneuploidy for 29% of pituitary, 25% of thyroid, 26% of parathyroid, and 53% of adrenal adenomas without sign of invasive growth. Others[21,22] have confirmed their findings. Thus, unlike colorectal adenomas, DNA aneuploidy in neuro-endocrine adenomas appears not to be associated with a premalignant condition[23].

A correlation between DNA aneuploidy and the age of the patients with paragangliomas similar to that described by Joensuu[21] for other benign endocrine tumours was not found in the present study.

DNA ploidy distribution was not associated with familiality of the disease, which suggests that tumour-ploidy evolution did not differ essentially between the two categories of patients. In 13/21 (62%) of the patients with a double tumour, the two lesions had similar, predominantly (peri-)diploid, DNA indices (Table 2.). With respect to the relatively low prevalence of DNA aneuploidy, the a priori probability of a combination of two (peri-)diploid stemlines is high. However, the observation that three out of five double tumours with DNA-aneuploid stemlines had closely similar DNA indices at first sight seems more puzzling. In one case (no 107), the confluence of two tumour sites cannot be excluded. This does not hold for the other two patients (no 017 and 048) who had bilateral tumours. In case no 048 there is only a partial agreement since the hypodiploid stemline is lacking in the T2 tumour. Therefore, there is not enough evidence to suggest a non-random ploidy evolution of multicentric tumours in patients with hereditary disease even when a common, predisposing genetic condition can assumed to be present. Such a 'programmed' ploidy evolution process, leading to identical karyotypes with the same specific and non-specific (secondary) chromosomal aberrations and hence identical DNA contents, would be at variance with the concepts on the stochastic nature of DNA ploidy evolution[23-27].

The relatively high proportion (15%) of peridiploid tumours with elevated G_2M fraction compares with the 23% diploid-tetraploid tumours found in the series of Barnes and Taylor[19]. This probably indicates a tendency of paragangliomas to develop genetically relatively stable tetraploid subpopulations which do not or only slowly progress to overt aneuploidy by chromosome loss.

Unlike the situation for several malignant tumours, no relationship was found between DNA ploidy and clinical signs of tumour progression.

Since the life expectancy of patients with paragangliomas does not differ significantly from that of the general population, we attempted to express tumour progression on the basis of parameters such as age at diagnosis, duration of symptoms, and size of the tumor. Since the introduction of CT scanning during the late seventies, it has been possible to make accurate and standardized estimations of tumour extension. In our retrospective study covering a period of 32 years, CT scans were not available for all of the patients and therefore paraganglioma size had to be classified semi-quantitatively, i.e. less objectively, particularly for skull-base tumours.

The absence of correlation between DNA ploidy and clinical signs of tumour progression indicates that at least at present, analysis of the DNA content is of no help in reaching a clinical decision as to whether or not extensive surgery should be performed in cases of young patients with paragangliomas. A similar conclusion was reached by Barnes and Taylor[19].

It remains an intriguing problem, that a variety of tumours of neuro-endocrine origin, including paragangliomas, can develop a quite pronounced DNA aneuploidy, that is indicative of numerical and probably also structural chromosomal aberrations, without showing overt signs of clinical malignancy. Recently, the presence of somatostatin receptors have been demonstrated in paragangliomas[28]. The presence of these receptors in other neuro-endocrine tumours and breast tumours with neuro-endocrine characteristics appears to be associated with a differentiated type of tumour with rather low malignancy[29]. One could hypothesize that the high somatostatin receptor content of paragangliomas may have some relationship with their usually indolent biological behaviour.

Lastly, our results show that in contrast with the situation for colorectal adenomas[25], genetic predisposition does not lead to a higher incidence or more extensive development of aneuploidy in paragangliomas.

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CHAPTER 5

IMMUNOHISTOCHEMICAL ANALYSIS OF HEAD AND NECK PARAGANGLIOMAS

(a series of 175 glomus tumors seen in a 32-year period)

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ABSTRACT

Immunohistochemical analysis was performed on 114 head and neck paragangliomas from 68 of which the disease was hereditary. These tumors were the subject of one of the five largest clinical studies in the literature dealing with 175 glomus tumors (108 patients) seen in a 32-year period. A broad panel comprising eight chief cell markers (PGP 9.5, NSE, Leu-Enkephalin, Met-Enkephalin, Chromogranin, Serotonin, Somatostatin, HNK-Leu 7) and two sustentacular cell markers (S100, GFAP) were applied to paraffin embedded slides. We were able to distinguish between chief cells and sustentacular cells on the basis of their marker specificity but did not detect any significant correlation between immunohistochemical reactivity and clinical criteria indicative of tumor progression, DNA-ploidy, or tumor localization. In contrast with other studies, a relative decrease in the staining of sustentacular cells was not indicative of recurrent and/ or locally aggressive tumor behavior. More specifically, the distribution of S100-positive cells was, for all head and neck tumor sites, irrespective of their clinical outcome, equal to that of aggressive and/or malignant cases. This observation led us to formulate the hypothesis that the underlying tumor-biological cause of the clinically different behavior of head and neck paragangliomas is not reflected by diminished differentiation as might be reflected by decreased production of tumor associated antigens or hormones.

INTRODUCTION

Paragangliomas (*syn.*: glomus tumor, chemodectoma) are rare, predominantly benign hypervascular tumors originating from the tiny glomus bodies which occur throughout the body. They belong to the extra-adrenal paraganglion system and are composed mainly of chief (type I) cells, containing the dense-core neurosecretory granules and in smaller numbers sustentacular (type II) cells, which are situated peripherally.
Microscopically a glomus tumor imitates the normal glomus body structure with the presence of clusters of tumor cells (*Zellballen*) interspersed among an extensive capillary network which can best be visualized by silver impregnation of the reticulin fibres[1].
The clinically most relevant paragangliomas in the head and neck are the carotid body tumor (CBT), the glomus jugulo-tympanicum tumor (GJTT), and the vagal-body tumor (VBT), named after their site of origin.
These tumors can also occur as an autosomal dominant hereditary disease for which our group has shown that genomic imprinting may account for the finding that tumor expression was restricted to inheritance via the paternal line[2]. Multicentricity (at least 30% of cases), either uni- or bilateral, is an important feature of hereditary glomus tumors[3].
In general, the growth pattern of these benign tumors is characterized by extremely slow progression and associated with a low mortality rate. Sometimes, however, the tumor develops rapidly with intracranial extension threatening a number of functions

or even the life of a patient. The morbidity is related not only to the variable growth pattern, but also to the mode of treatment applied. Especially for tumors located at the skull base, there is a considerable risk that morbidity will increase significantly as a result of surgical intervention[4]. However, many authors still consider surgery as the treatment of choice[5].
These reports underscore the need for a tumor-biological marker that gives information about the clinical behavior of paragangliomas, since routine histological evaluation does not provide such information[6]. In a recent study we attempted to find out whether flow cytometric tumor ploidy determinations could promote to discriminate between clinically indolent and aggressive tumors. Although 37% of the tumors showed frank aneuploidy supporting views concerning the neoplastic nature of these tumors, no association with the clinical course of the disease was found[7]. In a study done with immunohistochemical techniques, Kliewer et al.[8] found that a low proportion of type II cells was correlated with aggressive behavior and malignant degeneration, which was measured as a decrease or absence of the type II cell specific antigens S100 and GFAP mainly observed in GJTT's.
The present study was performed to detect any correlation between a specific pattern of immunohistochemical staining and the clinical outcome in large-scale series of 114 head and neck glomus tumors in 68 of which the disease was hereditary. Furthermore, we wanted to know whether GJTT differs biologically from other paragangliomas, and therefore looked for a clue to the clinically less favorable outcome observed in GJTT's as compared with other head and neck glomus tumors.
Finally, we used a broad panel of markers to compare antigen expression and the DNA ploidy status of the tumor was made to obtain insight into the relation between production of immunohistochemical markers and DNA-ploidy.

MATERIALS & METHODS

Patients
During the past 32 years (1956-1988), the diagnosis glomus tumor was made in 108 patients seen in the Departments of Otolaryngology and Surgery of the Leiden University Hospital. A positive family history was found in 58 cases (53%). The clinical data used in this retrospective study were retrieved from the status reports. For most of the cases they supplied information on the following items: family history of paragangliomas; tumor localization (GJTT, VBT, CBT); and data providing an indication about the extension of the tumor and the rate of growth, e.g. age at first diagnosis (average: 39 years), duration of symptoms (average: 3 years), and the size of the tumor.

The size of the temporal bone glomus tumors (GJTT) was estimated according the following semiquantitative categories defined by Rosenwasser[9]: *small* tumors, i.e. those confined to the middle ear space without extension in the hypotympanum. These are usually GTT's arising from the promontory or floor of the tympanum (n=6). Tumors

of *intermediate* size have penetrated beyond the middle ear in the direction of the mastoid and with involvement of the hypotympanum. This indicates that the process originated in the jugular bulb (n=3). Finally, *large* tumors show considerable extension into the base of the skull or intracranially (n=11).

The volume of the VBT's and CBT's (n=74) was calculated from the dimensions given in the histopathological reports[10]. Tumor volume ranged from 1 to 224 cm^3. Since no established or uniform classification was available before the introduction of the CAT scan, we arbitrarily subdivided tumor volume into three size classes, each of which covered approximately one-third of the size ranges. Tumors measuring between 1 and 18 cm^3 (n=33) were called *small*; those between 18 and 60 cm^3 *intermediate* (n=32), and those of 60 cm^3 or more *large* (n=9).

The 108 patients accounted for 52 GJTT's, 32 VBT's and 91 CBT's, giving a total of 175 glomus tumors. Multicentricity was present in 25 patients (i.e., 22 patients had two tumors; 3 patients even had three tumors). The diagnosis paraganglioma was histologically confirmed in almost all of the 132 excised tumors. The present study was limited to the 114 paraffin-embedded tissue blocks that were available for immunohistochemical analysis (35 GJTT, 14 VBT, and 65 CBT found in 87 patients in 47 of which the disease was familial.

No histological signs of malignancy or clinical evidence of distant metastases were found in any of these cases. Prior to surgical removal, none of the tumors had been irradiated and none showed evidence of vasoactivity.

Immunohistochemistry

Immunohistochemical staining was done for the ten antigens that were most frequently mentioned in the literature as markers for paragangliomas.

For the antigen detection with the polyclonal antibodies we used a two step method. In short, the following staining procedure was used: 5 μm thick sections were cut from formalin-fixed and paraffin-embedded tissues. Sections mounted on slides were deparaffinized in a series xylene, via alcohol to water. Endogenous peroxidase activity was inhibited with 0.3% H_2O_2 in water for 20 min at room temperature. After being washed with PBS, the sections were incubated with 100μl PBS/BSA1%/NGS10% (Normal Goat Serum buffer) for 10 min Excess NGS buffer was tipped off before the sections were incubated with 100 μl of the primary antibody diluted in NGS buffer. After overnight incubation, sections were rinsed with PBS and incubated with NGS buffer. NGS buffer-incubated sections served as negative controls. Appropriate tissue sections served as positive controls.

After 10 min., NGS buffer was tipped off and 100μl of peroxidase-conjugated swine-anti-rabbit-Ig-antibody (P217, Dakopatts, Denmark), both diluted in NGS buffer supplemented with 10% NHS, was add to the sections before incubation for 60 min. After three washes with PBS, the sections were washed once with 0.1 M sodium acetate buffer (pH 5.0) for 5 min. The staining reaction was then performed with a freshly prepared solution of 3-amino-9-ethylcarbazole (A-5754, Sigma, USA) in 0.1 M sodium acetate buffer (pH 5.0) containing 0.03% H_2O_2. The reaction was blocked by rinsing

the sections in demineralized water. Sections were counterstained with hematoxylin and mounted with Kaiser's glycerol gelatin (9242, Merck, BRD).

For the monoclonal antibodies, Leu-7 and GFAP a three-step method was applied. In this procedure, marker sections were incubated with peroxidase-conjugated swine-anti-rabbit-Ig-antibody (P217, Dakopatts, Denmark) after incubation with peroxide-conjugated rabbit-anti-mouse-Ig-antibody (P161, Dakopatts, Denmark).

For optimal dilution, all primary poly or monoclonal antibodies (table 1.) were tested on appropriate formalin-fixed and paraffin-embedded tissue sections, before tumor examination.

Table 1. Characteristics of the immunohistochemical markers used.
MCI = monoclonal CC = chief cell marker
PCI = polyclonal SC = sustentacular cell marker

ANTIGEN			DILUTION	CONTROLS	SOURCE	
PGP 9.5	PCI	CC	1:800	carcinoid	UltraClone	RA95101
NSE	PCI	CC	1:200	carcinoid	Dakopatts	A589
Leu-Enkephalin	PCI	CC	1:250	adrenal	Sorin Biomed	20066
Met-Enkephalin	PCI	CC	1:250	adrenal	Sorin Biomed	20065
Chromogranin A	PCI	CC	1:400	melanoma	Dakopatts	A430
Serotonin	PCI	CC	1:1000	carcinoid	Sorin Biomed	20080
Somatostatin	PCI	CC	1:800	pancreas	Dakopatts	A566
HNK-Leu 7	MCI	CC	1:20	adrenal	B-D	7390
S100	PCI	SC	1:800	carcinoid	Dakopatts	Z311
GFAP	MCI	SC	1:20	brain	Dakopatts	M761

The following markers were used to identify chief cells that are of neuroectodermal origin:

(1) enzymatic markers: NSE (neuron-specific enolase);
(2) granule-specific proteins: HNK-1 (anti-Leu-7) which reacts with the cell surface of the natural killer cells and chromogranin;
(3) indolamines: serotonin;
(4) neuropeptides: eutopic Leu-Enkephalin, Met-Enkephalin, and ectopic somatostatin;
(5) a non-granule-dependent protein of unknown function: PGP 9.5.;

The sustentacular cells are glia-like mesodermal structures and can be identified by the use of S100 protein (a calcium-binding protein of unknown function) and the cytoskeletal protein GFAP (glial fibrillary acidic protein).

This panel of markers was also used on 10 physiologic glomus bodies derived by autopsy from the carotid bifurcation.
All immunohistochemical slides were reviewed and scored independently (without knowledge of the clinical data) by three of the authors (A.G.M, W.C, and G.J.F). Immunohistochemical reactivity for PGP 9.5, NSE, Leu-Enkephalin, Met-Enkephalin, Chromogranin A, Serotonin, Somatostatin, and HNK-leu 7 was graded semiquantitatively, on basis of the percentage of cells that were stained. Absent reactivity was graded as negative (-), moderate (1-50%) cell reactivity was graded as +, and extensive (>50%) cell reactivity was graded as ++. Sustentacular cell immunoreactivity (antibodies: S100 and GFAP) was determined semiquantitatively as cell density per unit area relative to the maximum obtainable amount as described elsewhere by Kliewer[11]: absent reaction was graded as (-), moderate (1-25%) reactivity was graded as +, extensive reactivity (26-50%) as ++.

Flow cytometry
The procedures and terminology used in the flow-cytometric analysis of the material has been described elsewhere[7]. In short, tumors were classified as DNA-diploid (n=14), DNA peridiploid (n=33), and DNA-aneuploid (n=37). The peridiploid category includes tumors with an abnormally elevated G2M fraction as well as 'wide CV diploid' tumors.

Statistics
For the association between variables we used Pearson's correlation coefficients. For all tumor markers, the Mann-Whitney U or Kruskal-Wallis test was used to compare the age of onset of disease, the duration of symptoms, familiality of disease, tumor size, and DNA ploidy status in the categories 'absent', 'moderate', and 'extensive'.

RESULTS

The results of the semiquantitative evaluation of the immunohistochemical staining reaction for paragangliomas are detailed in table 2. Extensive immunoreactivity for chief cells was found for the following markers: in order of decreasing frequency PGP 9.5, NSE, Leu-Enkephalin, Met-Enkephalin, Chromogranin and Serotonin.
Somatostatin and HNK-Leu 7 gave less staining than the other markers (see fig 1-4.).
We regularly observed a marked heterogeneity in staining intensity in different parts of a single tumor as well as an often 'patchy' distribution of positively reacting cells. This patchiness was present mainly in slides stained with NSE and Met-Enkephalin. These differences were similarly encountered between tumors, but neither effect proved to be related to aggressive tumor behavior. Sustentacular cells stained exclusively with S100 and GFAP, but the number of cells and intensity was generally smaller than that for antigens of chief cells. The majority of tumors was GFAP-negative.

Table 2. Results of immunohistochemical staining in 114 head and neck paragangliomas for the 10 markers used. The missing slides* (0-7 for each marker) were of such an inferior quality that reliable scoring was impossible.

MARKER	IMMUNOREACTIVITY			TOTAL*
	absent	moderate	extensive	
PGP 9.5	2	14	91	107
NSE	12	19	79	110
Leu-Enkephalin	21	24	65	110
Met-Enkephalin	30	34	48	112
Chromogranin A	28	41	41	110
Serotonin	49	22	39	110
Somatostatin	87	26	0	113
HNK-Leu 7	94	19	1	114
S100	17	86	7	110
GFAP	74	9	30	113

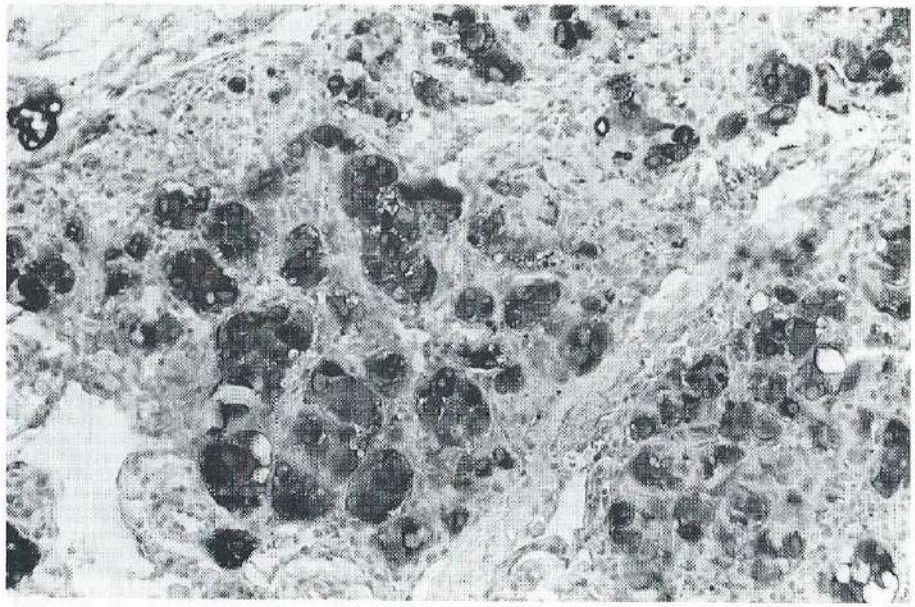

Figure 1. Clusters of chief cells with extensive NSE immunoreactivity in a CBT.

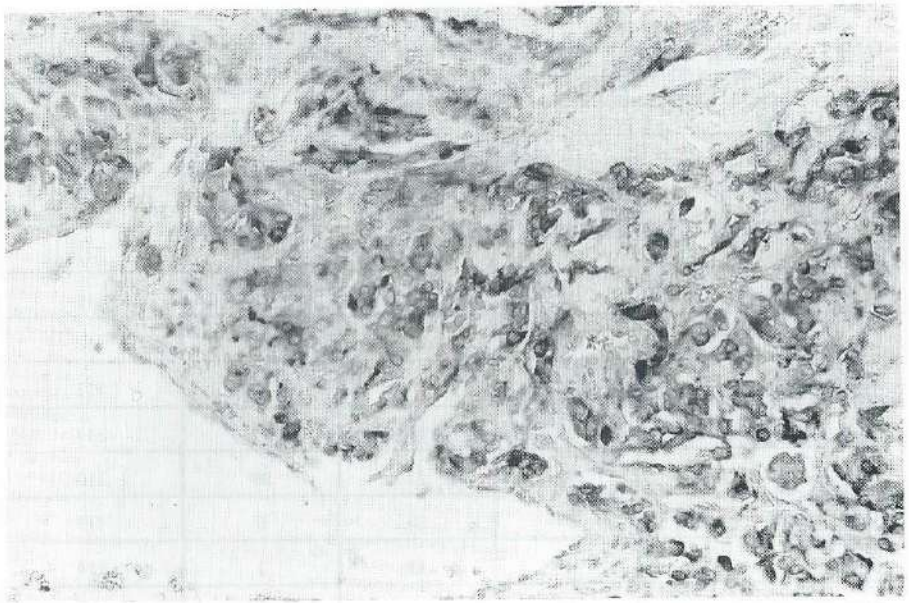

Figure 2. Extensive heterogenous staining for the chief cell marker Leu-Enkephalin in a GJTT.

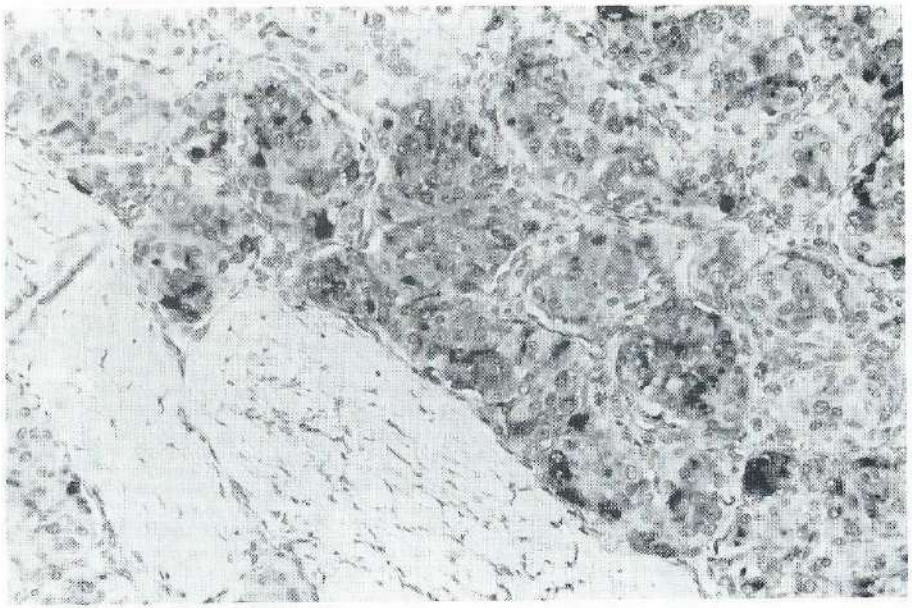

Figure 3. 'Patchy' distributed staining for chromogranin (CBT); clusters of chief cells with and without staining are clearly demonstrated.

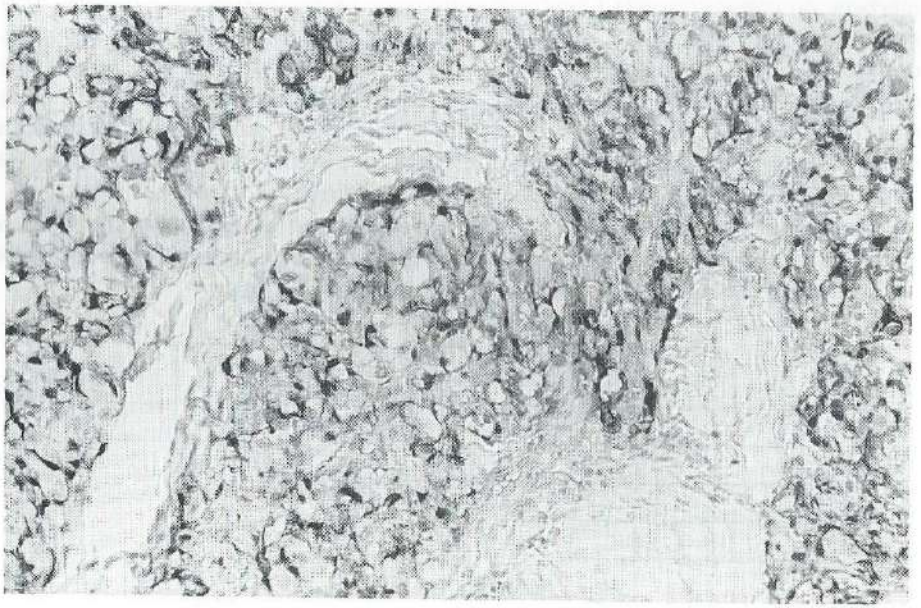

Figure 4. Extensive sustentacular cell staining for S100 (VBT).

The interrelation between the expression of the various markers is shown in table 3. For the sake of clarity only, associations with a Pearson's correlation coefficient exceeding 0.40 are indicated. Markers, which demonstrated almost always extensive (PGP 9.5) or absent staining (Somatostatin, HNK-Leu 7) are not included in this table, i.e., showed no correlation with the other markers, because of their skewed distribution. The expression of the three chief cell markers NSE, Met-Enkephalin, and Leu-Enkephalin showed correlation. No significant correlation was found between the sustentacular cell markers GFAP and S100. Remarkably, a relatively high degree of correlation was found between the sustentacular cell marker S100 and three chief cell markers.

Eight (8/10) 'glomus bodies' could be used to demonstrate staining for the CC-markers: NSE, Chromogranin, and PGP 9.5 resp. 95%, 20%, and 90%; for the SC-marker S100 staining was found in 100%. The other six markers did not show immunohistochemical staining in glomus bodies.

Immunohistochemical findings and clinical characteristics

Familiality of disease (68 tumors; 47 patients) showed no association with any of the investigated tumor markers. Interestingly, no correlation was found between the immunoreactivity of multicentric tumors (22 patients).

S100 and GFAP reactivity showed a very weak positive correlation with age of onset of disease ($r = 0.15$, $p = 0.06$ and $r = 0.19$, $p = 0.02$, respectively). For the other

markers, no correlation was found with either age of onset of disease or duration of symptoms. Met-Enkephalin and S100 immunoreactivity showed a weak inverse correlation with tumor size ($r = -0.21$, $p = 0.013$ and $r = -0.28$, $p = 0.0016$, respectively). We did not detect any significant difference between the staining characteristics of GJTT's, CBT's, and VBT's. In fact, all three tumor localizations showed no staining or moderate staining intensity with S100. For GFAP no staining was found in 65% of tumors, irrespective of the localization.
The correlation coefficient between marker expression and tumor ploidy did not exceed 0.30. The same holds when tumor ploidy is subdivided into two instead of three groups, i.e., when peri-diploid tumors are included in the diploid group.

	Serotonin	S100	Met-Enkephalin	Leu-Enkephalin	Chromogranin
NSE	-	0.44	0.41	0.40	0.44
Leu-Enkephalin	-	0.47	0.55		
Met-Enkephalin	-	0.45			
GFAP	0.40				

Table 3. The correlation coefficients between the immunoreactivity of the various markers. Only values of 0.40 or above ar shown ($p<0.0005$).

DISCUSSION

For paragangliomas (glomus tumors) it is controversial whether or not both type I and type II cells are proportionally present. Some authors have reported that proliferation of type II cells is the main feature of the carotid-body hyperplasia occurring in Peruvians living at high altitude[12,13]. On the other hand, a decrease in the proportion of type II cells has been claimed as a marker for malignant progression of tumors of the paraganglion system, i.e., pheochromocytoma[14] and paragangliomas.
Use of a panel of antibodies rather than a single marker to evaluate cellular composition by Kliewer[11] showed the cytologic heterogeneity of paragangliomas by which a decline in the relative proportion of type II cells was correlated with aggressive behavior and possibly genesis of metastases.
In the present study on head and neck paragangliomas we chose a panel of markers most frequently used (NSE, Chromogranin, Met-Enkephalin, S100 and GFAP) and added five other markers previously mentioned in the literature on paragangliomas[15-20]. Our results support the concept of a dual-cell population of the paraganglion system, and confirm the presence of large numbers of chief cells on the basis of their morphology and immune reactivity for the established chief cell markers such as NSE, Chromogranin, Met-Enkephalin, and Leu-Enkephalin. The staining for Serotonin was comparable, whereas PGP 9.5 gave more pronounced chief cell staining. In our series, Somatostatin and HNK-Leu 7 were inferior markers which were frequently absent, in contrast to the findings of other authors[17,21].

Surprisingly was that, at least in glomus bodies at the carotid bifurcation, we could only demonstrate staining for NSE, PGP 9.5, Chromogranin, and S100. The other six markers showed no staining which demonstrates that during proliferation, the glomus cells develop the ability to produce hormones and enzymes which were not present in the physiologic situation.
With the described set of markers we failed to detect any distinct correlation between the observed variety in staining patterns and tumor localization or clinical signs indicative of tumor progression, i.e., early age at first diagnosis, short duration of symptoms, or size of the tumor. This means that in our material (including 59 hereditary cases), at least at present, the clinical behavior of paragangliomas could not be predicted from a specific pattern of immunohistochemical staining.
It is possible that the occurrence of a patchy staining pattern reflects loss of differentiation or extreme fluctuations of antigen production. Both might indicate a more aggressive phenotype. Our results do not, however, support this hypothesis, since the patchiness was observed as often in cases with a clinically favorable outcome as it was in more serious cases.
Because of the largeness of our series ($n=114$) the semiquantitative grading of all markers was based on the percentage of cells that stained rather than the intensity of staining as done by others. For type-I markers, this difference hampers a realistic comparison of the results obtained in various series. Moreover in these slow growing tumors, the definition of recurrent and locally aggressive disease is usually arbitrary which also complicates comparison.
The presence of sustentacular cells in the majority of tumors was established in 85% of cases by positive staining (percentage of cells) for S100. However, a clear cut divergence in the staining of S100 and GFAP was found: in many cases S100 gave extensive staining, whereas GFAP was expressed by only 35% of the tumors. This is far less than the 90-95% GFAP positive reaction in benign and multifocal tumors reported by Kliewer[11]. He found in 12 head and neck paragangliomas (CBT, VBT, and GJTT) a low level of both GFAP and S100 expression in recurrent and locally aggressive tumors while we (in a series of 110 paragangliomas of the head and neck) with exactly the same dilution and panel of antibodies observed moderate staining for GFAP in all three head and neck tumor localizations and were not able to demonstrate any significant correlation with tumor site or clinical behavior. Our criteria for clinical (aggressive) behavior are given in the materials and methods of this paper. The possibility that the differences in staining are due to fixation, is unlikely, however can not be excluded.
Some studies noted that the apparent GFAP negativity of type II cells may arise from considerable biochemical and antigenic dissimilarity between proteins isolated from the central and peripheral nervous systems[8,22,23].
The distribution of the staining pattern for S100 in our series was in agreement with the results of Kliewer et al. for GJTT's: the great majority of these tumors (22 out of 32) showed a moderate staining, while extensive staining was just observed once. If Kliewer's '1+' and '2+' staining are considered to be comparable to our term

'moderate' and '3+' and '4+' are equivalents to our term 'extensive', they found 10 out of 11 GJTT's with moderate staining, while their series did not contain any case of extensive S100 staining in GJTT's.

A remarkable difference with the findings of Kliewer[11], is the fact that in the present series the distribution of S100 staining was similar to the pattern described for GJTT's, for CBT and VBT localizations, indicating a similar cellular composition of all three head and neck paragangliomas. Lloyd[14] correlated a similar S100 staining pattern of pheochromocytomas with malignant degeneration. One might hypothesize that head and neck glomus tumors are biologically different from paragangliomas elsewhere in the body. An argument in favor of this concept is the fact that head and neck paragangliomas seldom lead to clinically manifest syndromes due to catecholamine secretion, as contrasted to abdominally located paragangliomas including pheochromocytomas[24].

Obviously, there are also great clinical differences between paragangliomas located in the head and neck and their thoracic and abdominal counterparts. As was already mentioned, recurrent and/or locally aggressive paragangliomas are not a rare entity in the head and neck. In our series of 175 head and neck paragangliomas in 108 patients we demonstrated that the results of radical surgical treatment over the last 32 years are disappointing for tumors located at the skull base i.e., GJTT's. We found 59% local recurrences at this specific anatomic localization[4].

Also with regard to proven malignancy there are clear differences in clinical behavior of paragangliomas of different sites. Death due to metastatic disease occurs in up to 42% of cases of retroperitoneal paragangliomas[25] whereas this is relatively seldom the case in CBT's[1]. The capacity of paragangliomas to produce certain hormones and enzymes does not give an explanation for the observed differences in clinical behavior. Further studies are necessary to investigate the tumor biological origin of clinical differences between the three paragangliomas of the head and neck and those localized in the thorax or abdomen.

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CHAPTER 6

I-123 METAIODOBENZYLGUANIDINE SCINTIGRAPHY IN PATIENTS WITH CHEMODECTOMAS OF THE HEAD AND NECK REGION

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ABSTRACT

While studying the uptake of Iodine-123 metaiodobenzylguanidine (^{123}I MIBG) in chemodectomas, we coincidentally detected catecholamine secreting tumors in five out of fourteen patients. In three of these cases a norepinephrine secreting abdominal paraganglioma was subsequently removed. One patient had a norepinephrine secreting chemodectoma and one had a dopamine secreting chemodectoma. Prior to ^{123}I MIBG imaging and urinary catecholamine measurements, endocrine activity was suspected in only one of these five patients. Apart from these five cases, two other patients showed elevated catecholamine secretion and abnormal abdominal ^{123}I MIBG concentrations. However, these two patients were not surgically explored, because of normal computed tomographic (CT)- and magnetic resonance (MRI) studies. We suspect that catecholamine secreting tumors are more common in patients with chemodectomas than is assumed in the literature and we therefore recommend urinary catecholamine screening for all patients with chemodectomas. In case of elevated catecholamine secretion, MIBG scintigraphy is indicated.

INTRODUCTION

Chemodectomas, also known as glomus tumors, arise from paraganglionic tissue in the carotid body, the jugular fossa, the middle ear and superior mediastinum. Chemodectomas are extremely rare and of unknown incidence[1]. About 30% of the cases are familial in origin. The heriditary pattern of familial chemodectomas appears to be autosomal dominant[1]. Multiple chemodectomas occur in approximately 25% to 35% of the patients with familial chemodectoma but in less than 5% of those with the non-familial type[2]. Malignancy occurs in approximately 10%[3]. Together with the aortico-sympathetic, visceral autonomic and intravagal paragangliomas and pheochromocytomas they form a class of tumors known as paragangliomas[4]. Paraganglion cells are derived from the neural crest and migrate in close association with the autonomic ganglion cells. The common feature of these cells is the presence of numerous neurosecretory granules containing catecholamine in their cytoplasm. The highest concentration of these paraganglion cells is in the adrenal medulla, however they are also found in abundance along the aorta and great vessels[7,8].
Functioning paragangliomas may arise wherever paraganglion tissue exists but are called pheochromocytomas when they develop in the adrenal gland[4]. The proportion of hormonally active paragangliomas is thought to be high for adrenal pheochromocytomas, intermediate for aortico-sympathetic and visceral-autonomic paragangliomas and low for chemodectomas[9]. The percentage hormonally active chemodectomas has been estimated to be about 1%[10]. The association of head and neck chemodectomas with other paragangliomas (usually pheochrocytomas) as well as several other tumors (carcinoid, pituitary adenoma, thyroid carcinoma) derived from the neural crest has been reported[2,9,11-18], but these combinations are considered to be very rare[16].

The uptake of radioactive labeled metaiodobenzylguanidine (MIBG) has been demonstrated in pheochromocytomas[19] and neuroblastomas[20] as well as carcinoids[21], medullary carcinomas of the thyroid and chemodectomas[22-24]. The largest study on chemodectomas so far comprised five patients, imaged with ^{131}I MIBG[22]. We performed a ^{123}I MIBG study in 15 patients with a total of 24 chemodectomas. Urinary catecholamines and vanillylmandelic acid (VMA) levels were measured for correlation with the findings of ^{123}I MIBG imaging. Patients with elevated urinary catecholamine secretion and/or unexpected ^{123}I MIBG concentrations outside the head and neck region underwent further investigations to detect the source of catecholamine production.

METHODS

Between January and November 1988 fifteen patients, ranging in age from 22 to 65 years (mean age 45 years) were referred for endocrinological analysis and ^{123}I MIBG scintigraphy by the Department of Otolaryngology. All patients gave informed consent. In the head and neck region of these patients twenty-four chemodectomas were present: eleven jugular glomus tumors, seven carotid body tumors and six vagal body tumors. In this series no tympanic glomus tumors were seen. Nine patients were followed because of incomplete tumor removal, attributable mainly to the technical limitations encountered in the safe excision of these tumors from the cranial base. Four patients were analysed preoperatively and in two cases surgery was either not performed or not planned, for technical reasons.
The patients exhibited the common presenting symptoms of the carotid and vagal body tumors, with a painless pulsating lateral cervical mass near the angle of the mandible. The glomus jugulare tumors gave rise to aural symptoms, such as conductive hearing loss, pulsating tinnitus, a discoloured eardrum and sometimes cranial nerve palsy. In all 15 patients the diagnosis of chemodectoma was based on these clinical symptoms and characteristic findings on angiograms, as described elsewhere[3]. Except for patient no. 10 histological confirmation of the chemodectoma(s) was present in all patients. Twelve cases were familial, involving nine kindreds. Ten patients showed multiple chemodectomas on angiography. In two of these ten patients, one or more chemodectomas had already been removed prior to scintigraphy. A list of all drugs recently used was obtained to rule out interference with ^{123}I MIBG uptake; special attention was paid to drugs as reserpine, tricyclic anti-depressants, phenylpropanolamine, labetolol and sympatholytic agents[25].

Scintigraphy
Each patient was injected intravenously with 10 mCi ^{123}I MIBG while in the supine position. ^{123}I MIBG was obtained from 'Cygne' bv (Eindhoven, The Netherlands). The synthesis of ^{123}I MIBG was performed by the method of Wieland et al[26]. ^{123}I was produced by the 124Xe (p,2n) reaction with specification of a maximum ^{125}I impurity of 0.01% at the time of calibration. ^{123}I MIBG (specific activity at least 25 mCi per

milligram) was diluted in bacteriostatic phosphate buffer (pH 6.0-6.5) to a specific concentration of at least 2.0 mCi/ml. The free iodine concentration was less than 0.2%. Thyroidal uptake was blocked by the administration of Lugol's solution, ten drops three times daily (50 mg of iodine) for five days, starting the day before the injection.
A large field-of-view gamma camera (Toshiba GCA 90B, Toshiba, Tokyo, Japan) equipped with a low energy general purpose collimator and interfaced to a dedicated computer (Toshiba GMS-55, Toshiba, Tokyo, Japan) was used. A 20% window was centred at 159 KeV. In all cases anterior and posterior digitized images of the total body and four images of the head and neck were obtained 24 hr and, in most cases, 48 hr after the injection. Additional single photon emission computer tomography (SPECT) of the head and neck of all patients was performed 24 hr after the injection. From the SPECT study transaxial, sagittal and coronal slices, 5.3 mm thick, were reconstructed.

Computer tomography
Seven patients with elevated catecholamine levels (no.1,3,6,7,10,12 and 14) of whom five had abnormal ^{123}I MIBG concentrations outside the head and neck region were subsequently examined with a Tomoscan 350 (Philips, Best, The Netherlands) scanner. Initial screening of the abdomen was performed using 10 mm thick adjacent slices. If this routine scan was equivocal or negative, a more meticulous examination of the region with abnormal ^{123}I MIBG uptake was carried out. Such a procedure included narrow collimation in combination with geometric enlargement and back projection magnification. Care was taken that the entire region was visualized and that no major anatomical gaps were introduced by inconsistent expiration between individual slices. In three cases the CT examination was repeated after intravenous injection of 50 cc meglumine ioxitolamate (Telebrix 350, Guerbet, Aulnay-sous-bois, France).

Magnetic resonance imaging
Patients no.1,3,6,7,10,12 and 14 were examined at 0.5 T using a Gyroscan-S5 (Philips, Best, The Netherlands) scanner. In all cases a body coil was used. Imaging technique included multisectional acquisition of the adrenal area with 1 cm-thick transverse slices, intersection gaps of approximately 1 mm, an acquisition matrix of 179 x 256 and a display matrix of 256 x 256. The field of view was 400 mm. Patients were examined with a spin echo sequence TR300/TE 20 and a spin echo sequence TR2000/TE 50-100. After imaging of the adrenal area, T2- weighted coronal images of the lower abdomen and mediastinum were taken in two series using a field of view of 500 mm. If this routine scan was equivocal, a more meticulous examination of the area of interest was carried out using transverse T1 and T2-weighted images. In all cases plane resolution was less than 3 mm. The number of measurements per data line was two (T2-weighted sequences) or six (T1-weighted sequences). Software for motion compensation was not used. Total procedure time varied from 1.5 to 2 hours.

Catecholamine measurements
The urinary excretion of free norepinephrine, epinephrine, dopamine and vanillylmandelic acid was assessed in 24-hour urine samples collected on three consecutive days. Free norepinephrine, epinephrine and dopamine levels were assayed by high performance liquid chromatography (HPLC) and electrochemical detection (Coulochem 5100 A ESAR). VMA levels were measured by colorimetry after paperchromatography. For four patients (no.3,6,7 and 14) the levels of norepinephrine, epinephrine and dopamine in the tumor tissue were determined by the HPLC method and expressed as mmol/g tumor tissue.

RESULTS

Fourteen of the fifteen patients completed the study. One patient (no.5) underwent the clinical examination and scintigraphy but failed to supply the 24 h urine samples (lack of cooperation). The clinical examinations in the fifteen patients rendered the following additional information. Only one of the fifteen patients (no.14) had a history, that was indicative of a functioning paraganglioma, i.e. hypertension, episodic headaches, palpitations and heavy perspiration. This had not been recognized for more than 20 years.
On physical examination five patients (no.1,8,9,10,14) had hypertension, according to the definition of the W.H.O[27]; only one patient (no.14) received medication (verapamil chloride) for it. The other patients had no signs or symptoms suggesting a hormonal active tumor. None of the patients experienced any side effects of the ^{123}I MIBG injection. table 1 lists the patient data, the scintigraphic findings and the urinary levels of free norepinephrine, free epinephrine, dopamine and VMA. ^{123}I MIBG uptake in one or more chemodectomas in seven patients (no.1,2,8,10,13,14 and 15) was visualized on planar views and SPECT images. In one patient (no.9 ~fig 1.) ^{123}I MIBG uptake by a chemodectoma was detected only by SPECT.
In total 13 of the 24 chemodectomas were visualized. Neither the planar views nor the SPECT images revealed any uptake in the chemodectomas of the other seven patients. For each patient showing ^{123}I MIBG uptake all known chemodectomas were visualized. Most chemodectomas showed low to moderate uptake of ^{123}I MIBG. Only patient no.1 showed a high uptake in a sporadic chemodectoma which proved to be a norepinephrine-secreting chemodectoma.
In five patients (no.3,6,10,12 and 14) abnormal locations of ^{123}I MIBG were noted in the abdomen. Only one of them (no.14) showed simultaneous moderate ^{123}I MIBG uptake in a chemodectoma (fig 2.).
Seven patients (no.1,3,6,7,10,12 and 14) with elevated catecholamine levels of whom five had abnormal ^{123}I MIBG concentrations in the abdomen underwent further examination. The results of the CT and MRI investigations in these seven patients and the histopathological data are shown in table 2. Patients no.3 and no.6 had a pheochrocytoma of the left adrenal gland that was excised under labetolol prophylaxis without adverse effects. In patient no.14 an aortico-sympathetic paraganglioma was

removed. Histologically these tumors resembled the paraganglionic tissue found in pheochrocytomas. The tumors contained only norepinephrine. In all three cases (no.3,6,14) cathecolamine secretion normalized postoperatively. Patient no.14 became free of symptoms.

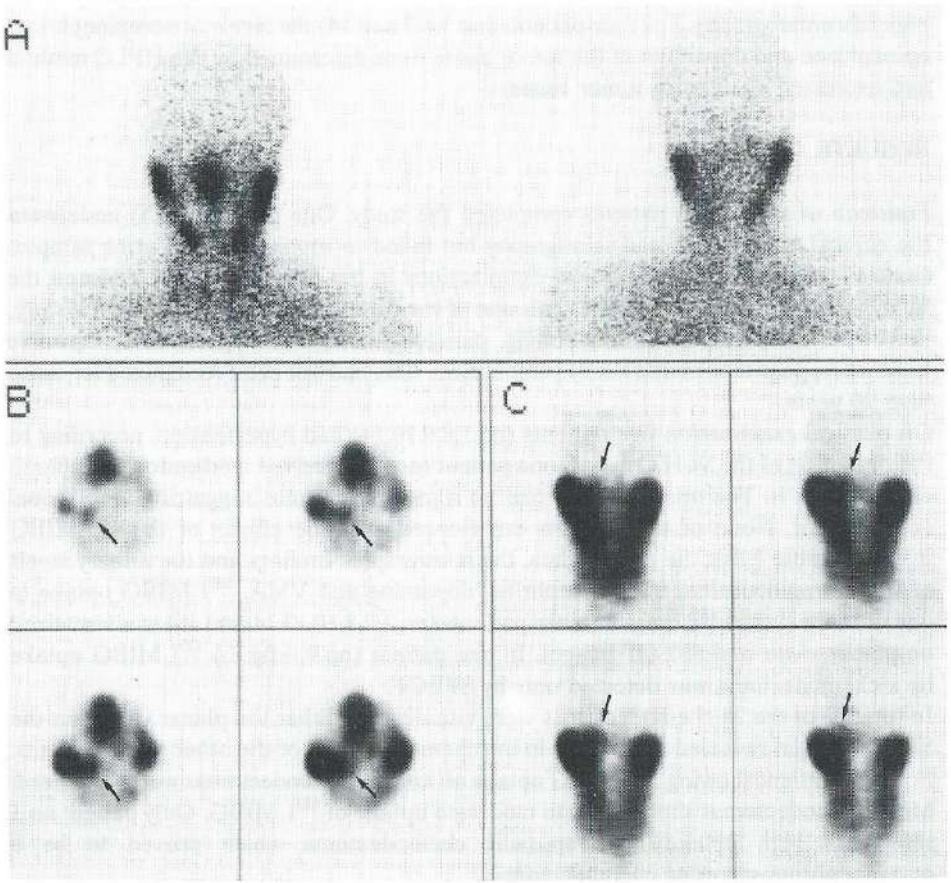

Figure 1. (Patient 9) Anterior and posterior MIBG images of the head do not reveal any uptake in a chemodectoma (A). Transaxial (B) and coronal SPECT images (C) show uptake of MIBG in a right GJT.

Patient no.1 had a left-sided glomus jugulare tumor that was partially removed. During and after the operation periods of severe hypertension developed but could be managed with 400 mg labetolol three times a day. Postoperatively, norepinephrine excretion normalized, but VMA excretion was elevated on one follow-up visit. In two patients (no.10 and 12) no lesions were revealed by either CT or MR, although distinct

abdominal deposits of ^{123}I MIBG had been seen on two consecutive days. Patient no.7 underwent partial resection of a vagal body tumor that was not revealed by ^{123}I MIBG imaging. The tumor contained only dopamine. Postoperatively, the average dopamine excretion decreased but did not normalize, probably because a large part of the tumor remained in situ.

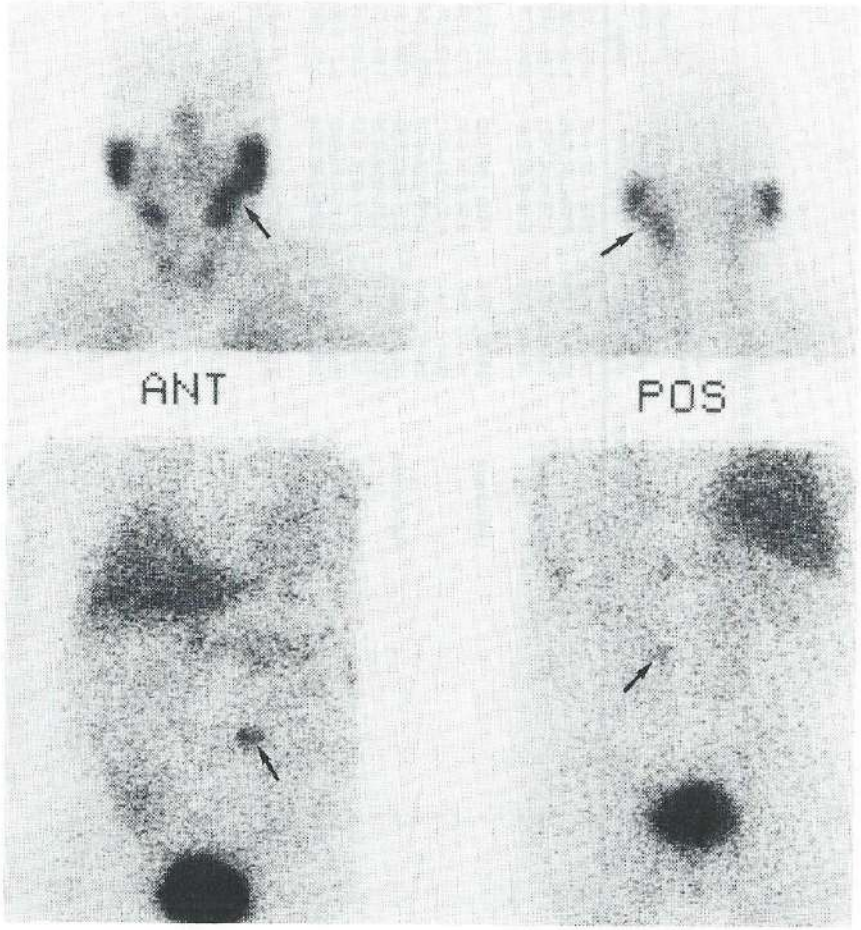

Figure 2. (Patient 14) Detail MIBG images show uptake in left carotid chemodectoma (arrow) and in the associated aortico-sympathetic paraganglioma (large arrow). A right CBT had been removed 20 yr earlier.

TABLE 1
Demographic, Scintigraphic, and Hormone Data on Patients with Chemodectoma

Patient no.	Age, sex	Kindred*	Multiple chemodectomas	MIBG uptake in chemodectoma	Abnormal MIBG uptake elsewhere	Urinary excretion			Number of measurements
						Vanillylmandelic acid μmole/24 hr range, mean	Norepinephrine μmole/24 hr range, mean	Dopamine μmole/24 hr range, mean	
1	41, F	—	—	+	—	22–35, 28.5	0.72–1.42, 1.02	0.76–1.78, 1.40	5
2	36, M	A	+	+	—	15–22, 18.0	0.13–0.34, 0.25	1.58–1.90, 1.72	3
3	24, M	B	—	—	Left adrenal	36–102, 73.0	4.35–6.50, 5.27	1.69–7.42, 3.22	7
4	31, M	A	+	—	—	14–28, 20.7	0.18–0.42, 0.32	0.91–1.73, 1.54	3
5	22, F	C	+	—	—				
6	49, F	D	+	—	Left adrenal	21–27, 25.0	1.04–1.82, 1.53	1.70–2.50, 1.98	3
7	39, F	E	—	—	—	19–27, 23.0	0.24–0.34, 0.30	2.52–5.88, 4.66	3
8	65, F	F	+	+	—	20–26, 23.0	0.20–0.21, 0.21	0.69–0.76, 0.72	3
9	50, F	F	—	+	—	25–37, 29.0	0.20–0.26, 0.23	0.21–0.90, 0.59	3
10	62, F	—	+	+	Right adrenal	10–24, 18.3	0.31–0.82, 0.56	1.08–1.44, 1.30	3
11	48, F	G	+	—	—	17–18, 17.3	0.26–0.32, 0.28	1.89–2.09, 2.01	3
12	41, F	H	+	—	Left adrenal	10–17, 13.3	0.41–0.75, 0.53	2.31–3.34, 2.68	3
13	58, F	—	+	+	—	15–18, 16.7	0.11–0.20, 0.16	0.21–0.90, 0.59	3
14	64, M	I	+	+	Left paramedian mid-abdominal	20–26, 25.0	0.35–0.88, 0.56	0.35–3.57, 2.14	4
15	26, M	B	—	+	—	21–27, 23.3	0.22–0.38, 0.28	1.94–2.44, 2.13	3
Normal values						<30	0.06–0.47	0.46–3.40	

*Letters A–I denote separate kindreds.

TABLE 2
Analysis of Patients with Elevated Catecholamine Excretion

Patient no.	Vanillylmandelic acid elevated/total no. of measurements	Norepinephrine elevated/total no. of measurements	Dopamine elevated/total no. of measurements	CT/MRI abdomen	Histological diagnosis	Tumor content	
						Norepinephrine μmole/g	Dopamine nmole/g
1	2/5	5/5	0/5	Normal	Jugular chemodectoma	(Measurement not performed)	
3	7/7	7/7	1/7	Pheochromocytoma in left adrenal	Pheochromocytoma	2.35	0
6	0/3	3/3	0/3	Pheochromocytoma in left adrenal	Pheochromocytoma	22.10	0
7	0/3	0/3	2/3	Normal	Vagal chemodectoma	0.00	15
10	0/3	2/3	0/3	Normal	—	—	—
12	0/3	1/3	0/3	Normal	—	—	—
14	0/4	2/4	1/4	Paraaortic Paraganglioma	Paraganglioma (ectopic pheochromocytoma)	3.40	0

DISCUSSION

The standard for diagnosis of chemodectomas is angiography. However, this is an invasive procedure and therefore only performed preoperatively to demonstrate the vascular supply of these lesions[3]. For screening purposes and postoperative control contrast enhanced CT is used[28]. A scintigraphic method offers several potential advantages such as better detection of recurrent lesions in a scarred field, the absence of artifacts from clips, and the possibility to detect metastases in the entire body. A further advantage is the absence of the small but appreciable risk of contrast reactions. For these reasons we evaluated the use of ^{123}I MIBG in patients with chemodectomas. Because normal ^{123}I MIBG uptake in parotid glands and submandibular glands interferes with the visualization of chemodectomas, planar views were supplemented by SPECT of the head and neck area. SPECT made the delineation of chemodectomas easier and demonstrated a chemodectoma in one patient that were not visible on planar views.

In total we noticed uptake ranging from low to high intensity in 13 of 24 chemodectomas accounting for 54%. Von Moll et al. using ^{131}I MIBG found uptake in 2 out of 5 chemodectomas (40%)[22]. The number of chemodectomas in the latter study is too small to make a valid comparison between the two isotopes. It is interesting to notice that the norepinephrine secreting tumor in patient no.1 showed the highest uptake. Shapiro et al reported two catecholamine secreting chemodectomas showing no ^{131}I MIBG uptake[19]. Whether this discrepancy is caused by the lower dose of ^{131}I MIBG, or by differences in norepinephrine kinetics is not clear. As already stated by von Moll et al. the ability of a tumor to take up MIBG can be independent of it's ability to secrete catecholamines[22]. The moderate sensitivity, found in our study suggests a limited role of ^{123}I MIBG in patients with chemodectomas.

However, performing ^{123}I MIBG scintigraphy and urinary screening for catecholamines we unexpectedly found a high number of surgically proved catecholamine-secreting tumors of the paraganglia (functioning paragangliomas). ^{123}I MIBG uptake provided an important clue to the hormonal activity of four of the five functioning paragangliomas. This was particularly the case in patient no.14 who initially showed only marginally elevated urinary catecholamine levels despite his obvious symptoms. Hormonal activity in patients with chemodectomas can be caused by the chemodectoma itself or by an associated catecholamine-secreting tumor in the thorax or the abdomen.

The percentage hormonally active chemodectomas has been estimated to be about 1%[10]. Approximately 2000 patients with one or more chemodectomas have been described. There are 17 documented cases of associated functioning paragangliomas outside the head and neck region suggesting an overall incidence of less than 1%[9,10,29]. The high incidence of functioning paragangliomas (functioning chemodectomas included) in our study may be attributable to several factors. First, we screened all patients for hormonal activity. In the past this was probably only performed in the event of clinical symptoms that suggested a hormonally active tumor. In our group, only one patient (no.14) had symptoms suggesting a catecholamine secreting tumor. Yet these symptoms had not been appreciated prior to this study. For the four other patients with a functioning paraganglioma, symptoms of a hormonally active tumor were equivocal or absent.

Secondly, not only the VMA levels in 24 h urine samples were measured, as in the past[10], but also the free catecholamine levels. According to Duncan et al. this is the most sensitive method for detecting pheochromocytomas[30]. The VMA levels were elevated in only one patient in our series and marginally elevated in a second. Even when elevated, the possibility of a functioning chemodectoma is sometimes disregarded. Smit et al. described in this journal a patient with a chemodectoma showing intense ^{131}I MIBG uptake and modestly elevated urinary VMA levels, which they attributed to cardiac failure rather than to excessive secretion of the chemodectoma[24].Thirdly, we performed whole body scintigraphy with ^{123}I MIBG, which is probably more sensitive in detecting pheochromocytomas and provides better image quality than ^{131}I MIBG[31]. To our knowledge whole body imaging has never been performed before in such a large group of patients with chemodectomas.

Of course some selection may have occurred. A high percentage of the patients studied were known to have a familial history of multiple chemodectomas. Since the aim of our study was to evaluate ^{123}I MIBG scintigraphy of chemodectomas in the head and neck region, we could only investigate patients who had not been operated upon for technical reasons, i.e. those with multiple chemodectomas or those who had undergone only a partial resection. Patients with multiple chemodectomas may be more prone to develop an associated functioning paraganglioma. We reviewed the case reports collected by Dunn et al. and found that of the 16 patients with a functioning paraganglioma and chemodectomas, at least 10 had multiple chemodectomas and 6 had a familial history[9].

In our patient population there was one patient with a solitary chemodectoma associated with a pheochromocytoma, but chemodectomas occurred in his family history. Retrospective analysis of the data on 20 patients with chemodectoma examined in our hospital in the past five years revealed another patient with a solitary, non-familial chemodectoma and a functioning mediastinal paraganglioma.

The familial occurrence of the association of chemodectoma with a paraganglioma has been described[2,16]. As shown in table 1 the high incidence of functioning paragangliomas in this study was not due to the enrollment of several members of one family with both types of tumor.Two patients (no. 10 and 12) exhibited an increased uptake of ^{123}I MIBG in an adrenal gland with elevated levels of norepinephrine; however, these findings were not confirmed by CT or MRI. These two patients will be followed because pheochromocytomas are known to develop gradually in the course of decades and an increase in the uptake of ^{123}I MIBG may be the earliest evidence of adrenal medullary disease[32,33].

The patient with the dopamine-producing chemodectoma is the fourth patient to be reported in the literature[34]. The tumor was not visualized by ^{123}I MIBG. Proye et al. have described two cases of dopamine-producing pheochromocytomas that were not

revealed either by ^{131}I MIBG[35]. Interestingly neither the chemodectoma nor the two pheochromocytomas, described by Proye et al. contained any norepinephrine. The most likely explanation for the non-visualization of these dopamine secreting paragangliomas is the absence of a specific norepinephrine uptake mechanism and/or a defective storage mechanism.

Although the number of chemodectomas taking up ^{123}I MIBG is limited this does not imply that ^{131}I MIBG cannot play an important role in the treatment of irresectable chemodectomas, showing the capacity to store MIBG. Patient no.1 is currently considered for treatment with therapeutic doses of ^{131}I MIBG, because the chemodectoma showed its capability for MIBG uptake and is not susceptible for further surgical treatment.

In conclusion, this study suggests that functioning paragangliomas in patients with chemodectomas are more common than is estimated in the literature. Because of the selection the high percentage of functioning paragangliomas cannot directly be applied to solitary, nonfamilial chemodectomas. Nevertheless with long-term follow-up and appropriate screening, functioning paragangliomas will be seen with increasing frequency in patients with chemodectomas. For this reason and because of the minimal symptoms we feel that patients with chemodectomas should be screened for elevated 24 h urinary catecholamine levels. In those patients with chemodectomas in whom urinary catecholamines are elevated, MIBG scintigraphy should be performed.

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CHAPTER 7

SUMMARY AND CONCLUDING REMARKS

SUMMARY AND CONCLUDING REMARKS

Paragangliomas (syn.: glomus tumors, chemodectomas) are rare, usually benign hypervascular tumors originating from the tiny glomus bodies present throughout the body. The most common types in the head and neck are the carotid body tumor, the glomus jugulo-tympanic tumor and the vagal body tumor.
The studies on hereditary and sporadic glomus tumors reported in this thesis were focussed on the 'natural' course of the disease as well as on the genetic and tumor-biological aspects of paragangliomas.
Opinions concerning the management of these vascular lesions remain controversial. Although paragangliomas are extremely slow-growing and rarely malignant, they can have a critical localization, which means that, especially for tumor localizations at the skull-base, the proposal to treatment is difficult. Surgical treatment for these benign lesions in the temporal bone has been criticized because of the difficulty of performing a radical resection as well as because of the risk of (post)-operative complications and morbidity, i.e., cranial nerve palsy and decreased quality of life. Radiation therapists have focussed on the surgery-related operative complications and advocate for non-surgical management of these tumors in spite of the question as to whether these tumors are really radiosensitive. Advocates of both modalities make good use of the unknown and usually indolent natural course of the disease to support their results. This means that there is a need to identify patient and tumor-related factors that can give information about the growth pattern of these tumors for definition of the indication of treatment. The investigation of intrinsic tumor characteristics may give insight into the biology of the disease.
This **Chapter (7)** gives a summary of our results, and discusses several questions arising from our findings.

Chapter 1 gives a review of the literature and thus contains general information on the disease and data on the current diagnostics, histopathology, and modalities of treatment.
Because the present clinical study is comparable to the five largest studies in the literature with respect to the number of cases reported and the duration of follow-up, **Chapter 2**, deals with the results of management of 108 glomus tumor patients with a total of 175 head and neck paragangliomas seen at the Departments of Otolaryngology and Vascular Surgery of the Leiden University Hospital in the last 32 years (1956-1988).
The general epidemiologic data, presenting signs, and symptoms, and the results of treatment of 53 glomus jugulo-tympanic (GJTT), 32 vagal body (VBT) and 91 carotid body tumors (CBT) are in accordance with earlier reports. Due to the high incidence of cases of familial disease (50%) in the present study we registered an unusual high percentage of multicentric tumors (55%).
Our surgical results for GJTT's (59% non-radical) were disappointing but comparable to those of other series. However, a few authors of reports on series recruited

from Centers of Cranial Base Surgery[1,2] steadily on claim better results for complete resection of temporal bone paragangliomas as well as diminishing morbidity. These improved results are also attributed partially to the introduction of modern imaging facilities, selective embolization, micro-surgical techniques, and approaches to the skull-base. The concept of conservation skull-base surgery was recently introduced by Jackson[3] in 1989 and clearly shows that the aim of temporal bone paraganglioma (GJTT) surgery remains radical tumor resection with minimal and acceptable morbidity. Because of it's uncertain therapeutic effect, radiation therapy for GJTT's is generally to be considered preferable for the elderly and patients in poor health with the aim to slow down tumor progression.
The results of radical surgical treatment for cervical paragangliomas (i.e., CBT's and VBT's) have become much more favorable. Today, the vascular and neurological post-operative complications of CBT and VBT surgery are minimal and the resection is generally radical.
To achieve a better basis for the choice to treatment of glomus tumors, it is essential to obtain more insight into the 'natural' course of disease, since the growth pattern of paragangliomas is usually characterized by extremely slow progression. In such cases, it is very difficult to decide whether and when to perform extensive tumor resection, which frequently results in considerable morbidity with invalidating cranial nerve palsy.
The contribution to the most likely approximation of the natural course of this disease could be made by the history of disease of patients treated and followed at our hospital (n=25) as well as by information obtained from patients that had lived with such a tumor for a long time without seeking medical help. From the material used in a previous study (discussed in Chapter 3) on hereditary glomus tumors we traced 16 persons or 'patients'. The course of the disease (n=41) could be reconstructed from (a) patients that were left untreated, (b) those with residual tumor after surgery, or (c) who had undergone irradiation or (d) a combination of surgery and irradiation. The results of treatment and the duration of follow-up of the clinical series in addition to the data on the reconstruction of the ('natural') course of disease in both hereditary and non-familial disease indicated that the average course of the disease is about 20 years (see **Chapter 2**).
These data indicated that no treatment can in fact improve survival and it also justifies consideration of a 'monitored wait-and-see' policy for especially GJTT's as part of any proposal for treatment. In fact, treatment of GJTT's should be performed to reduce morbidity rather than to improve survival rates. However, it is important to note, that there are exceptions too, i.e., in cases in which tumor progression is far more rapid. This makes it even more difficult to refrain from intervention, especially in young patients with multifocal disease. These observations also underline the need for tumor-biological markers that can predict the growth pattern.

Because of the high incidence of familial disease in our series, we were able to study 15 different pedigrees. **Chapter 3** shows that familial glomus tumors are

inherited almost exclusively via the paternal line, a finding inconsistent with the autosomal dominant transmission. This pattern of inheritance was also encountered in earlier reports on familial glomus tumors. These findings, can be explained in terms of genomic imprinting, i.e., the maternally derived gene is inactivated during female oogenesis and can only be reactivated by passage through the male germline.

The term genomic imprinting is used to describe modifications of gene expression by parental origin of the gene i.e., the expression of genes may be modified by the sex of the parent from whom the gene is inherited. Recently, this type of non-Mendelian inheritance has received wide attention[4-8]. Results of animal experiments indicate that imprinting of some parental alleles is critical for normal embryonic development and that neither the male nor the female genome is by itself toti-potentential[8,9].
At present, little is known about the exact mechanism of genomic imprinting, although DNA methylation may play a role in it[12]. Identification and characterization of the involved disease genes may provide insight into these mechanisms.
Now work is in progress to further delineate the locus, the purpose to clone and characterize the involved gene. Once the gene has been identified, the accuracy of genetic counselling can become maximal.
For non-carriers, this information will remove the fear of being affected, for carriers it will give the opportunity to perform selective and efficient screening (MR). Moreover the value of early diagnosis will also improve the chances of radical excision with less invalidating morbidity.
In the meantime, this new genetic theory already has considerable implications for genetic counselling in hereditary glomus tumor disease. The risk for the offspring of affected males remains 50%, for those off affected females the risk is very low, and for the children of sons of woman with glomus tumors the risk is 25%.

For a variety of solid tumor types, aberrations of total nuclear DNA content are associated with increasing clinical aggressiveness. In **Chapter** 4 the determination of the nuclear DNA content of 99 paragangliomas is reported.
The results show that DNA aneuploidy occurs relatively frequently (37%) in clinically and histologically benign paragangliomas. This finding, however, unlike the situation for several malignant tumors, has no clinical relevance for definition of the indication and the timing of surgical treatment but supports the concept that these tumors are true neoplasia cytogenetically. It remains an intriguing problem, that a variety of tumors of neuro-endocrine origin, including paragangliomas, can develop quite pronounced DNA aneuploidy, that is indicative of numerical and probably also structural chromosomal aberrations, in the absence of overt signs of clinical malignancy.
Since routine histological evaluation does not provide information about the clinical behavior of paragangliomas, immunohistochemical analysis (**Chapter 5**) was performed on aa ample series of 114 head and neck glomus tumors. According to

earlier reports, a decrease in the proportion of type-II cells has been claimed as a marker for aggressive behavior. This technique clearly shows the difference between chief (type I) and sustentacular (type II) cells but did not confirm any significant correlation between immunoreactivity (10 markers used) and clinical criteria indicative for tumor progression, tumor localization or familiality of disease. The distribution of the S100 expression indicates that the cellular composition of head and neck paragangliomas are similar but differing from that of pheochromocytomas. On the basis of the relative low level of catecholamine secretion, the absence of proven malignancy, clinical differences in recurrent and locally aggressive disease, it is evident that head and neck paragangliomas differ biologically from other paragangliomas.
The common feature of paraganglion cells is the presence of numerous neuro-secretory granules containing catecholamine in their cytoplasm. The proportion of hormonally active paragangliomas is thought to be high for adrenal pheochromocytomas but low for head and neck chemodectomas (1%). In the study reported in **Chapter 6**, the functional activity of glomus tumors (15 patients with 24 glomus tumors) was detected from the uptake of iodine-123-metaiodobenzylguanidine (MIBG). In total, we noticed uptake ranging from low to high intensity in 13 (54%) chemodectomas. However, MIBG scintigraphy and urinary screening for catecholamine, led to the detection of an unexpectedly large number (n=5) of histologically confirmed catecholamine-secreting tumors of the paraganglia, e.g. three surgically proved pheochromocytoma. Especially in familial disease, appropriate screening will detect multicentric functioning paragangliomas in or outside the head and neck region with increasing frequency. Patients should be screened for elevated 24-hr urinary catecholamine levels, MIBG scintigraphy being performed when elevated levels are found. Because of the potential peri-operative complications, vasoactivity should be excluded prior to surgical treatment.

Finally, in terms of the aims of the study, it may be concluded that the results offered a realistic approximation of the natural course of glomus tumor disease.
The usually indolent growth pattern of these tumors can clearly influence the choice of treatment for skull base tumors which, for several reasons, will remain difficult.
At present, it is not possible to identify aggressive tumor behavior from histological patterns, by DNA flow-cytometry, or immunohistochemistry, since functioning paragangliomas are not frequently diagnosed.
The results of the study on hereditary glomus tumors suggested strong evidence for the new genetic theory concerning genomic imprinting, which will affect genetic counselling essentially. Awareness of this non-Mendelian pattern of inheritance can be expected to stimulate molecular genetic research to localize and identify the predisposing gene defect, and will probably provide further proof and help to elucidate the mechanisms underlying genomic imprinting.

SAMENVATTING

Glomustumoren (syn.: chemodectomen, paragangliomen) ontstaan doordat er groei optreedt in het glomus weefsel van de zgn. 'glomuslichaampjes'. Deze 'glomuslichaampjes', vaak niet groter dan een rijstkorrel, kunnen zich overal in het lichaam bevinden, doch meestal zijn ze te vinden langs de banen van het autonoom zenuwweefsel en nabij de grote vaten. De functie van de glomuslichaampjes is niet duidelijk, aangenomen wordt dat ze als chemoreceptor betrokken zijn bij de regulatie van de arteriële O_2 en CO_2 spanning.
In het hoofd/hals gebied zijn de drie meest bekende tumorlocalisaties: de glomus caroticum tumor (CBT), gelegen in de splitsing van de hals slagader, de glomus vagale tumor (GVT), naast de 10e hersenzenuw, glomus tympanicum tumor (GTT) en de glomus jugulare tumor (GJT), beide in of nabij het oor gelegen.
Het is niet bekend waarom de 'glomuslichaampjes' zich ontwikkelen tot glomustumoren. Wel is bekend dat het groeipatroon van deze tumoren sterk kan wisselen. Sommige tumoren blijven jarenlang onveranderd aanwezig en geven weinig klachten, andere hebben een meer agressief groeipatroon of zijn zelfs een enkele keer kwaadaardig.
In het algemeen worden glomustumoren operatief behandeld. De afweging om tot een chirurgische, niet zelden uitgebreide operatie te besluiten wordt bemoeilijkt omdat er geen gegevens bestaan over het natuurlijk beloop van deze goedaardige, meestal extreem langzaam groeiende tumoren. Een operatie van een glomustumor aan de schedelbasis, waarbij de tumor meestal niet volledig verwijderd kan kan worden, veroorzaakt vaak ernstige en blijvende beschadiging (zenuwuitval). Als een patient meerdere tumoren heeft (één-of twee-zijdig) wordt de afweging tot behandeling nog moeilijker. Juist bij de erfelijke glomustumoren wordt vaak meer dan één glomustumor (multifocaliteit) gevonden.
Juist omdat de tumoren zo langzaam groeien is er dringend behoefte aan tumor-biologische markers of graadmeters, die het groei patroon van een glomustumor zouden kunnen voorspellen.
De studies, die in dit proefschrift aan de orde komen zijn enerzijds gericht op het 'natuurlijk' beloop en de erfelijke aspecten van de aandoening, anderzijds op de tumor-biologische eigenschappen van glomustumoren.

Hoofdstuk 1 betreft een beknopt literatuur overzicht aan de hand waarvan algemene informatie over het ziektebeeld wordt gegeven. De huidige stand van zaken over diagnostiek, histopathologie en behandeling wordt besproken.
Om meer inzicht te krijgen in het 'natuurlijk' beloop van glomustumoren **(Hoofdstuk 2)** werden de behandelingsresultaten in de periode 1956 - 1988 (32 jaar) bestudeerd. In totaal werden 175 glomustumoren gediagnostiseerd (52 glomus jugulo-tympanicum tumoren, 32 glomus vagale tumoren en 91 glomus caroticum tumoren) bij 108 patienten. Bij 58 patienten (50%) kwam de aandoening in de familie voor. Het Leidse patientenbestand van de afdelingen keel-, neus-, en

oorheelkunde en algemene heelkunde is wat betreft aantal patienten en duur van follow-up vergelijkbaar met de vijf grootste series in de literatuur. In het algemeen waren ook de lengte van de anamnese (duur van de ziekte) en de symptomatologie (klachtenpatroon) in overeenstemming met de literatuur. De behandelingsresultaten van het operatief verwijderen van glomustumoren aan/in de schedelbasis (GJTT) waren teleurstellend t.w. in 59% (n=23) van de gevallen was de operatie niet radicaal. Daarentegen was de operatie van tumoren uit de hals (GCT en GVT) in 96% van de gevallen succesvol. Door de ontwikkeling van moderne beeldvormende en micro-chirurgische technieken worden de laatste jaren de resultaten van operaties aan de schedelbasis steeds beter[1,2]. In het algemeen wordt er de voorkeur aangegeven deze vaatrijke tumoren (ook de GJT's) operatief te behandelen[3]. Radiotherapie zou overwogen kunnen worden als de patient op leeftijd is of in een slechte algemene conditie verkeerd.

Om een zo goed mogelijk beeld van het 'natuurlijk beloop' van de ziekte te krijgen zijn niet alleen de behandelde en vervolgde patienten (n=25) van belang. Informatief zijn met name 'patienten' of familieleden van patienten, die oud zijn geworden met een glomustumor zonder dat ze er voor behandeld zijn geweest. Er werden vier groepen bestudeerd; personen of 'patienten' die (a) geen behandeling hadden ondergaan (b) restant tumor hadden na chirurgie (c) bestraald waren of (d) combinatie behandeling (chirurgie en bestraling) hadden ondergaan.
Het 'natuurlijk' beloop van de ziekte kon voor zowel familiaire als niet-familiaire glomustumoren met deze gegevens gereconstrueerd worden. De duur van het ziektebeloop en/of de follow-up voor de vier groepen (a-d) bedroeg gemiddeld 20 jaar. Geconcludeerd werd, dat 'je oud kunt worden met een glomustumor' en dat het gezien deze bevindingen de vraag is of behandeling van glomustumoren de overleving van glomus patienten doet verbeteren. Daarom dient bij elk voorstel tot behandeling van m.n. GJTT's een 'monitored wait and see policy' overwogen te worden. De behandeling van GJTT's dient er eerder op gericht te zijn om de klachten van de patiënt te verminderen, dan de overleving te verbeteren.
Men moet zich echter wel realiseren dat er uitzonderingen bestaan waarbij de tumor veel sneller groeit. Vooral bij jonge patienten met multifocale tumoren bemoeilijkt dit de beslissing om af te zien van behandeling.

Het familieonderzoek (**Hoofdstuk 3**) begon in twee Zuid-Hollandse dorpen waar drie met elkaar verwante families met glomustumoren te vinden waren. Met hulp van de familieleden en foto's (zie omslag) konden in totaal 15 verschillende stambomen worden opgesteld die geschikt waren voor verder onderzoek.
Bij het bestuderen van de stambomen viel op dat de overerving vrijwel uitsluitend via de vaderlijke lijn plaatsvond, ongeacht of de vader nu wel of niet was aangedaan. Het meest opvallend was dat overerving via de moeder niet werd gezien. Ook in de 15 uit de literatuur beschikbare stambomen kwam overerving via de moeder niet voor, met uitzondering van enkele dubieuze gevallen. In de literatuur werd het

erfelijksheidspatroon van familiaire glomustumoren beschouwd als strikt autosomaal dominant. De door ons beschreven overerving kon echter niet worden verklaard met de klassieke wetten van Mendel. Met het concept van 'genomic imprinting' zou de gevonden overerving bij glomustumoren echter wel verklaard kunnen worden[4-8].
Bij genomic imprinting gaat men er vanuit dat de expressie van bepaalde genen wordt bepaald door het feit of ze van de moeder dan wel van de vader afkomstig zijn[8,9]. Hoe het 'imprint' mechanisme precies werkt is niet duidelijk. Een hypothese is dat bij glomustumoren tijdens de vorming van de eicellen het ziekte gen door methylering geinactiveerd wordt, zodat de eerstvolgende generatie niet is aangedaan. Dit betekent dat een vrouw het ziekte gen doorgeeft in een uitgeschakelde (gemethyleerde) vorm zodat haar kinderen niet zijn aangedaan. Vervolgens wordt bij haar zonen, tijdens de vorming van de zaadcellen, het van moeder afkomstige gen ge-demethyleerd c.q. gereactiveerd of aangeschakeld, zodat (pas) bij nakomelingen van de zonen, de tumor weer tevoorschijn kan komen[5]. Het lijkt er dus op, dat het verantwoordelijke gen, het 'stempel' of 'imprint' van zijn herkomst (vader of moeder) draagt. Het maakt dus verschil of het gen, dat verantwoordelijk voor de aandoening is, van de vader of van de moeder afkomstig is[12].
Omdat bij erfelijke glomustumoren de overerving via de moeder ontbreekt moet het erfelijkheids advies herzien worden. Het risico om een glomustumor te krijgen voor kinderen van aangedane mannen blijft 50%, terwijl het risico voor kinderen van aangedane vrouwen nihil is. Kinderen van zoons van deze vrouwen hebben vervolgens wel weer een risico van 25% om de tumor te krijgen.
Verder (DNA) onderzoek zal zich concentreren op het lokaliseren van het chromosoom en vervolgens het gen. Als het gen in kaart gebracht is, kan worden aangetoond dat 'imprinting' van genen echt bestaat en niet slechts een hypothese is[12]. Dragerschap van het ziekte gen zal dan aantoonbaar zijn en het zal mogelijk zijn om exact aan te geven wie van de nakomelingen de ziekte zullen krijgen. Voor niet gen-dragers zal deze optimale vorm van erfelijkheids advies de angst om ooit glomus patient te worden wegnemen en dragers kunnen hierdoor efficient gecontroleerd worden met beeldvormende technieken zoals CT of MR. Door efficiënte vroeg-diagnostiek zal de kans op het volledig operatief verwijderen van een glomustumor met minimale beschadiging van hersenzenuwen aanzienlijk vergroot worden.

Bij een aantal types solide tumoren (bijv. ovarium) zijn afwijkingen in het totale DNA-gehalte van de tumoren geassocieerd met agressief tumorgedrag en een slechte prognose. In **Hoofdstuk 4** worden de resultaten van DNA-flowcytometrie bij 99 glomustumoren beschreven. Het was opvallend dat een afwijkend DNA gehalte (aneuploidie) relatief vaak (37%) gevonden werd bij glomustumoren. Deze bevinding is echter niet in overeenstemming met met het niet agressieve biologisch gedrag van glomustumoren. DNA-flowcytometrie levert derhalve geen bijdrage in de indicatiestelling tot operatie. Het steunt echter wel de gedachte dat een

glomustumor beschouwd moeten worden als een echte nieuwvorming en niet als hyperplasie. Het blijft een intrigerend probleem dat een glomustumor en sommige andere tumoren van neuro-endocriene oorsprong aanzienlijke aneuploidie kunnen ontwikkelen. Dit pleit voor numerieke en mogelijk structurele chromosoom veranderingen, zonder duidelijke klinische manifestatie als kwaadaardige tumor.

Aangezien het klinisch gedrag van glomustumoren niet voorspeld kan worden op basis van routine weefsel-microscopie werden 114 glomustumoren geanalyseerd met immunohistochemie (**Hoofdstuk 5**). Met deze techniek werden chief (type I) cells en sustentacular (type II) cells onderscheiden. Bij tien geteste markers kon geen significante correlatie worden aangetoond tussen immunoreactiviteit en die klinische criteria die representatief waren voor tumorprogressie, tumorlocalisatie, en familiariteit. Tevens bleek, in tegenstelling tot eerdere rapporten, dat afname van het percentage type II cells niet gerelateerd was aan agressief tumor gedrag. Op basis van de gevonden resultaten bij immunohistochemie, de matige catecholamine productie en het feit dat de tumoren zelden kwaadaardig worden, blijkt dat glomustumoren uit het hoofd/hals gebied zich onderscheiden van paragangliomen, die daarbuiten zijn gelocaliseerd.

Glomustumoren behoren tot de non-chromaffine paragangliomen en samen met de pheochromocytomen zijn ze te rangschikken onder de neuro-endocriene tumoren. Paragangliomen bevatten catecholamines die zijn opgeslagen in het cytoplasma van de neuro-secretoire granula. Glomustumoren zijn zelden (1%) klinisch hormonaal actief, i.t.t. het catecholamine producerende pheochromocytoom (90% van de gevallen vasoactief). Verschillende auteurs benadrukken het belang van screening op vasoactiviteit. In het in **Hoofdstuk 6** beschreven onderzoek, werd bij 15 patienten (24 glomustumoren) functionele activiteit aangetoond met behulp van I^{123}MIBG (methylbenzylguanidine) scintigrafie. In totaal lieten 13 (54%) glomustumoren opname van MIBG, zien. Gezien het potentiële gevaar op complicaties tijdens operatie dient vasoactiviteit voor de operatie te worden uitgesloten. Elke patient met een glomustumor moet worden onderzocht op functionele activiteit middels het bepalen van catecholamines in de 24 uurs urine en MIBG scintigrafie.

Gezien de resultaten van het in dit proefschrift beschreven onderzoek kan worden geconcludeerd dat er een realistisch beeld is verkregen over het 'natuurlijk' beloop van glomustumoren. Het groeipatroon van deze tumoren is meestal zo langzaam, dat dit m.n. voor glomustumoren aan de schedelbasis (GJTT) het behandelingsvoorstel aanzienlijk kan beinvloeden.
Op dit moment kan met de routine weefselonderzoek, DNA-flowcytometrie en/of immunohistochemische markers (pre-operatief) nog geen indicatie voor het groeigedrag verkregen worden.
Door bestudering van de stambomen van glomustumor families bleek dat bij glomustumoren de overerving via de moeder ontbreekt.

Dit overervingspatroon (niet-Mendeliaans) heeft vermoedelijk te maken met genomic imprinting. Alhoewel deze hypothese nog bewezen moet worden, is het erfelijkheids advies aan jonge mensen uit een glomustumor familie inmiddels essentieel gewijzigd.
Met genetische koppelingsanalyse zijn recent vorderingen gemaakt in de localisatie van het chromosoom met het voor de aandoening verantwoordelijke gen. Moleculair genetisch onderzoek zal zich nu concentreren op de identificatie van het gen zodat de mechanismen die een rol spelen bij genomic imprinting kunnen worden opgehelderd.

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CURRICULUM VITAE

The author of this thesis was born on the 10th of November 1952 in the city of Groningen. After completing his secondary education (HBS-b) at the Montessori Lyceum in Rotterdam, he completed military service. He started his medical studies in 1974 at the School of Medicine of the University of Leiden and obtained medical qualification in 1981. From 1981 to 1984, he received training in surgery under the supervision of Dr R.K.J. Koumans at the Westeinde Hospital in The Hague. In 1984, he became resident in Otolaryngology at the Leiden University Hospital (head: Prof.Dr. P.H. Schmidt). Since 1988 he has been a member of the Otolaryngology staff of the same hospital.

STELLINGEN

1. Het concept van genomic imprinting is een belangrijke toevoeging aan de klassieke wetten van de genetica en vergroot hun klinische toepasbaarheid.

2. Een vrouw uit een familie met glomustumoren heeft geen kans op aangedane kinderen hetgeen tot uiting moet komen in het genetisch advies.

3. Door genomic imprinting kunnen erfelijke aandoeningen generaties lang verborgen blijven.

4. De behandeling van glomustumoren is primair chirurgisch en dient er eerder op gericht te zijn de morbiditeit te verminderen dan de overleving te verbeteren.

5. Het opofferen van de nervus vagus is inherent aan het verwijderen van een glomus vagale tumor.

6. Het onvoldoende bekend zijn met de anatomie van het voorste epitympanum verhoogd de kans op residu cholesteatoom.

7. Het toxic shocksyndroom kan ook optreden na het inbrengen van een neustampon.

8. Een diepe halsinfectie kan zich snel uitbreiden omdat de verschillende compartimenten in de hals slechts door losmazige fasciebladen van elkaar zijn gescheiden.

9. 'Bijschildklieren leiden een eigen leven' (H. Muller, 1967 - 1977).

10. Tegenwoordig wordt in Nederland het promoveren in de geneeskunde gezien als het obligate middenstands - diploma van de medisch specialist.

11. Serendipity - 'Chance favors only the prepared mind' (L. Pasteur, 1848).

12. This thesis is a small step for mankind but a giant step for the author.

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Andel G.L. van der Mey