2. Studies of Cancer in Humans

2.1 Burkitt's lymphoma

2.1.1 *Clinical features and pathology*

2.1.1.1 *Clinical features*

The jaw is the most frequently involved site and the commonest presenting feature in patients with Burkitt's lymphoma in equatorial Africa (Burkitt, 1958, 1970a) and Papua–New Guinea (ten Seldam *et al.*, 1966; Burkitt, 1967; Magrath *et al.*, 1992). The tumour frequently affects multiple jaw quadrants (Figure 5). Jaw involvement is age-dependent, occurring much more frequently in young children, since it arises in close proximity to the developing molar tooth buds. In series of cases of Burkitt's lymphoma in Uganda, 70% of children under five years of age and 25% of patients over 14 had jaw involvement (Burkitt, 1970a). Very young children who do not have overt jaw tumours often have orbital involvement (Olurin & Williams, 1972; Figure 6); some of these orbital tumours may arise in the maxilla. In general, jaw involvement appears to be more frequent in regions of higher incidence, even within equatorial Africa; however, patients from, for example, highland regions in Africa, in which the annual incidence rate of Burkitt's lymphoma is much lower, are also of higher median age, and this probably accounts for the lower frequency of jaw tumours (Burkitt & Wright, 1966; Kitinya & Lauren, 1982). It has been suggested that the frequency of jaw tumours is decreasing in some regions of equatorial Africa, with a corresponding increase in the fraction of abdominal tumours but with no clear change in the age-related incidence (Nkrumah, 1984).

Abdominal involvement is found in a little more than half of equatorial African patients at presentation (Burkitt & Wright, 1963; Burkitt, 1970b; Williams, 1975) and as many as 80% of patients in other countries (Magrath, 1991, 1997). There appear to be differences in the intra-abdominal sites of involvement in endemic countries (e.g. equatorial Africa and Papua–New Guinea) and in those where the disease is sporadic (Europe, Australia and North America). Presentation with a resectable mass in the right iliac fossa or with intussusception (arising from intraluminal tumours), for example, both of which are common in sporadic tumours, is uncommon in African patients. Thus, Wright (1970)

Table 6. Cellular origin and patterns of viral gene expression in EBV-associated malignancies

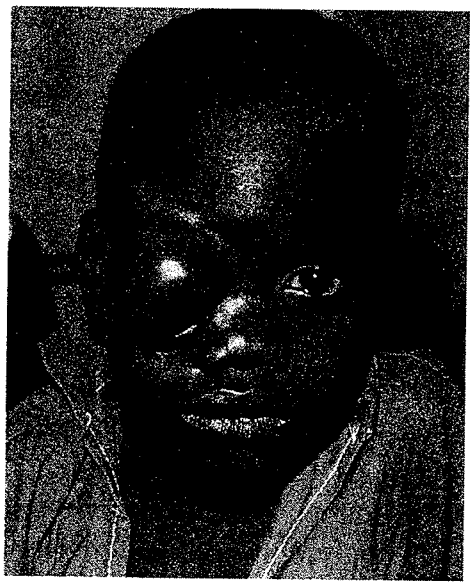
Cell type	Tumour	EBV association (%)	EBV gene expression			Latency type	Reference
			EBNA-1	*EBNA-2, -3A, -3B, -3C, -LP*	*LMP-1/2*		
B Lymphocyte	Immunoblastic B lymphoma	100	+	+	+	III	Thomas *et al.* (1990)
B Lymphocyte	Endemic Burkitt's lymphoma	> 95	+	–	–	I	Rowe *et al.* (1987a)
T Lymphocyte	Immunoblastic T lymphoma	50–90	+	–	+	II	Jones *et al.* (1988); Kikuta *et al.* (1988)
T lymphocyte	Midline granuloma	100	+	–	+	II	Minarovits *et al.* (1994a)
HRS cells	Hodgkin's disease	40–50	+	–	+	II	Herbst *et al.* (1991a); Pallesen *et al.* (1991c); Deacon *et al.* (1993); Grasser *et al.* (1994)
Nasal epithelium	Nasopharyngeal carcinoma	100	+	–	+ (60%)	II	Fåhraeus *et al.* (1988); Brooks *et al.* (1992); Busson *et al.* (1992a); Smith & Griffin (1992)
Thymic epithelium	Thymic carcinoma	Case report	+	–	+	II	Leyvraz *et al.* (1985)
Gastric epithelium	Gastric carcinoma	90	+	–	–	I	Imai *et al.* (1994a)
Smooth muscle	Leiomyosarcoma	100	+	+	ñ	IV	Lee *et al.* (1995b)

Viral antigen expression was demonstrated by immunohistochemistry and/or reverse transcriptase–polymerase chain reaction.
HRS, Hodgkin and Reed-Sternberg

Figure 5. Ugandan patients with Burkitt's lymphoma involving the jaw

From WHO (1969); left, mandibular tumour; right, maxillary tumour

Figure 6. Orbital Burkitt's lymphoma in a Ugandan child



From WHO (1969)

reported no case of intussusception in Uganda among over 500 cases. In contrast, involvement of the mesentery and omentum (i.e. extraluminal) is common in African cases of Burkitt's lymphoma, hence the rarity of Burkitt's lymphoma-associated intussusception in Africa. Ascites may be a manifestation of abdominal disease regardless of geographic location. Precise figures for the involvement of various intra-abdominal and

retroperitoneal structures at presentation are not available from Africa, as relatively few centres have adequate radiological facilities. The available data are based largely on the results of clinical examinations, sometimes supplemented by laparotomy, or by autopsy studies dating from the 1960s (O'Conor, 1961; Wright, 1964, 1970; Williams, 1975; see below).

Bone-marrow involvement is seen in some 7–8% of Ugandan patients at presentation and relapse but in about 20% of patients in Europe and the United States. An additional fraction of patients in Europe and the United States presents with a leukaemic syndrome, referred to as the French–American–British subtype 'L3' or acute B-cell Burkitt's lymphoma (Magrath & Ziegler, 1980).

Central nervous system involvement — including cerebral spinal fluid pleocytosis, cranial nerve palsy and paraplegia due to paraspinal disease — is relatively common in Africa, being found in about one-third of patients at presentation (Ziegler *et al.*, 1970), but is much less common in regions of sporadic incidence (Magrath, 1997). Interestingly, cranial nerve involvement (usually due to direct infiltration by tumour cells) is frequently not associated with cerebral spinal fluid pleocytosis at presentation, but malignant cells are nearly always detectable in cerebral spinal fluid of patients with cranial nerve palsy at relapse (Magrath, 1991). The optic nerve is frequently compressed and the surrounding subarachnoid space involved, however, when an intraorbital tumour is present (Ifekwunigwe *et al.*, 1966). Cranial nerves and meninges have been described as the only sites of disease at presentation (Osuntokun *et al.*, 1973). Paraplegia is the presenting feature in 15% of Ugandan patients but in less than 1% of patients in the United States (Magrath, 1991, 1997). In Ugandan patients, the spine is quite frequently the only evident site of disease, such that lamininectomy is required to make a diagnosis (and to relieve spinal cord pressure). Intracerebral disease is very uncommon and usually occurs in patients who have had persistent or multiple relapsed cranial nerve palsy or cerebral spinal fluid pleocytosis (Wright, 1970; Magrath *et al.*, 1974); however, presentation with raised intracranial pressure from parenchymal brain infiltration is observed, albeit very uncommonly (Odeku *et al.*, 1973; Osuntokun *et al.*, 1973). Other sites of disease that are occasionally observed include the salivary glands, thyroid, breast (in pubertal girls or lactating women), testis, bone, pleura and heart, with involvement of the pericardium or, infrequently, cardiac muscle (Burkitt, 1970a; Aderele *et al.*, 1975; Durodola, 1976; Magrath, 1991, 1997). Interestingly, pharyngeal involvement, peripheral lymphadenopathy and splenic involvement are rare in the African patient (although there is frequently splenomegaly from malaria) and rather more common in patients in regions of sporadic disease, although both splenic and lymph-node involvement are significantly more frequently observed in cases of Burkitt-like lymphoma.

2.1.1.2 *Gross pathology*

Burkitt's lymphoma, unlike follicular lymphomas and diffuse large B-cell lymphomas, arises predominantly at extranodal sites. The kidneys are a frequent site of disease in sub-Saharan Africa, being the organ involved in 80% of cases at autopsy; the ovaries are frequently bilaterally involved in young females, representing the site seen in

81% of autopsies in females. The adrenals are the third most commonly involved intra-abdominal organ, are more often involved at autopsy than the liver or spleen and are about as frequently involved in children as the jaw (58%). Bowel involvement is common, but other organs are involved less frequently. The lung parenchyma is very rarely involved, but serosal infiltration of the pleura and peritoneum, resulting in effusion, is frequent. Wright (1964, 1970) never observed involvement of the thymus. Fatal Burkitt's lymphoma is usually accompanied by widespread dissemination.

Grossly, the tumour is fleshy, creamy and soft. Areas of necrosis and haemorrhage are seen only in very large tumours. The tumours can locally infiltrate surrounding tissues and may spread via the lymphatics or blood vessels. In the central nervous system, choroid plexus involvement is not uncommon and is usually associated with cerebrospinal fluid pleocytosis. Haematogenous dissemination may result in parenchymal involvement of the brain and spinal cord, in which case cerebrospinal fluid pleocytosis may be difficult to detect.

2.1.1.3 *Histological characteristics*

Burkitt's lymphoma (small non-cleaved-cell lymphoma, Burkitt's type) is classified as a non-Hodgkin's lymphoma and is characterized by a monomorphic cytoarchitecture composed of medium-sized cells (between those of large B-cell lymphoma and small-cell lymphocytic lymphoma). These cells do not have the characteristics of plasmacytoid cells or mature lymphocytes; they have a high nucleus to cytoplasm ratio, a round or oval nucleus with a coarse or 'open' chromatin pattern and usually two to five readily discernible nucleoli. A few cells may have a single large nucleolus, but when more than a few such cells are present the tumour is probably a Burkitt-like lymphoma. The cytoplasm is intensely basophilic, staining strongly with methyl-green pyronine (pyroninophilic) because of the abundant free ribosomes, which are readily visible on transmission electron microscopy. Intracytoplasmic lipid vacuoles which stain with oil-red O and Sudan black are usually apparent on imprint or smear cytology (Berard *et al.*, 1969). Histological sections often reveal the presence of tingible body macrophages scattered among the tumour cells, giving rise to a 'starry sky appearance' in which nuclear debris from apoptotic tumour cells is discernible. This appearance is not, however, pathognomonic of Burkitt's lymphoma and may be seen in other lymphomas (O'Conor, 1961).

Burkitt-like (small non-cleaved, non-Burkitt's) lymphomas are similar in appearance to Burkitt's lymphoma, but are distinguishable by a greater degree of pleomorphism, a fraction of the cells being similar in size to those of large-cell or centroblastic lymphomas, and a higher frequency of cells with a single large nucleolus in the neoplastic population. This entity may simply represent the borderline between Burkitt's lymphoma and large-cell lymphoma.

Burkitt's lymphoma is invariably of B-cell origin, the presence of surface immunoglobulin being first shown in 1967 (Klein *et al.*, 1967), and has the immunophenotypic characteristics of a subset of germinal-centre cells; hence, the cells do not or very uncommonly express terminal deoxyribonucleotide transferase. B-Cell lineage markers such as CD19, CD20, CD22 and CD79a and surface immunoglobulin are always

demonstrable. The surface immunoglobulin is usually IgM, but IgG and IgA are occasionally present and kappa or lambda immunoglobulin light chains are nearly always detected. Other surface antigens that are expressed in most Burkitt's lymphomas include CD10 and CD77, but CD23 and CD5 are absent (Harris *et al.*, 1994). Burkitt's lymphoma cells express low levels of HLA class I adhesion and activation molecules such as CD54, CD11a/18 and CD58 (Masucci *et al.*, 1987; Billaud *et al.*, 1989; Andersson *et al.*, 1991). Some EBV-negative Burkitt's lymphomas of American origin synthesize and secrete immunoglobulin (Benjamin *et al.*, 1982).

2.1.2 *Descriptive epidemiology*

2.1.2.1 *Historical aspects*

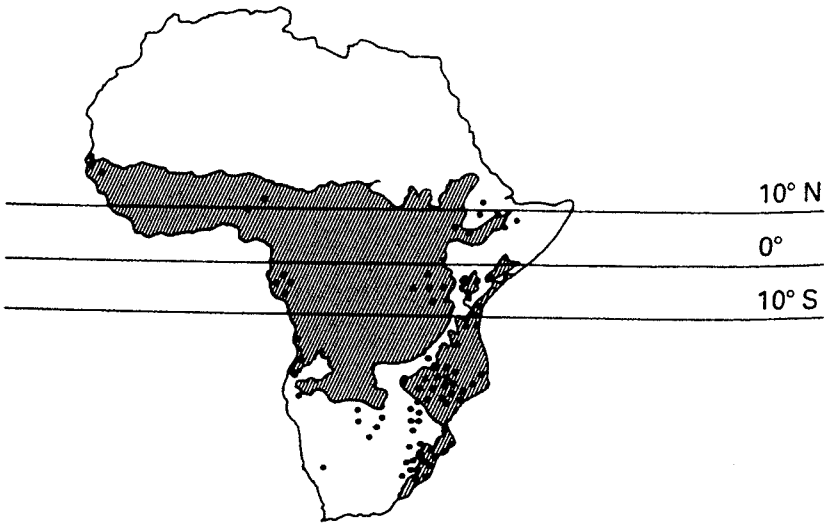
Burkitt's lymphoma was first identified in Africa, where there is every reason to believe that it has existed for millennia. Its presence prior to its description by Europeans is attested to by wooden masks depicting jaw and orbital tumours (Pulvertaft, 1965). It seems probable that the environmental factors relevant to its pathogenesis, with the possible exception of HIV, were relatively constant prior to the lifestyle changes brought about by the technological revolution of this century. The first known medical description is that of Sir Albert Cook who, with his brother, established the first mission hospital in Uganda in 1897. Cook's meticulous records were analysed many years later by Davies *et al.* (1964a,b), who reproduced in their report a drawing made by Cook in 1910 of a malignant jaw tumour in a child. Subsequently, a number of expatriate pathologists working in Africa noted that facial 'sarcomas' and lymphomas occurred at high frequency in African children. Most of these tumours were probably Burkitt's lymphomas.

Smith and Elmes (1934) described a series of 500 malignant tumours collected in Lagos, Nigeria, which included 16 jaw tumours recorded as sarcomas, three of which were in children, and 10 'round-cell sarcomas of the orbit', all in children under 10 years of age. More than a decade later, Davies (1948) observed that neoplasms of the reticuloendothelial system occurred at high frequency in Uganda, and Edington (1956; Edington & Giles, 1968) working in Ghana, then known as the Gold Coast, commented on the relatively high frequency of maxillary lymphosarcoma in children. Thijs (1957) reported from the Belgian Congo that 74 of 145 children with malignant tumours had lymphosarcoma. Interestingly, jaw tumours accounted for only 11 of the latter cases. De Smet (1956), also working in the Belgian Congo, mentioned in his report on children with lymphosarcoma that multiple organ sites, including the maxilla, orbit, abdomen and thyroid, were frequently involved.

Denis Burkitt and his pathologist colleagues, Davies and O'Conor noted, like De Smet, that children with jaw tumours often had histologically similar tumours at multiple organ sites, particularly in the abdomen (Burkitt, 1958; O'Conor & Davies, 1960). They subsequently demonstrated that the tumour occurred at high frequency in a broad belt across Africa, extending approximately 15° N and S of the equator, with a southern prolongation on the eastern side of the continent (Burkitt, 1962a,b,c; Figure 7). While Burkitt was initially under the impression that the tumour he was studying was a

sarcoma, O'Conor and Davies, in a review of the malignant tumours of children in the Kampala Cancer Registry in Uganda, recognized, as had Thijs, that malignant lymphoma accounted for some 50% of all childhood malignant tumours in the registry (O'Conor & Davies, 1960; O'Conor, 1961).

Figure 7. Distribution of Burkitt's lymphoma in Africa



From Haddow (1963)
The shaded area represents the area in which, on climatological grounds, Burkitt's lymphoma might be expected to occur. The black points show the distribution of the series of cases compiled by D. Burkitt. The method used was to fill in any degree-square in which the condition had been recorded, irrespective of the number of cases.

Soon after these observations in Africa, O'Conor, Wright (who had also worked in Uganda) and others reported histologically indistinguishable tumours in children in Europe and the United States (Dorfman, 1965; O'Conor *et al.*, 1965; Wright, 1966). At about the same time, strong similarities between the distribution of Burkitt's lymphoma and that of yellow fever were seen (Burkitt & Davies, 1961; Haddow, 1963), and it was therefore suggested that Burkitt's lymphoma might be caused by a vectored virus.

2.1.2.2 *Incidence*

Estimates made some 30 years ago suggested a relatively high incidence rate of Burkitt's lymphoma in Africa, with about 5–10 cases per 100 000 children below the age of 16 years and a peak incidence between five and 10 years of age. In Nigeria, the incidence in 1960–63 was reported to be 22 per 100 000 in 5–9-year-old boys and 10 per 100 000 in girls (Edington & MacLean, 1964). Williams (1975) found that Burkitt's lymphoma accounted for 682 of 1325 tumours in a hospital-based tumour registry in Ibadan, Nigeria, in 1960–72.

Burkitt's lymphoma now accounts for 30–70% of childhood cancers in equatorial Africa. In the United States, the incidence of Burkitt's lymphoma is 2–3 per million children per year. Recent estimates of incidence rates worldwide range from zero, which

may be due to differences in nomenclature or diagnostic practices, to 3.6 per 100 000 per year.

2.1.2.3 *Climatic determinants*

The very high frequency of Burkitt's lymphoma in equatorial Africa and its climatically determined distribution, first described in the early 1960s, focused attention on the possibility of a causal association with an environmental agent. The evidence that malaria is such an agent is based largely on the similarity of the distribution of holoendemic malaria and African Burkitt's lymphoma and on associations between rates of parasitaemia and the likelihood of developing Burkitt's lymphoma (see section 2.1.4.1).

The peri-equatorial distribution of patients in Africa with a clinical syndrome consistent with Burkitt's lymphoma was established by the initial investigations of Burkitt and several colleagues throughout Africa and on responses on questionnaires administered in a large number of hospitals on the continent (Burkitt, 1962a,b,c; Haddow, 1963; Burkitt, 1985). These investigators observed that the tumour occurred in a belt across equatorial Africa, with a prolongation to the south-east (Mozambique and Natal) (see Figure 7). Within this region, however, the tumour was rare or absent in a number of densely populated areas (Burkitt, 1963, 1969, 1970a). These included south-west Uganda, Rwanda and Burundi, the highlands of Kenya and the United Republic of Tanzania, the islands of Pemba and Zanzibar and some parts of Zaire. Several sharply delineated transitions from high to low incidence were observed, the majority between river valleys or lake shores and highland regions. In Nigeria, the relative rarity of the tumour in the arid (30 in [76 cm] of rainfall per year between the months of June and September) but densely populated northern region, Kano, contrasted with its much greater prevalence in wetter (up to 400 in [10 m] of rainfall per year), more sparsely populated regions less than 400 miles [640 km] to the south. Interestingly, this pattern applied to West Africa in general, the tumour being common throughout the southern parts of these countries (Accra in Ghana being an exception) but rare in the north. With some exceptions (e.g. the islands of Pemba and Zanzibar, Kinshasa, Brazzaville and Lambarene), the distribution was clearly determined by altitude in East Africa and by rainfall in West Africa. Thus, in East Africa, the tumour did not occur at any notable frequency above 1500 feet (450 m), or in regions where the mean temperature fell below 15.5 °C in any month (Haddow, 1963), and in West Africa the tumour did not in occur in regions in which the annual rainfall was less than 50 cm.

Burkitt and Wright (1966) showed in their series of histologically proven cases that the incidence of the tumour was much higher in the northern, eastern and central regions of Uganda. In Kigezi, in the south-west, for example, the incidence of 0.6 per 100 000 was estimated to be one-twentieth of that in the north-west. The low-incidence areas are highlands over 5000 feet (1500 m) above sea level, e.g. Kigezi, which continues westwards into the high central plateau that forms much of Rwanda and Burundi and extends into north-western Tanzania. Less than 4% of the cases in the Kampala registry occurred in children living in the south-western districts, which accounted for 25% of the population of Uganda; however, some 20% of the population of the central region of Uganda,

Buganda, is comprised of peoples from the low-incidence south-western region (Rwanda, Burundi and Kigezi), and about 19% of the cases of Burkitt's lymphoma recorded at Mulago Hospital up to the time of the report occurred in these immigrant peoples. Moreover, the average age of patients in the immigrant groups was 16.5 years, while that of patients from high-incidence areas was 8.1 years. Some of the immigrants with Burkitt's lymphoma had been living in the area for fewer than three years. The average age of patients from low-incidence areas was also higher than that of patients from high-incidence areas. These data strongly suggested that differences in the geographical distribution of the tumour were not a consequence of genetic differences among the tribes of Uganda but were related to environmental differences, being entirely consistent with the previously described barriers of temperature (altitude) and rainfall.

Haddow (1963) subsequently refined the map of the distribution of Burkitt's lymphoma in relation to climatic parameters and pointed out that the high-frequency regions were those in which the rainfall was more than 20 in (50 cm) per year (Figure 7). This finding is consistent with the hypothesis that Burkitt's lymphoma is caused by a vectored virus, since investigators at the East African Virus Research Institute in Entebbe, which Haddow directed, had shown that the yellow fever virus cannot replicate in mosquito vectors when temperatures fall below 60 °F (15 °C), accounting for the virtual absence of yellow fever in people living above 5000 feet (1500 m). In addition, adult mosquitoes do not survive in dry weather, but rely upon drought-resistant eggs in regions prone to dry spells. In West Africa, such regions were defined as those in which the annual rainfall was less than 20 in (50 cm). By using maps in which the distribution of Burkitt's lymphoma was superimposed on isotherms and isohets, it was possible to determine that only 5% of cases of Burkitt's lymphoma fell outside these limits of temperature and rainfall.

The distribution of Burkitt's lymphoma was also shown (Burkitt, 1963) to be very similar to that of a mosquito-vectored arbovirus disease, *o'nyong nyong* fever, in Uganda (Shore, 1961). Thus, it seemed highly probable that insect vectors were in some way involved in transmission of the disease. Tumour-free areas were readily explained on the basis of the absence of the vector, the vectored microorganism or both.

Wright and Roberts (1966), who studied 324 histologically proven cases of Burkitt's lymphoma seen in Uganda between 1959 and 1964 and 425 cases of other types of lymphoma seen during the same period, showed that the remarkably precise climatic determinants of the distribution of Burkitt's lymphoma did not apply to the distribution of other types of lymphoma in Uganda, which varied, instead, solely with the density of the population. Similar data were reported from northern Nigeria by Edington (1978) in respect of other malignant lymphomas.

Dalldorf *et al.* (1964) reported on the distribution of Burkitt's lymphoma in Kenya. The lowest incidence was found in the Kalenjin tribe, living in the highland belt (above 5000 feet [1500 m]), and the highest incidence in coastal or lake-shore dwelling tribes (below 5000 feet). There was no significant difference in the incidence of squamous-cell carcinoma among these tribal groups. These authors also reported on exposure to natural radiation, to arboviruses (by serology) and to malaria. Children of the Kalenjin tribe had

a variable rate of splenomegaly, this region being considered to be free of malaria in the recent past, in contrast to coastal tribes and the Luo, in regions where malaria was considered to be holoendemic. The prevalence of malaria in these tribes was confirmed by the rates of sickle-cell trait (negatively correlated) and glucose-6-phosphate dehydrogenase deficiency (positively correlated) (Bienzle *et al.*, 1981).

Eshleman (1966) reported on 31 cases of Burkitt's tumour seen at the Shirati Hospital in the United Republic of Tanzania. All of the patients came from lowland areas, less than 4500 feet (1350 m) above sea level, although the other patients seen at the hospital came from the Mara region with a height above sea level varying from 3700 to 5500 feet (1110–1650 m).

Goma (1965) carried out a survey of the environment in Uganda within a two-mile zone of 21 huts in which cases of Burkitt's lymphoma had occurred. He found permanent or semi-permanent water nearby and usually dense vegetation — ideal breeding conditions for mosquitoes. Goma easily trapped mosquitoes from the vicinity of the huts and was able to identify 42 different species. Williams (1967) pointed out that, among the large variety of arthropods known to transmit human diseases, only *Mansonia* and *Anopheles* species had similar distributions in Uganda to Burkitt's lymphoma.

More recent data (Kitinya & Lauren, 1982) confirm the relationship between the incidence of Burkitt's lymphoma and altitude. On Mount Kilimanjaro in northern Tanzania, the relative frequency of Burkitt's lymphoma (2.2% of all malignancies) was less than that in the coastal and low-lying regions of Uganda and Tanzania. Moreover, on the slopes of Mount Kilimanjaro, cases of Burkitt's lymphoma occurred predominantly in the sparsely populated areas below 1000 m, although some cases were found in densely populated areas up to 1500 m. As in earlier studies in East Africa, the average age of patients from this low-incidence area was higher (16.4% were over 20 years of age) than that of patients from high-incidence areas (in Uganda, for example, only 5% of patients were over 20), while the frequency of jaw tumours was lower (22%, compared with 55% or more in Uganda).

2.1.2.4 *Time–space clustering*

Pike *et al.* (1967) first reported on time–space clustering of Burkitt's lymphoma in the West Nile District of Uganda between 1961 and 1965. They concluded that patients whose dates of onset were close tended to live close together. This finding was highly statistically significant and suggested an 'epidemic' character.

Williams *et al.* (1969) provided further evidence of time–space clustering in the West Nile District, refining previously collected information and extending the study period to 1967. While the new data confirmed the results of the earlier analysis, the authors reported that an unpublished analysis conducted in the East and West Mengo districts in central Uganda did not show a similar phenomenon.

Baikie *et al.* (1972) subsequently reviewed the evidence for clustering of Burkitt's lymphoma. Of particular interest is a cluster observed in Bwamba County, in a low-incidence area in the south-west of Uganda, close to the border with the Congo. The only seven cases known to have occurred in the county were diagnosed between October 1966

and December 1968. Two of these cases were in a brother and sister, and five occurred during a six-month period from July to December 1968. No other clusters, apart from those in the West Nile, were observed in either Uganda or Tanzania (Brubaker *et al.*, 1973).

2.1.2.5 *Familial cases*

X-Linked lymphoproliferative syndrome, characterized by an abnormal response to EBV, predisposes males to the development of lymphomas, some of which may resemble Burkitt's lymphoma. Familial Burkitt's lymphomas, apparently unrelated to this syndrome and in some cases associated with a familial predisposition to other tumours, such as chronic myeloid leukaemia and nasopharyngeal carcinoma, have also been described.

Brubaker *et al.* (1980) reported the occurrence of Burkitt's lymphoma in North Mara District, Tanzania, in five families in which several members were afflicted, sometimes with another malignancy. The families in which multiple cases of Burkitt's lymphoma had occurred included two brothers and a half-brother and two brothers. In two other families, there were cases of chronic myeloid leukaemia and of Burkitt's lymphoma, in young siblings in one family and in a father and a son in the other; in another family, one woman who had nasopharyngeal carcinoma had a son with Burkitt's lymphoma. Nasopharyngeal carcinoma and chronic myeloid leukaemia are both rare tumours in the North Mara region.

Stevens *et al.* (1972) described two siblings with Burkitt's lymphoma, one of whom had Burkitt's leukaemia, in the United States. Joncas *et al.* (1976) described Burkitt's lymphoma in first-degree cousins, a young woman aged 16 and a young man of 17, in a large French-Canadian family. Two of the siblings of one of the patients with Burkitt's lymphoma had nasopharyngeal carcinomas, and an additional first-degree cousin had a plasmacytoma. Several siblings and cousins had low IgA values. Poulsen *et al.* (1991) described a case of EBV-negative Burkitt's lymphoma of the breast in two sisters aged 18 and 21 years in Denmark.

Familial Burkitt's lymphoma has also been described in three families in Papua–New Guinea (Winnett *et al.*, 1997). The first family had three cases of Burkitt's lymphoma between 1964 and 1965 in full brothers. In the second, two cases of Burkitt's lymphoma occurred between 1983 and 1984 in a full brother and sister; and in the third family, three cases arose between 1980 and 1983 in two sons and one daughter of one man and his two wives.

2.1.3 *Epidemiology of Burkitt's lymphoma in association with EBV*

The EBV was identified in an attempt to confirm the hypothesis that African Burkitt's lymphoma is caused by a vectored virus. Epstein (1985) examined tumour samples sent by Burkitt by electron microscopy, as the viral culture systems available at the time gave negative results. No virus was found, however, despite the most exhaustive searches, until he and his colleagues, Achong and Barr, succeeded in growing cell lines from the tumours. They believed that the virus could grow in cells that did not have host defences,

as is the case for avian tumour virus. Viral particles with a morphology consistent with that of the herpes family were identified in the first grid square (Epstein *et al.*, 1964). Subsequently, antigenic relationships with other herpesviruses, including the Lucké virus, Marek's disease virus and infectious bovine rhinotracheitis, were demonstrated by immunological methods (Ono *et al.*, 1970; Stevens *et al.*, 1971; Evans *et al.*, 1972). Evidence that this virus — EBV, as it came to be known — is frequently associated with Burkitt's lymphoma was based initially on serological evidence, made possible by the development of immunofluorescence tests for viral antibodies by Werner Henle and Gertrude Henle (1966). This test was later shown to reflect the level of VCA in the cells; cells that gave positive results in immunofluorescence assays were shown to contain viral particles by electron microscopy (Henle & Henle, 1967; Epstein & Achong, 1968). It was found, however, that control sera also generally showed anti-EBV antibodies, and the late viral antigens detected by the fluorescence antibody tests were rarely, if ever, detected in fresh tumour cells, although they were detected in a small percentage of cells in cell lines derived from tumours. Confirmation that viral genomes are present in every tumour cell therefore had to await the development of fluorescence tests for latent antigens and in-situ hybridization techniques.

zur Hausen and Schulte-Holthausen (1970) were the first to detect EBV DNA, using a nucleic acid hybridization technique, in a cell line in which viral particles could not be demonstrated. This result was then confirmed by Nonoyama and Pagano (1971). These results strongly suggested that viral DNA is present in cells in which late viral antigens or viral particles cannot be detected, indicating that the virus must be able to latently infect cells, i.e. remain within the cell without replicating viral particles.

2.1.3.1 *Case series*

Differences in the frequency of the association of Burkitt's lymphoma with EBV, as determined by the presence of EBV DNA (or RNA) or EBV antigens, are found in different regions of the world, as reflected in differences in serology. Table 7 gives the results found in case series in endemic and non-endemic areas of the world with respect to the association between Burkitt's lymphoma and markers of infection with EBV.

(*a*) *African patients*

Some 95% of African Burkitt's lymphomas are associated with EBV, as demonstrated by the presence of either EBNA or EBV DNA in the tumour cells, and such patients have higher geometric mean titres of antibodies against EBV-associated antigens. For some time, investigators had recognized the presence of complement-fixing antibodies in the sera of patients with Burkitt's lymphoma (Armstrong *et al.*, 1966). Pope *et al.* (1969) subsequently identified an EBV-associated complement-fixing antigen in soluble extracts of EBV-transformed lymphoblastoid cell lines and the Raji cell line, then thought to be 'virus free'. Reedman and Klein (1973) identified these antigens at the cellular level with an anticomplement immunofluorescence test in the nuclei of over 90% of the cells of EBV-transformed lymphoblastoid cell lines, regardless of whether they produced virus or not, and in a similar fraction of cells from two Burkitt's lymphoma biopsy samples. Comparison with other antibodies against EBV in 52 sera used to demonstrate the

antigen showed that the 32 with anti-VCA and complement-fixing antibodies also showed anticomplement immunofluorescence. Five of six sera which had no anti-VCA but had complement-fixing antibodies gave positive or equivocal results for anticomplement immunofluorescence, and 14 sera that had no anti-VCA and no complement-fixing antibodies gave negative results. A variety of control cells also gave negative results. This complement-fixing antigen became known as the EBV nuclear antigen (EBNA).

Table 7. Association between Burkitt's lymphoma and EBV in endemic and non-endemic regions

Country	No. of cases	Association with EBV	Reference
Endemic areas of Africa			
	23	EBV DNA in 22 (low level in 1 case)	Nonoyama *et al.* (1973)
	13	11 EBNA-positive	Reedman *et al.* (1974)
	27	EBV DNA in 26; 25 EBNA-positive	Lindahl *et al.* (1974)
Cameroon and Gabon	10	EBV DNA in 10	Prévot *et al.* (1992)
Ghana	4	EBV RNA identified	Dambaugh *et al.* (1979)
	23	EBV DNA in 23	Shiramizu *et al.* (1991)
Uganda	27	EBNA-positive; satisfactory biopsies	Olweny *et al.* (1977)
	15	EBV DNA in 14; all EBNA-positive	
	5	All EBNA-positive; IF on extracted DNA	Luka *et al.* (1978)
Malawi	44	42 EBER-1- or *Bam*HI W-positive by ISH	Labrecque *et al.* (1994)
Non-endemic areas			
Americas			
Argentina	16	EBER-1 in 4	Drut *et al.* (1994)
Argentina, Chile	27	EBV DNA in 13 (48%)	Gutiérrez *et al.* (1992)
Brazil	12	EBV DNA in 7 (58%)	
Brazil	24	EBER-1 in 17 (71%)	Bacchi *et al.* (1996a)
Brazil	54	47 EBER-1 positive (87%)	Araujo *et al.* (1996)
Cuba	7	6 VCA and EA(R)-positive	Riverend *et al.* (1984)
USA	4	No EBV DNA	Pagano *et al.* (1973)
USA	1	EBV DNA present	Gravell *et al.* (1976)
USA	12	EBV DNA in 5	Andersson *et al.* (1976);
	20	EBV DNA in same 5	Ziegler *et al.* (1976)
USA	3	EBV DNA in 3	Judson *et al.* (1977)
USA	32	EBV DNA in 12 (38%)	Shiramizu *et al.* (1991)
Asia			
China	18	EBER-1 in 5 (28%)	Chan *et al.* (1995a)

Table 7 (contd)

Country	No. of cases	Association with EBV	Reference
Non-endemic areas (contd)			
Egypt	41	30 EBER-1-positive (73%)	Anwar *et al.* (1995)
India	2	EBNA-positive	Venkitaraman *et al.* (1983)
Japan	14	2 EBNA-positive	Miyoshi (1983)
Japan	7	EBV DNA in 4	Okano *et al.* (1992)
Europe			
France	67	EBV DNA in 24%	Philip (1985)
Germany	1	EBV DNA present	Bornkamm *et al.* (1976)
Germany	1	EBNA-positive	Kachel *et al.* (1980)
Italy	15	EBV DNA in 1/12	Tirelli *et al.* (1984)
Sweden	1	EBV DNA present	Biberfeld *et al.* (1981)
Turkey	15	EBV DNA in 14	Çavdar *et al.* (1994)
Oceania			
Australia	1	EBNA-negative	Roeser *et al.* (1977)

EBNA, EBV nuclear antigen; EBER, EBV-encoded RNA; IF, immunofluorescence; ISH, in-situ hybridization

Nonoyama *et al.* (1973) reported that 22 of 23 biopsy samples from African patients with Burkitt's lymphoma contained EBV DNA as ascertained by nucleic acid hybridization, most containing 15–113 genome copies per cell (average, 38). Reedman *et al.* (1974) subsequently examined biopsies from 19 African Burkitt's lymphomas by complement-fixation tests and anticomplement immunofluorescence for EBV-associated antigens. Extracts from 12 of these tumours reacted in complement fixation tests with EBV-seropositive sera but not with EBV-seronegative sera. Eleven of 13 biopsy samples showed anticomplement fluorescence, and the reactive antibodies could be absorbed out with lymphoblastoid cell lines. In all cases, the fluorescence was coarsely granular and present in almost all the tumour cells. The remaining biopsy samples were considered not to contain EBV. None of 13 samples from tumours other than Burkitt's lymphoma showed anticomplement immunofluorescence.

Lindahl *et al.* (1974) reported on the correlation between the presence of EBNA and the detection of EBV DNA by nucleic acid hybridization with complementary RNA probes. Twenty-six of 27 histologically confirmed cases of African Burkitt's lymphoma were shown to contain 10–101 viral genomes per cell (average, 39) by hybridization, and 25 of these 26 cases also contained EBNA.

Magrath *et al.* (1975) reported on a patient who presented with Hodgkin's disease and then Burkitt's lymphoma. The patient had a high titre of anti-VCA (> 1:320) but not anti-EA at the time of presentation with Hodgkin's disease. The anti-VCA titres continued to rise, albeit at a slow pace, over the ensuing months, to > 1:1250, and those of anti-EA and anti-EBNA increased rapidly to 1:1250 shortly before presentation of widespread

Burkitt's lymphoma nine months later. Anti-membrane antigen antibodies, although continuously present, did not change in titre in association with the onset of Burkitt's lymphoma. This case demonstrates that antibody titres to EBV are raised many months before the onset of Burkitt's lymphoma, consistent with the results of the prospective study in the West Nile District, and also that rapid changes in EA titres may occur at the time of tumour growth.

Olweny *et al.* (1977) examined the expression of EBNA in 34 patients with Burkitt's lymphoma in Uganda and 25 patients with other malignancies (predominantly other lymphomas). All 27 biopsy samples from cases of Burkitt's lymphoma considered to be in satisfactory condition contained EBNA. Fourteen of 15 of these samples examined by nucleic acid hybridization were shown to contain EBV DNA, with a mean genome copy number of 39. The one case in which fewer than two genome copies (the level of detection of the test) were found was serologically negative for EBV antibodies and was in a patient who had lived in a malaria-free highland area before migrating two months before the diagnosis of Burkitt's lymphoma to a malaria-endemic region. None of the 25 non-Burkitt's lymphoma biopsy specimens contained EBNA, and all 15 subjected to nucleic acid hybridization showed no EBV DNA. The results of EBV serology were consistent with those reported previously, the anti-VCA titre being fourfold higher and anti-EA being detectable in 59% of the group with Burkitt's lymphoma.

Luka *et al.* (1978) examined five biopsy samples from Burkitt's lymphoma patients in Africa previously shown to contain EBNA by standard anticomplementary immunofluorescence, DNA extraction, separation on DNA–cellulose columns and fixation to chicken erythrocytes before immunofluorescence testing. All five samples contained EBV DNA.

Dambaugh *et al.* (1979) studied four Burkitt's lymphoma biopsy samples from Accra, Ghana, and demonstrated RNA homologous to at least 3–6% of the DNA of EBV; they also identified the general regions of the EBV genome from which the RNA was transcribed.

Prévot *et al.* (1992) studied tissues from 12 cases of Burkitt's lymphoma and 12 cases of non-Burkitt's lymphoma from Cameroon and Gabon by in-situ hybridization with a DNA probe derived from the *Bam*HI W repeat region of EBV. All 10 samples from Burkitt's lymphoma considered technically satisfactory were shown to contain EBV DNA, and all 12 of the non-Burkitt's lymphoma samples gave negative results.

Shiramizu *et al.* (1991) studied tissues from 23 cases of Burkitt's lymphoma from Ghana by hybridization with a probe derived from the *Bam*HI K fragment of the EBV genome. All 23 samples showed the presence of EBV DNA, which was confirmed with probes from the terminal repeat region of EBV.

Labrecque *et al.* (1994) in Malawi used in-situ hybridization on material obtained from Burkitt's lymphomas by aspiration biopsy, with two probes: EBER-1 and the *Bam*HI W fragment of EBV. Of 66 cases of suspected Burkitt's lymphoma, 44 were confirmed cytopathologically, and 42 showed the presence of EBV DNA with EBER-1 and/or *Bam*HI W. Of the 22 cases considered not be Burkitt's lymphoma, only one contained EBV.

(*b*) *Non-African patients*

Some 10–30% of European and American tumours appear to be associated with EBV, while 50–90% of tumours in developing countries are EBV-associated. The fact that only a proportion of cases are associated with EBV should be borne in mind when interpreting the results of serological studies, as the available data indicate that only patients with EBV present in the tumour cells have raised antibody titres to EBV. Thus, serological results reflect the fact that these series include both EBV-associated and EBV-negative cases. Furthermore, age may be an important factor in the frequency of association with EBV.

Goldberg and Drut (1986) reviewed their 16-year experience of Burkitt's lymphoma in Argentina; 73% of patients had elevated antibody titres to EBV. Drut *et al.* (1994) examined 16 Argentine patients with Burkitt's lymphoma aged 2–8 years for EBV by PCR and in-situ hybridization (using a NotI/PstI fragment). Four cases gave positive results in both tests, 70–90% of the cells giving a signal in in-situ hybridization. Interestingly, all of these cases were seen in the same year, 1984. Gutiérrez *et al.* (1992) reported 39 cases of Burkitt's lymphoma from Argentina, Chile and Brazil, mostly in children, the age range being 1–28, with only two patients over 15 years of age. Overall, 51% of these cases (48% from Argentina and Chile and 58% from Brazil) were EBV-associated, as determined by Southern blotting. The EBV in these tumours was shown with the EBV terminal-repeat region probe to be monoclonal.

Bacchi *et al.* (1996a) reported the results of in-situ hybridization for EBER-1 in 24 cases (mostly in children, but some ages unknown) of Burkitt's lymphoma from Campinas and São Paulo in Brazil. Seventeen (71%) gave positive results, the signal being present in virtually all of the neoplastic cells of each case. Only one of these patients had jaw involvement. Araujo *et al.* (1996) described 54 children (median age, six years) with Burkitt's lymphoma, four of whom had mandibular tumours, in Bahia, in tropical Brazil and reported the results of in-situ hybridization with an EBER-1 probe. Forty seven (87%) of the cases showed the presence of EBV DNA. Interestingly, the fraction of positive cases appeared to be related to age: 39 of 41 patients under nine years of age and six of 11 aged nine years or more had EBER-1-positive tumours. Essentially 100% of the cells showed positive results. LMP-1 was not expressed in the tumours, except in a few cells in proximity to ova of *Schistosoma mansoni* in two cases. Of 27 EBV-positive tumours tested for EBV subtype, 20 (74%) were type 1 and the remaining seven (26%) were type 2. This result is intermediate between that of African Burkitt's lymphoma (50% type 1) and North American Burkitt's lymphoma (10% type 2) (Goldschmidts *et al.*, 1992).

Riverend *et al.* (1984) described seven children with Burkitt's lymphoma in Cuba. Five of the patients had high antibody titres to VCA (1:640–1:5120) and to EA(R) (1:10–1:2560), one had low titres to these antigens (1:20 and 1:5, respectively) and one had negative titres. Interestingly, three of the patients with high antibody titres had jaw tumours.

In spite of the serological evidence that Burkitt's lymphoma in the United States is also EBV-associated, Pagano *et al.* (1973) failed to detect hybridizable EBV DNA in

tumour-infiltrated tissue from four cases of Burkitt's lymphoma; however, Gravell *et al.* (1976) detected EBV DNA in an American patient with Burkitt's lymphoma.

Andersson *et al.* (1976) and Ziegler *et al.* (1976) studied an additional 12 American patients with Burkitt's lymphoma. Nine of the patients had low anti-VCA titres, but two had titres of 1:320 and 1:160 and one gave a negative result. Five of the tumours had EBV DNA that was readily detectable by liquid hybridization studies; the remainder had less than one genome copy per cell. These data suggested that the majority of American Burkitt's lymphomas lack EBV DNA. This suggestion was confirmed by Shiramizu *et al.* (1991), who studied tissues from 32 cases of Burkitt's lymphoma in the United States by hybridization with a probe derived from the *Bam*HI K fragment of the EBV genome. Twelve samples (38%) showed the presence of EBV DNA, which was confirmed with probes from the terminal repeat region of EBV. Judson *et al.* (1977), however, described four patients with Burkitt's lymphoma living within 50 km of each other in the United States. All showed anti-EBV antibodies similar to those observed in African patients (e.g. anti-VCA titre > 1:640 in all cases). Similarly, viral DNA and EBNA were found in tumour samples from three of the patients; the fourth was not tested.

Chan *et al.* (1995a) reported on the association between EBV and Burkitt's lymphoma in a Chinese population in Hong Kong. Among 18 patients who ranged in age from 4 to 85 years (median, 35.5), five aged 4, 6, 18, 57 and 58 years showed EBER-1 by in-situ hybridization in essentially all tumour cells. There were no cases of jaw tumour. Two cases (a T cell-rich large B-cell lymphoma and an anaplastic large-cell lymphoma) out of 54 of non-Hodgkin's lymphoma also showed positive results, the Reed-Sternberg-like cells found in one case being positive.

Anwar *et al.* (1995) found positive results by in-situ hybridization in 30 of 41 patients with Burkitt's lymphoma in Egypt.

Venkitaraman *et al.* (1983) reported on two Indian cases of Burkitt's lymphoma in children aged 5 and 11 years. Their anti-VCA titres were 1: 640 and 1:80; those of anti-EBNA were 1:1280 and 1:320, and those of anti-EA(R) were 1:80 and < 1:5, respectively. The presence of EBV in the tumour cells was not assessed.

Miyoshi *et al.* (1978) reported a case of EBNA-positive Burkitt's lymphoma in a person aged 29 years in Okayama, Japan, and referred to an earlier EBV-negative case (Tanaka *et al.*, 1976; Miyoshi *et al.*, 1977). Miyoshi (1983) subsequently described 14 patients (age range, 4–52 years), including these two cases, with Burkitt's lymphoma, five of which were jaw tumours. Two were EBV-seronegative, nine had anti-VCA titres of 1:40–1:1280, and three were not tested. The anti-EBNA titres in the six tested ranged from 1:20 to 1:80. Four of these patients had bone-marrow involvement at the time of presentation. Two of 14 tumours tested for EBNA by anticomplement immunofluorescence showed positive results. Okano *et al.* (1992) reported on seven patients in Hokkaido, Japan, aged 4–39 years. Five had anti-VCA titres of ≥ 1:320, including three cases with anti-VCA titres of 1:2560 and one with a titre of 1:320, with correspondingly high titres of antibodies to EA; two had titres of 1:40 and 1:80 and no anti-EA antibodies.

Four of these seven cases were EBNA-positive and three of the six tested showed EBV DNA by nucleic acid hybridization.

Philip (1985) mentioned the unpublished results of G. Lenoir showing that 16 of 67 Burkitt's lymphomas in Caucasian patients were EBNA-positive; 42 of the patients were from Lyon, France, of whom 6% had EBNA. Bornkamm *et al.* (1976) studied a single patient among a series of lymphoma patients in Germany and detected EBV DNA by reassociation kinetics. A single case of EBV-associated Burkitt's lymphoma that was EBNA-positive, with high titres to anti-VCA and anti-EA (1:2056), was reported in a 34-year-old woman in Germany (Kachel *et al.*, 1980). Among 15 patients, five of whom were 15 years of age or less, with Burkitt's lymphoma reported in north-east Italy, only one was serologically positive for EBV among 12 patients tested (Tirelli *et al.*, 1984). Biberfeld *et al.* (1981) reported an EBV-associated case in a 25-year-old man in Sweden. Çavdar *et al.* (1994) found EBV DNA by Southern blotting in 14 of 15 tumours examined in Turkey.

Roeser *et al.* (1977) studied a single Australian case of Burkitt's lymphoma and found the patient to be EBV-seronegative; no EBNA was found in the tumour cells.

In summary, the frequency of EBV association in Burkitt's lymphoma varies with geographical location. In most developed countries, some 20% or less of tumours are positive in most series, while in equatorial Africa about 95% are positive. In temperate regions in South America, such as Argentina and Chile, the rate of EBV association is lower than in tropical regions in the north of the continent; however, differences in the climate and in socioeconomic circumstances may account for the apparent differences in EBV association. If age at EBV infection is a primary determinant of association, socioeconomic or lifestyle factors are likely to be of paramount importance. Too few data are available to assess the importance of age as a determinant of EBV status in countries in which the frequency of EBV-negative cases is sufficiently high to make such an investigation worthwhile.

2.1.3.2 *Case–control studies*

Because the prevalence of antibodies to EBV changes with age (see section 1.4), cases and controls should be closely matched for age; however, in most of the studies described below, the closeness of age-matching is not stated.

(*a*) *African patients*

Henle *et al.* (1969) compared antibody titres to EBV (using an indirect immunofluorescent test for IgG antibodies to Burkitt's lymphoma tumour cells) in sera from Burkitt's lymphoma patients and various comparison groups, including 94 'recent onset' cases from East Africa (58 from Nairobi, Kenya and 36 from Kampala, Uganda). The comparison groups [selection criteria unspecified] comprised 62 children who were matched to cases for age, sex and tribe, 72 siblings and neighbours aged 1–15 years, 62 children from 'regions of high Burkitt's lymphoma incidence', 50 children from 'regions of low Burkitt's lymphoma incidence', 113 children aged six months to five years from 'well-baby' clinics in Nairobi and 130 patients without cancer from the paediatric wards

of Kenya Hospital, Nairobi. All of the Burkitt's lymphoma patients had EBV antibody titres ≥ 1:10, 87.2% having titres of ≥ 1/160. The geometric mean titre (GMT) was 1:326 (1:382 in Kenya, 1:243 in Uganda). In the 'control' groups, 18% had no antibody (13% in age-, sex- and tribe-matched controls), and only 14% had titres ≥ 1:160 (11% in age-, sex- and tribe-matched control [odds ratio, 54]). The GMT in the control children was 1:37 (1:36 in age-, sex- and tribe-matched controls).

Klein *et al.* (1970) investigated the presence of anti-EBV antibody in sera from 19 confirmed cases of Burkitt's lymphoma and 27 controls [selection criteria unspecified, but probably not matched in any way to the cases], using intracellular immunofluorescence in fixed smears of EBV-carrying lymphoblastoid (P3J) cell lines (anti-EBV), blocking of membrane fluorescence or immunoprecipitation against a soluble antigen from an EBV-carrying cell line. Of the 19 patients with confirmed Burkitt's lymphoma, 15 were highly reactive in all three tests, while only three of the 27 controls were; 19 of the controls had low titres in all three tests.

Henle *et al.* (1971b) compared the anti-EA antibody titres in sera from 156 Burkitt's lymphoma patients recruited from hospitals in Kampala, Uganda, and Nairobi, Kenya, with those in sera from 200 control children from the West Nile District of Uganda. The controls were selected to include children with low or absent titres of anti-VCA titre [but presumably were not matched to the cases]. At each level of anti-VCA titre, more of the Burkitt's lymphoma cases were anti-EA-positive. [The Mantel-Haenszel odds ratio (stratified for anti-VCA titre) associated with positive anti-EA is 19 (95% confidence interval [CI], 9.6–39)].

Hirshaut *et al.* (1973) studied cases of Burkitt's lymphoma from Africa (as well as from the United States, see below) with respect to anti-EBV antibody (measured by immunofluorescence and immunodiffusion) and compared them with age- and sex-matched controls from the same district and parents and siblings. When tested by immunofluorescence, EBV antibody was present in all 21 cases tested and in 21/27 of the age- and sex-matched controls, 76% of cases and 12% of controls having titres > 1:640 (odds ratio, 26; [95% CI, 5.3–120]). When tested by immunodiffusion, 67 of 73 cases and 19/30 controls gave positive results (odds ratio, 6.5; [95% CI, 2.1–20]).

Nkrumah *et al.* (1976) examined sera from 141 patients with Burkitt's lymphoma in Ghana. For a subset of 75 patients, they compared the anti-VCA antibody titres with those of 54 siblings and 50 age- and sex-matched neighbourhood controls. The GMT of antibody in the cases was significantly higher (1:424; $p < 0.001$) than that in either siblings (1:56) or neighbours (1:62).

(*b*) *Non-African patients*

Levine *et al.* (1972) examined patients with histologically identified Burkitt's lymphoma in the United States for anti-VCA antibodies against EBV. Twenty-four of 29 cases gave positive results, in comparison with 31/57 age- and sex-matched controls, and the antibody GMTs were higher (1:94) than in controls (1:11) or in 26 patients with lymphoblastic leukaemia (1:16), but were not as high as in African Burkitt's lymphoma patients (1:3338). A subset of patients under eight years of age all had anti-VCA anti-

bodies, however with a GMT of 1:425 (in comparison with 1:2848 in 11 African children), which was markedly higher than that in the 34 controls (1:4), 62% of whom had no anti-VCA.

Hirshaut *et al.* (1973) compared the EBV antibody titres of 15 patients with Burkitt's lymphoma in the United States with those in 15 age- and sex-matched controls and 25 parents and siblings of the cases. As ascertained by immunofluorescence, 10 cases and seven controls had antibodies. Although two patients and one control had antibody levels ≥ 1:640, the GMT was not significantly different between the two groups (5.2 versus 4.4). When 16 cases and 16 controls were ascertained by immunodiffusion, seven cases and six controls were found to have anti-EBV antibodies.

The data of Levine *et al.* (1972) were extended by Ablashi *et al.* (1974), who studied a wider range of antibodies in 21 patients in the United States with Burkitt's lymphoma, and compared them with 10 control subjects. [It is not clear how the controls were selected; one was a relative of a case.] All 15 sera tested for anti-VCA, anti-EA, anti-EBNA and complement-fixing antibodies had titres of at least one of the antibodies, whereas only one of seven control sera did. Five of the case sera did not show anti-VCA antibodies, but three of these sera had antibodies against EBNA. The GMTs for the patients were 1:111 for VCA, 1:17 for EA, 1:4.2 for complement fixation and 1:59 for EBNA; those of controls were 1:24, 1:2.7, 1:2.6 and 1:1.5, respectively.

Gotlieb-Stematsky *et al.* (1976) reported on 16 children with Burkitt's lymphoma in Israel. Although some of the 10 Arab children did not have elevated antibody titres against EBV (only one had negative serology), elevated titres against VCA were observed in the Burkitt's lymphoma patients, four having titres ≥ 1:160 (GMT, 1:26), whereas controls matched for age, sex and ethnicity showed no such increase, except for one subject with a titre of 1:160 (GMT, 1: 5.6).

Çavdar *et al.* (1994) reported on 81 children, of a median age of five years, with Burkitt's lymphoma in Turkey. In 32 of these patients in whom antibody titres against EBV were examined, they were found to be high, with 100% positive for anti-EBNA and a GMT of 1:320 for VCA. In 311 healthy children [unmatched for age or sex], the GMT for VCA was 1:93. [The proportion that was anti-EBNA positive was not reported.]

2.1.3.3 *Cohort study*

Between February 1972 and September 1974, serum samples were collected from about 42 000 children aged four to eight years in four counties in the West Nile District of Uganda. As of November 1977, 13 incident cases of histologically confirmed Burkitt's lymphoma and one case of 'unclassified lymphoma' had developed among the cohort members. The interval between initial serum collection and diagnosis of Burkitt's lymphoma ranged from seven to 54 months. EBV was found in seven of nine tumours tested by nucleic acid hybridization and in eight of these tumours by testing for EBNA. For each case of Burkitt's lymphoma, five control subjects from the cohort matched for age, sex and locality to the index case were chosen for a nested case–control analysis (de Thé *et al.*, 1978a). The 14 Burkitt's lymphoma patients had significantly higher pre-diagnostic anti-VCA titres than control subjects (GMT, 425.5 versus 125.8), but no

difference was observed between cases and controls in the titres of anti-EA and anti-EBNA. No difference in anti-VCA antibody titres was seen before and after diagnosis in the Burkitt's lymphoma patients, but seven of them developed anti-EA(R) antibodies; only one patient had anti-EA(D) antibodies before developing Burkitt's lymphoma. Similar temporal changes were not observed in the controls. Antibody titres to herpes simplex virus, cytomegalovirus and measles were unchanged, and the malaria parasitaemia rates before development of the tumour did not differ in patients and controls. This study clearly demonstrated that antibodies to EBV are present months to years before the development of Burkitt's lymphoma.

In 1982, Geser *et al.* reported the final results of this study. Two additional EBV-associated, histologically confirmed cases were detected up to 1979, both of which had high anti-VCA titres before the onset of Burkitt's lymphoma. One had anti-EA antibodies and the other did not, both before and after onset of tumour; anti-EBNA antibodies were also found before and after development of the tumour in both patients. This study showed that anti-VCA titres can be elevated as long as six years before the onset of Burkitt's lymphoma and as early as three months after birth. The relative risk for developing Burkitt's lymphoma increased multiplicatively by a factor of 5.1 for each two-fold dilution in anti-VCA titre for all cases of Burkitt's lymphoma and by a factor of 9.2 when the analysis was confined to cases in which EBV DNA was present in the tumour.

2.1.4 *Cofactors*

2.1.4.1 *Malaria*

Morrow (1985) summarized the data that suggest that malaria is a cofactor in the development of Burkitt's lymphoma:

- The incidence of Burkitt's lymphoma correlates within countries and internationally with the incidence of malaria and with parasitaemia rates.
- The age at which peak levels of antimalarial antibodies are acquired (5–8 years) corresponds to the peak age incidence of Burkitt's lymphoma.
- Individuals who live in urban areas where malarial transmission rates are lower also have a lower incidence of Burkitt's lymphoma.
- In regions in which death rates due to malaria have declined, there is a corresponding decline in the incidence of Burkitt's lymphoma.
- The age at onset of Burkitt's lymphoma in immigrants from malaria-free areas to malarious areas is higher than that of the original inhabitants.
- There is an inverse relationship between the age at onset of Burkitt's lymphoma and the intensity of infection with *Plasmodium falciparum.*
- There is an apparently reduced incidence (though not statistically significant) of Burkitt's lymphoma in individuals with the sickle-cell trait, which also protects against malaria.
- There is some evidence for a seasonal variation in the onset of Burkitt's lymphoma and for time–space clustering (see section 2.1.2.4).

(*a*) *Ecological studies*

Dalldorf (1962) first suggested that malaria is relevant to the development of Burkitt's lymphoma. Subsequently, he examined the distribution of Burkitt's lymphoma in Kenya (Dalldorf *et al.*, 1964). Because of marked variability in the endemicity of malaria in Kenya, primarily related to the suitability of various environments for breeding of the mosquito vector, he was able to show that the highest incidence of Burkitt's lymphoma occurred in areas where malaria was holoendemic, namely in the coastal and lakeside regions (see Figure 7). The major vectors in these areas are *Anopheles gambiae* and *A. funestus*. Dalldorf pointed out that malaria affects the reticuloendothelial system and as such could well influence the development of Burkitt's lymphoma. Infestation rates by malarial parasites rise rapidly during the first year of life and often persist up to the age of three. Similarly high rates of infestation occurred in Papua–New Guinea (ten Seldam *et al.*, 1966), the only other region in the world where malaria was known to be holoendemic and where the incidence of Burkitt's lymphoma was similarly high.

Burkitt (1969) also noted that high incidences of Burkitt's lymphoma occurred only in regions where malaria was holo- or hyperendemic (equatorial Africa, Papua–New Guinea and parts of Malaysia) and that the disease was rare in regions in which malaria eradication campaigns had been successful (the islands of Zanzibar and Pemba, Singapore, Sri Lanka, the West Indies and India) or showed a marked decrease in incidence in regions where malaria eradication had been undertaken only recently.

Not all areas believed in the 1960s to be lymphoma-free, however, were known to be malaria-free. Kinshasa, for example, and Lambarene appeared to have a markedly lower incidence of Burkitt's lymphoma than surrounding areas (Kafuko & Burkitt, 1970); however, similar anomalies of distribution apply to known vectored viral diseases, such as yellow fever, and could be related to the paucity of suitable mosquito breeding grounds (e.g. in many urban regions) or even differences in exposure to mosquitoes related to differences in life style.

Interestingly, ten Seldam *et al.* (1966) were not convinced that similar climatic determinants of the distribution of Burkitt's lymphoma pertained in the territories of Papua–New Guinea, largely because of their observation that four of their 35 cases occurred in highlands above 5000 feet (1500 m). Booth *et al.* (1967) found, however, that the distribution of the 37 cases they reported (many of which had been reported by ten Seldam *et al.*) was consistent with that observed in Africa. Thus, 34 of their cases came from the coastal regions or plains immediately adjacent to the coast, and only three came from the highlands. They also remarked that the capital (Port Moresby) appeared to be tumour-free. They equated this with the six dry months of the year in Port Moresby and drew a parallel with the situation in Accra, Ghana. They estimated that the incidence of Burkitt's lymphoma in the highlands of New Guinea, where 40% of the people live, was 1 per 442 000 children, as compared with 1 in 29 000 in children living in the coastal region — a difference of some 14- or 15-fold. These figures, are, however, based on rather small numbers of observed cases. Tefuarani *et al.* (1988) later estimated the incidence of Burkitt's lymphoma in Papua–New Guinea on the basis of 109 cases. The incidence of all childhood tumours was 36.5 per 100 000 (based on a total of 680 cases) per annum,

and that of Burkitt's lymphoma, representing 16% of all tumours, was about 6 per 100 000 — closely similar to that in Africa.

Kafuko and Burkitt (1970) summarized the available information on the influence of malaria on the risk for developing Burkitt's lymphoma. They stated that the disease is not common in any area where malaria transmission occurs for less than six months in the year. They quoted an unpublished report by P.J. Cook and D.P. Burkitt on the frequency of Burkitt's lymphoma in relationship to gastric cancer, liver cancer, Kaposi's sarcoma and epithelioma at the site of a chronic tropical ulcer in a large number of hospitals in Uganda, Kenya and Tanzania, who found that the hospitals with the highest relative fraction of Burkitt's lymphoma were all in highly malarious areas. A survey of spleen size and malarial parasite rates was carried out in Uganda between 1963 and 1966 by testing children in over 100 schools and conducting 86 mass surveys; the degree of malarial endemicity was calculated according to accepted criteria based on parasitaemia rates in children of various ages. A close correlation was found between malarial endemicity and the incidence of Burkitt's lymphoma, although no statistical analysis was performed. In addition, it was shown that the parasite density index was highest in the 0–4-year-old age group and higher in the 0–10-year-old age group than in older individuals. Thus, the peak incidence of Burkitt's lymphoma corresponds to the age range in which malarial infestation rates are highest. It was also noted that adults who emigrate to malarious regions from malaria-free regions develop an intense parasitaemia not seen in the resident adults, who have acquired immunity. This is consistent with the greater incidence of Burkitt's lymphoma in immigrants than in the regions from which they come.

Morrow *et al.* (1976) and Morrow (1985) studied the incidence of Burkitt's lymphoma in various counties in the Mengo districts of central Uganda. On the basis of 130 cases from East and West Mengo, 100 with a confirmed histological diagnosis and 11 with a typical clinical syndrome of Burkitt's lymphoma, seen at Mulago Hospital between 1959 and 1968, they recorded a marked variation in the incidence of Burkitt's lymphoma which, they stated, corresponded to the recorded incidence rates of malaria in those regions. They observed a gradual decline in the incidence rate of Burkitt's lymphoma throughout this period, in spite of improved case ascertainment, as evidenced by a tripling of the number of other cancers reported from the Mengo districts to the Kampala Cancer Registry during the same period. The decline was particularly noticeable among the Ganda tribe, native to this region, which they suggested was due to the greater availability and use of chloroquine from Government and private dispensaries, with a consequent decline in the incidence of severe malarial infection. They showed that immigrants into Mengo from highland regions with low malarial prevalence who developed Burkitt's lymphoma were significantly older (median, 12 years) at the onset of Burkitt's lymphoma than patients from meso-endemic Mengo (median, 8 years; $p < 0.008$), while patients from Mengo were significantly older than patients from hyper- or holoendemic regions at lower altitude (median, 6 years; $p < 0.04$). These observations were considered to be consistent with the hypothesis that Burkitt's lymphoma is most likely to occur within a few years of a first intense infection with malaria. The authors also showed a seasonal variation in the onset of Burkitt's lymphoma, more cases occurring in the first half of the year. No time–space clustering was observed.

In Ghana, Biggar *et al.* (1981) showed a significant difference in malaria parasitaemia (*P. falciparum*) rates in urban (1.4%) and rural populations (22%), accompanied by similar differences in antimalarial antibody titres. Persons who had taken chloroquine for treatment of suspected malaria had a lower antibody frequency and lower titres than those who did not use chloroquine. This difference correlated with the distribution of Burkitt's lymphoma in Ghana (Biggar & Nkrumah, 1979).

Morrow (1985) reported a significant correlation ($p < 0.001$ by Spearman rank coefficient) between malaria parasitaemia (*P. falciparum*) rates and the incidence of Burkitt's lymphoma in various districts in Uganda. The parasitaemia rates ranged from 7.9% (in Ankole) to 75.2% (in Madi), and the incidence of Burkitt's lymphoma in children aged 0–14 from 0 (0.09 in Ankole) to 6.0 (in Lango) per 100 000. He also suggested that differences in vectorial capacity, i.e. the rate of potentially infective contacts per person by a vector, and the consequent levels of parasitaemia may account for differences in the likelihood that Burkitt's lymphoma will occur. In holoendemic areas, the peak age of prevalence and of the density of falciparum parasitaemia is two to three years, whereas the maximal level of antimalarial and non-antimalarial immunoglobulin occurs two to five years later, coinciding with the peak age of incidence of Burkitt's lymphoma in these regions (Molineaux & Gramiccia, 1980). No differences have been reported in the levels of malarial antibodies between patients and controls.

(*b*) *Relationship between Burkitt's lymphoma and sickle-cell trait*

Since sickle-cell trait (AS haemoglobin) was shown to protect substantially against severe *P. falciparum* malaria (Allison, 1963), several attempts have been made to determine whether children with AS haemoglobin are less likely to develop Burkitt's lymphoma than others.

Williams (1966) compared the haemoglobin electrophoretic patterns of 100 children of the Yoruba tribe in Nigeria who had Burkitt's lymphoma (78% AA, 17% AS and 5% SC, SS, C or AC haemoglobin) with those of 331 similarly aged control patients from the same hospital (68% AA). Children with AA haemoglobin were more susceptible to Burkitt's lymphoma ($p = 0.03$). In another study of children over five years of age from a single Yoruba village near Ibadan, Nigeria, 66% had AA haemoglobin, 26% AS and 8% SC, SS, CC or AC, and there was no significant difference between patients and village controls (Gilles, 1963). Pike *et al.* (1970) conducted a case–control study in Uganda in which the controls were matched for age, sex, tribe and place of residence. Although the AS haemoglobin type appeared to be protective, the results were not statistically significant.

Studies in Uganda are difficult to conduct owing to the marked variation in the frequency of AS haemoglobin disease in different districts. Nkrumah and Perkins (1976) studied 112 patients with Burkitt's lymphoma, using the patients' nearest neighbours of the same age, sex and tribe as controls. Once again, no statistically significant difference in the frequency of patients with AS and other variations from AA haemoglobin was observed. The issue of whether individuals with variant haemoglobins are protected against the development of Burkitt's lymphoma therefore remains unanswered, largely

because of the lack of large enough studies. If there is a protective effect, it is presumably a modest one.

(*c*) *Intervention study*

Geser *et al.* (1989) undertook a study to determine whether suppression of malaria in the North Mara District of Tanzania by distributing chloroquine regularly to a cohort of children below the age of 10 years would result in a decrease in the incidence of Burkitt's lymphoma. This trial provided confirmation of the relationship between malaria prevalence rates and the incidence of Burkitt's lymphoma. Thus, before the trial (1964–76), all 85 cases of Burkitt's lymphoma in North Mara occurred in the lowlands (near Lake Victoria), with a high malarial parasitaemia rate (28–48%), and none occurred in the high plateau (over 1500 m) bordering Kenya, with a low malarial parasitaemia rate (5–24%). The prevalence of both malaria (parasitaemia rates of 11 and 13% in 1977 and 1978, with corresponding reductions in antimalarial fluorescence antibody titres) and Burkitt's lymphoma fell transiently during and after the period of chloroquine administration (1977–82), the latter to the lowest level ever recorded in the region: 0.5 per 100 000 in 1979 and 1981 in comparison with 2.6–6.9 per 100 000 before the trial. After 1979, the prevalence of malaria rapidly rose again to pre-trial levels, apparently because of problems in chloroquine distribution, although the incidence of Burkitt's lymphoma remained low until approximately two years after the distribution of chloroquine was stopped, when it reached a high of 7.1 in 1984. Interestingly, the prevalence of parasitaemia in South Mara, where chloroquine distribution was not conducted, rose throughout the trial from the pre-trial level of 23–28% to a high of 57% in 1985. Chloroquine resistance was not reported in the area until 1982 (Draper *et al.*, 1985). As anticipated, 90% of malaria detected was due to *P. falciparum*, the remainder being due to *P. malariae*. The authors reported some evidence of a trend towards a lower incidence of Burkitt's lymphoma in North Mara before the trial, although this did not reach statistical significance, whereas the reduction in the incidence of Burkitt's lymphoma between 1964 and 1982 was highly significant ($p < 0.001$).

2.1.4.2 Euphorbia tirucalli *and other medicinal plants*

Another possible cofactor in the pathogenesis of Burkitt's lymphoma in Africa is the plant *Euphorbia tirucalli*, which is used quite widely in equatorial Africa for medicinal purposes. Phorbol esters present in this plant have been reported to increase the ability of EBV to transform B lymphocytes and to increase the likelihood that a chromosomal translocation will develop in transformed cells (see section 4.2.4). Epidemiological information relevant to this issue is limited, although the distribution of *Euphorbia* in Africa has been examined.

Osato *et al.* (1987, 1990) found *Euphorbia tirucalli* around almost all houses, fields and reservoirs in villages surrounding Lake Victoria and in other high-incidence regions in Kenya and Tanzania. The plant was reported to be uncommon in areas in these countries in which Burkitt's lymphoma is uncommon.

van den Bosch *et al.* (1993) reported this plant to be frequent within the 'lymphoma belt' in equatorial Africa and to be used significantly more commonly in the homes of patients from Malawi with Burkitt's lymphoma than in control patients. A number of other plants of this family (Euphorbiaceae) and a variety of medicinal plants were also common. The tobacco plant was also significantly more commonly used in the homes of patients with Burkitt's lymphoma than those of controls, but use of other plants was equally distributed (Ito *et al.*, 1983).

van den Bosch *et al.* (1993) mentioned three patients who developed Burkitt's lymphoma after being treated for an illness with an extract of *Terminalia sericea*.

2.1.5 *Molecular epidemiology*

The identification of specific chromosomal abnormalities in patients with Burkitt's lymphoma (see section 4.2.1.1) permitted the examination of differences in the translocation-dependent structural alterations in *c-myc* in different regions of the world (Pelicci *et al.*, 1986; Shiramizu *et al.*, 1991; Gutiérrez *et al.*, 1992). In some tumours, the chromosomal breakpoint is quite distant from *c-myc* — often as much as several hundred kilobases upstream (in t(8;14)) or downstream (in variant translocations). This is the most frequent location of the breakpoint in African Burkitt's lymphoma, occurring in 75% of tumours, and is observed in about 50% of Brazilian tumours. Sometimes the breakpoint is close to the gene, i.e. within its 5′ flanking region, arbitrarily defined as extending from the upstream *Hind*III restriction enzyme site to exon 1. This is found in approximately half of the tumours occurring in Chile and Argentina (Gutiérrez *et al.*, 1992). Of tumours in North America, 60% have the breakpoints within the gene, i.e. within exon 1 or intron 1, and only 9% outside the *Hind*III fragment (Shiramizu *et al.*, 1991). Intron and exon breakpoints separate the coding region of *c-myc* from its major promoters, and transcripts are initiated from regions within the first intron. This is a marked difference from African Burkitt's lymphomas in which the *c-myc* gene is grossly intact, although point mutations in regulatory and coding regions are nearly always observed, implying different mechanisms leading to *c-myc* deregulation. Factors that are relevant to the induction of specific chromosomal breakpoint locations have not been identified but are likely to be environmental.

Interestingly, there is an apparent geographical association between the frequency of breakpoints outside the *c-myc* gene and the fraction of tumours associated with EBV (Magrath, 1997). In the United States, for example, only 15–20% of tumours are EBV-associated and only 9% have a breakpoint outside *c-myc*. In Africa, 95% of tumours are EBV-associated and 75% have breakpoints outside *c-myc*. Tumours from South American countries are intermediate in both respects. The nature of this relationship is uncertain, since, for example, non-African tumours with breakpoints outside *c-myc*, which occur at highest frequency (in countries for which data are available) in Brazil are not necessarily EBV-associated.

2.2 Non-Hodgkin's lymphomas other than Burkitt's lymphoma

2.2.1 *Pathology*

Non-Hodgkin's lymphomas are a numerous, heterogeneous group of malignancies that originate from lymphocytes. They can develop either from within organized lymphoid tissues, such as lymph nodes, or from other sites. Many lymphoid neoplasms pass through both solid tumour and circulating (leukaemic) phases.

Classification of non-Hodgkin's lymphomas is a complex and evolving process, and a thorough description of these diseases and their diagnostic criteria is beyond the scope of this volume. Several schemes of nomenclature have been proposed and used in different parts of the world, and many lymphoma entities that are recognized clinically and pathologically have been described by different names in these classifications. Lymphomas are divided into B-cell and T-cell neoplasms, according to their immunophenotypic characteristics. Within each group, there are numerous specific disease entities, many of which are associated with specific karyotypic abnormalities. Neoplasms of putative NK cell origin are provisionally grouped with the T-cell malignancies.

The International Lymphoma Study Group proposed in 1994 (Harris *et al.*, 1994) a 'Revised European–American Lymphoma (REAL) Classification' that attempted to link the major classification systems then in use in Europe and the United States. These included the Kiel classification, widely used in Europe (Stansfeld *et al.*, 1988), and the Working Formulation used in clinical trials in the United States (Anon., 1982). Table 8, adapted from Harris *et al.* (1994), is a list of non-Hodgkin's lymphoid neoplasms recognized by the International Lymphoma Study Group. The disease entities mentioned in section 2.2.2 are named in most cases according to the REAL classification; the pathology of Burkitt's lymphoma, a B-cell neoplasm, is presented in section 2.1.1. Hodgkin's disease, which is also considered within the REAL classification, is discussed separately in section 2.3.1.

2.2.2 *Epidemiology*

2.2.2.1 *Descriptive epidemiology*

Non-Hodgkin's lymphoma is estimated to account for 2.5% of all cancer cases worldwide (Pisani *et al.*, 1997). The incidence varies approximately sixfold, the highest reported rates being seen in whites in the United States and the lowest in Southeast Asia, India and sub-Saharan Africa (Parkin *et al.*, 1997). The incidence rises steeply with age. In the United States, the age-adjusted rates for 1990–94 were 9.2 cases per 100 000 annually among people under 65 years of age and 73.5 per 100 000 for those aged 65 and over (Ries *et al.*, 1997). Males are at higher risk, having incidence rates approximately 50–100% higher than females in most countries (Parkin *et al.*, 1997). The rates have been increasing steadily throughout the world, for reasons that are largely unexplained (Hartge *et al.*, 1994). Immunodeficiency of various etiologies, including HIV infection (see section 2.2.3), iatrogenic immunosuppression (see section 2.2.3) and congenital immunodeficiency (see section 2.2.4), is associated with a greatly increased risk, but the etiology

Table 8. The Revised European–American Lymphoma classification of non-Hodgkin's lymphomas

B-Cell neoplasms

I. Precursor B-cell neoplasm: Precursor B-lymphoblastic leukaemia/lymphoma
II. Peripheral B-cell neoplasms
  1. B-Cell chronic lymphocytic leukaemia/prolymphocytic leukaemia/small lymphocytic lymphoma
  2. Lymphoplasmacytoid lymphoma/immunocytoma
  3. Mantle-cell lymphoma
  4. Follicle-centre lymphoma, follicular
  5. Marginal zone B-cell lymphoma
     Extranodal (MALT-type +/– monocytoid B cells)
  6. Provisional entity: Splenic marginal zone lymphoma
     (+/– villous lymphocytes)
  7. Hairy-cell leukaemia
  8. Plasmacytoma/plasma-cell myeloma
  9. Diffuse large B-cell lymphoma[a] (centroblastic)
     Subtype: Primary mediastinal (thymic) B-cell lymphoma
  10. Burkitt's lymphoma (small noncleaved cell)
  11. Provisional entity: High-grade B-cell lymphoma, Burkitt-like[a]

T-Cell and putative NK-cell neoplasms

I. Precursor T-cell neoplasm: Precursor T-lymphoblastic lymphoma/leukaemia
II. Peripheral T-cell and NK-cell neoplasms
  1. T-Cell chronic lymphocytic leukaemia/prolymphocytic leukaemia
  2. Large granular lymphocyte leukaemia
     T-cell type
     NK-cell type
  3. Mycosis fungoides/Sezary syndrome
  4. Peripheral T-cell lymphomas, unspecified[a]
     Provisional cytological categories: Medium-sized cell, mixed medium and large cell, large cell, lymphoepithelioid cell
  5. Angioimmunoblastic T-cell lymphoma
  6. Angiocentric lymphoma
  7. Intestinal T-cell lymphoma (+/– enteropathy associated)
  8. Adult T-cell lymphoma/leukaemia
  9. Anaplastic large-cell lymphoma, $CD30^+$, T- and null-cell types
  10. Provisional entity: Anaplastic large-cell lymphoma, Hodgkin's-like

From Harris *et al.* (1994)

[a]These categories are thought likely to include more than one disease entity.

of most cases of non-Hodgkin's lymphoma remains unknown. As immunodeficiency-associated lymphomas differ in a number of respects, the remainder of this section addresses only lymphomas in individuals with no evidence of prior immunocompromise.

2.2.2.2 *Case reports and case series*

Non-Hodgkin's lymphomas are pathologically diverse, as noted above, and their association with EBV differs accordingly.

(*a*) *B-Cell non-Hodgkin's lymphoma*

Although the B cell is the usual target in latent infection, the rate of detection of EBV was relatively low in three large series of B-cell lymphomas. Hamilton-Dutoit and Pallesen (1992) found that four of 105 B-cell lymphomas expressed LMP-1 and only one expressed EBNA-2. Hummel *et al.* (1995b) found EBER transcription in 54 (26%) of 208 tumours; in half of the positive cases, this was localized to non-neoplastic bystander cells. Of 27 cases with EBER in the tumour cells, more than 80% of tumour cells in 17 cases expressed EBER. d'Amore *et al.* (1996) found EBER transcription in 25 (6.5%) of 386 tumours, but only 6 (2%) had more than 10 EBER-positive tumour cells per medium-power (× 200) field on light microscopic examination. The three studies showed no consistent association between histological subtype and the presence of EBV (Table 9).

Primary central nervous system lymphoma, which is nearly always of B-cell origin, has been of particular interest since the first description by Hochberg *et al.* (1983) of an EBV-containing tumour detected by Southern blot, as EBV is almost universally present in brain lymphomas secondary to immunodeficiency (see section 2.2.3). In the series of central nervous system lymphomas reported by Murphy *et al.* (1990) and by DeAngelis *et al.* (1992), nearly half of the tumours were shown to contain EBV DNA by in-situ hybridization or PCR. These two initial reports were not, however, confirmed by many other studies of DNA (Bashir *et al.*, 1990; Nakhleh *et al.*, 1991; Geddes *et al.*, 1992) and RNA (MacMahon *et al.*, 1991; Chang *et al.*, 1993a; Bashir *et al.*, 1994; Bergmann *et al.*, 1995) by in-situ hybridization, in which EBV was detected in no more than 12% of cases (Table 10).

B-Cell lymphoma of mucosa-associated lymphoid tissue (MALT), which is found in the stomach and elsewhere, warrants attention because of the association between gastric adenocarcinoma and EBV (see section 2.5). Liu *et al.* (1995) found one of 16 Japanese cases of gastric MALT lymphoma to contain EBER-1 by in-situ hybridization, and 20% of the tumour cells contained EBV. An additional case showed no EBV in the primary gastric tumour, but an EBER-positive tumour was found in a regional lymph node. d'Amore *et al.* (1996) found rare EBER-1/2-positive tumour cells in three of 28 MALT tumours in Denmark. Lee *et al.* (1997) found no EBER-1-positive cases among eight gastric MALT tumours in the Republic of Korea. A similarly low frequency of EBV-positivity has been found in MALT tumours outside of the stomach: using PCR, Diss *et al.* (1995) found EBV DNA in three of 36 parotid MALT tumours in the United

Kingdom; in the one case with EBV DNA detected by in-situ hybridization, less than 5% of tumour cells contained EBER.

Table 9. Presence of EBV in B-cell non-Hodgkin's lymphoma tissue

Reference	Study area	Detection method	Histology	No. of cases	No. with EBV	Tumour cells with EBV
Hamilton-Dutoit & Pallesen (1992)	Denmark	LMP-1, IHC	Diffuse large cell	37	0	
			Immunoblastic	54	3	< 50%
			Small non-cleaved cell	10	0	
			Anaplastic large cell	4	1	
Hummel *et al.* (1995b)	Germany	EBER-1/2, ISH	Low-grade	65	3	Variable
			Diffuse large cell	39	3	
			Immunoblastic	28	4	
			Small non-cleaved cell	36	7	
			Anaplastic large cell	16	3	
			Other high grade	24	7	
		LMP, IHC	Low grade	65	1	Low
			Diffuse large cell	39	2	
			Immunoblastic	28	2	
			Small non-cleaved cell	36	0	
			Anaplastic large cell	16	3	
			Other high grade	24	3	
d'Amore *et al.* (1996)	Denmark	EBER-1/2, ISH	Low grade	154	9	Few
			Diffuse large cell	74	3	
			Immunoblastic	21	1	
			Small non-cleaved cell	24	3	
			Anaplastic large cell	7	0	
			Other high grade	106	9	

All in non-immunocompromised individuals
EBER, EBV-encoded RNA; IHC, immunohistochemistry; ISH, in-situ hybridization; LMP, latent membrane protein

(*b*) *Angiocentric T-cell lymphoma*

Sinonasal T-cell lymphoma, which encompasses entities also referred to as lethal midline granuloma and midline reticulosis, is the non-Hodgkin's lymphoma most strongly associated with EBV. Series in Asia (Harabuchi *et al.*, 1990; Ho *et al.*, 1990; Chan *et al.*, 1994a; Ko & Lee, 1994; Lee *et al.*, 1994a; Peh *et al.*, 1995; Harabuchi *et al.*, 1996), South America (Arber *et al.*, 1993), Europe (O'Leary & Kennedy, 1995; Dictor *et al.*, 1996; Kanavaros *et al.*, 1996) and the United States (Weiss *et al.*, 1992a; Davison *et al.*, 1996) consistently showed the presence of EBV in the great majority of cases, EBV being present in most tumour cells (Table 11). CD56-positive tumours, which are suggested to be of NK-cell derivation, were uniformly EBV-positive, whereas CD56-negative tumours were more frequently EBV-negative (Chan *et al.*, 1994a; Harabuchi

et al., 1996; Kanavaros *et al.*, 1996). EBV-positive tumours consistently expressed the transforming EBV protein LMP-1 (Harabuchi *et al.*, 1996; Kanavaros *et al.*, 1996), and EBV terminal repeat sequences have been shown to have monoclonal or biclonal infection (Ho *et al.*, 1990; Harabuchi *et al.*, 1996).

Table 10. Presence of EBV in primary central nervous system non-Hodgkin's lymphoma tissue in HIV-negative cases

Reference	Study area	Detection method	No. of cases	No. with EBV
Hochberg *et al.* (1983)	USA	EBV DNA, SB	1	1
Bashir *et al.* (1990)	USA	*Bam*HI V, ISH	10	0
Murphy *et al.* (1990)	UK	*Bam*HI W, ISH	24	11
Rouah *et al.* (1990)	USA	EBV DNA, ISH	5	2
		EBV DNA, PCR	7	2
Bignon *et al.* (1991)	France	EBV DNA, PCR	9	1
MacMahon *et al.* (1991)	USA	EBER-1, ISH	14	1
Nakhleh *et al.* (1991)	USA	*Bam*HI W, ISH	17	2
Geddes *et al.* (1992)	UK	*Bam*HI W, ISH	43	2
DeAngelis *et al.* (1992)	USA	EBV DNA, PCR	13	7
Chang *et al.* (1993a)	USA	EBER-1, ISH	27	1
Bashir *et al.* (1994)	USA	EBER-1, ISH	9	0
Bergmann *et al.* (1995/96)	Germany	EBER, ISH	36	0

*Bam*HI V and *Bam*HI W, DNA restriction fragments; EBER, EBV-encoded RNA; ISH, in-situ hybridization; PCR, polymerase chain reaction; SB, Southern blot

In contrast, EBV is much less frequently found in sinonasal B-cell lymphoma. Chan *et al.* (1994a) and Peh *et al.* (1995) found one EBV-positive case among ten and nine tumours respectively, and Kanavaros *et al.* (1996) found one among 10 tumours; a somewhat higher frequency was found by Weiss *et al.* (1992a) (Table 11).

B-Cell lymphomas of the nasopharynx, tonsil and tongue ('Waldeyer's ring') less frequently contain EBV, the frequency ranging from 0 to 13% in six series (Chan *et al.*, 1994a; Ko & Lee, 1994; Lee *et al.*, 1994a; O'Leary & Kennedy, 1995; Peh *et al.*, 1995; Kanavaros *et al.*, 1996), with a slightly higher frequency in the report of Weiss *et al.* (1992a) (Table 11). T-Cell tumours of Waldeyer's ring have a more variable association with EBV: 0/8 and 3/6 tumours were found to contain EBV in the two largest series (Ko & Lee, 1994; Kanavaros *et al.* 1996).

Lymphomatoid granulomatosis, which resembles sinonasal lymphoma histologically, is another condition in which EBV is highly prevalent. In eight series, EBV was detected in the majority of cases (Katzenstein & Peiper, 1990; Medeiros *et al.*, 1991; Guinee *et al.*, 1994; Tsang *et al.*, 1994; Myers *et al.*, 1995; Nicholson *et al.*, 1996; Takeshita *et al.*, 1996; Wilson *et al.*, 1996b); in two other series it was detected at lower frequency (Sabourin *et al.*, 1993; Kobashi *et al.*, 1996) (Table 12). The diagnostic classification of

Table 11. Presence of EBV in sinonasal and Waldeyer's ring non-Hodgkin's lymphomas

Reference	Study area	Detection method	Immuno-phenotype	Sinonasal		Waldeyer's ring		Tumour cells with EBV	Comments
				No. of cases	No. with EBV	No. of cases	No. with EBV		
Harabuchi *et al.* (1990)	Japan	*Bam*HI W, ISH	T Cell	5	5			80–90%	
		EBNA, IFA	T Cell	5	5				
Ho *et al.* (1990)	Hong Kong	*Bam*HI W, SB	T Cell	7	7				EBV mono- or biclonal
			B Cell	3	2				
Weiss *et al.* (1989a; 1992a)	USA	*Bam*HI W, ISH	T Cell	3	3			Uniform	
			B Cell	5	2	10	2		
Arber *et al.* (1993)	Peru	EBER-1, ISH	T Cell	11	11			Many	
			B Cell	2	1				
			Indeter-minate	1	1				
Chan *et al.* (1994a)	Hong Kong	EBER-1/2, ISH	T Cell	30	25	1	0	Most	21/21 $CD56^+$ and 4/9 $CD56^-$ contained EBV
			B Cell	10	1	20	0		
Ko & Lee (1994)	Republic of Korea	EBER, ISH	T Cell	10	7	8	0		
			B Cell			12	1		
Lee *et al.* (1994a)	Republic of Korea	EBER-1/2, ISH	T Cell	12	12	2	2	Many	
			B Cell	1	0	16	2		
O'Leary & Kennedy (1995)	Ireland	EBER-1/2, ISH	T Cell	8	6			Many	
			B Cell	3	0	10	2		
Peh *et al.* (1995)	Malaysia	EBER-1, ISH	T Cell	10	9	3	2	70–100%	
			B Cell	9	1	7	0		
Davison *et al.* (1996)	USA	EBER-1, ISH	T Cell	30	29			Majority	
Dictor *et al.* (1996)	Sweden	EBER-1, ISH	T Cell	12	12			50–100%	

Table 11 (contd)

Reference	Study area	Detection method	Immuno-phenotype	Sinonasal		Waldeyer's ring		Tumour cells with EBV	Comments
				No. of cases	No. with EBV	No. of cases	No. with EBV		
Harabuchi *et al.* (1996)	Japan	EBER-1, ISH	T Cell	16	16			> 50%	EBV monoclonal; 9/9 $CD56^+$; includes 6 cases from Harabuchi *et al.* (1990)
		LMP-1, IHC		9	9			> 50%	
Kanavaros *et al.* (1996)	France and Greece	EBER-1/2, ISH	B Cell	17	16	6	3	> 50%	11/11 $CD56^+$ and 1/3 $CD56^-$ contained EBV
				10	1	22	1		
		LMP-1, IHC	T Cell	17	16	6	3	1–50%	
			B Cell	10	0	22	0		

All in non-immunocompromised individuals
EBER, EBV-encoded RNA; IHC, immunohistochemistry; ISH, in-situ hybridization; LMP, latent membrane protein; SB, Southern blot

Table 12. Presence of EBV in lymphomatoid granulomatosis tissue

Reference	Study area	Detection method	Immunophenotype	No. of cases	No. with EBV	Tumours with EBV	Comments
Katzenstein & Peiper (1990)	USA	EBV DNA, PCR		29	21		
Medeiros *et al.* (1991)	USA	*Bam*HI W, PCR	T Cell	5	3		
		EBV, SB	T Cell	7	2		
Sabourin *et al.* (1993)	France	EBER-1/2, ISH	T Cell	6	2	Numerous	
			B Cell	1	0		
		LMP, IHC	T Cell	6	2		
			B Cell	1	0		
Guinee *et al.* (1994)	USA	EBER-1, ISH	T and B cell	10	10	Only in B cells	B cells monoclonal, T cells polyclonal
Tsang *et al.* (1994)	Hong Kong	EBER-1/2, ISH, IHC	NK Cell	15	10	Most	
Myers *et al.* (1995)	USA	EBER-1, ISH	T Cell	6	0		
			T and B cell	11	10	Only in B cells	
Kobashi *et al.* (1996)	Japan	EBER-1/2, ISH	NK Cell	9	3	Most	EBV mono- or biclonal
		LMP-1, IHC	NK Cell	9	2		
Nicholson *et al.* (1996)	UK	EBER-1, ISH	T and B cell	7	4	Only in B cells	
Takeshita *et al.* (1996)	Japan	EBER-1, ISH	T and B cell	3	2	Some to many	1 only in B cells
			B Cell	9	4		
			NK Cell	2	2		
		LMP, IHC	T and B cell	3	2		1 only in B cells
			B Cell	9	4		
			NK Cell	2	1		
Wilson *et al.* (1996b)	USA	EBER-1, ISH	T and B cell	4	4	Only in B cells	

All in non-immunocompromised individuals

*Bam*HI W, DNA restriction fragment; EBER, EBV-encoded RNA; EBNA, EBV nuclear antigen; IFA, immunofluorescence assay; IHC, immunohistochemistry; ISH, in-situ hybridization; LMP, latent membrane protein; PCR, polymerase chain reaction; SB, Southern blot; NK, natural killer

this disorder has recently undergone revision. Guinee *et al.* (1994) found that despite a predominance of T cells in these tumours, a minor population of monoclonal B cells is present within a polyclonal population of T cells. Furthermore, in all 10 cases, EBV was present in the B-cell population only. Myers *et al.* (1995), Nicholson *et al.* (1996) and Takeshita *et al.* (1996) reported a similar restriction of EBV to B cells in tumours of mixed immunophenotype. Kobashi *et al.* (1996) found monoclonal or biclonal EBV by terminal-repeat fragment analysis in three of three EBV-positive tumours.

(*c*) *Other peripheral T-cell lymphomas*

These entities have been reviewed by Pallesen *et al.* (1993). Other peripheral T-cell lymphomas commonly contain EBV (Table 13). In 12 series from North America, Europe and Asia, the frequency of EBV positivity ranged from 18 to 70% in all but one study (Herbst *et al.*, 1991b; Lee *et al.*, 1991; Kanavaros *et al.*, 1992; Ott *et al.*, 1992; Weiss *et al.*, 1992b; Korbjuhn *et al.*, 1993; Tsang *et al.*, 1994; Zhou *et al.*, 1994; Lopategui *et al.*, 1995; d'Amore *et al.*, 1996; Hirose *et al.*, 1996). Hamilton-Dutoit and Pallesen (1992) found only 10% positivity, but used an assay with low sensitivity. The presence of EBV in tumour cells varies, some tumours showing its presence uniformly and others in only a fraction of cells. Terminal-repeat assays have been used to confirm the mono- or oligoclonality of latent EBV episomes (Ott *et al.*, 1992; Hirose *et al.*, 1996).

There is some variation by histological subtype. Most angioimmunoblastic lymphomas contain EBV (Ott *et al.*, 1992; Weiss *et al.*, 1992b; Zhou *et al.*, 1994; d'Amore *et al.*, 1996; Hirose *et al.*, 1996), although in one study tumour cells containing EBV were rare (Tsang *et al.*, 1994). Most cases of anaplastic large-cell lymphoma do not contain EBV (Herbst *et al.*, 1991b; Hamilton-Dutoit & Pallesen, 1992; Kanavaros *et al.*, 1992; Ott *et al.*, 1992; Zhou *et al.*, 1994; Lopategui *et al.*, 1995; d'Amore *et al.*, 1996). Pleomorphic and other tumour types, such as lymphoepithelioid and T-zone tumours, show an intermediate frequency, about one-third of the reported cases containing EBV (Hamilton-Dutoit & Pallesen, 1992; Ott *et al.*, 1992; Korbjuhn *et al.*, 1993; Tsang *et al.*, 1994; Zhou *et al.*, 1994; d'Amore *et al.*, 1996; Hirose *et al.*, 1996; Table 13).

Enteropathy-associated T-cell lymphoma is a distinct entity which has been investigated for the presence of EBV by EBER in-situ hybridization in four studies. Pan *et al.* (1993) detected EBV in four of 11 cases in the United Kingdom; in the cases with EBV, over 80% of tumour cells contained EBER, and the EBV was shown to be monoclonal by terminal-repeat analysis. These findings were not confirmed in other studies. Korbjuhn *et al.* (1993) found EBV in two of 10 cases in Germany; in both cases, less than 20% of the tumour cells contained EBER. Similarly, Ilyas *et al.* (1995) found EBV in none of seven cases in the United Kingdom, and Walsh *et al.* (1995) found EBV in only one of 16 cases in Ireland.

EBV was detected infrequently in low-grade cutaneous lymphomas (mycosis fungoides and Sézary syndrome) in three studies in which EBER-1/2 was analysed by in-situ hybridization. Anagnostopoulos *et al.* (1996) found EBER in four of 42 cases in Germany, but less than 1% of the tumour cells contained EBV. Angel *et al.* (1996) in the

Table 13. Presence of EBV in peripheral T-cell lymphoma tissue

Reference	Study area	Detection method	Histological type	No. of cases	No. with EBV	Tumour cells with EBV	Comments
Herbst *et al.* (1991b)	Germany	*Bam*HI W, PCR	Anaplastic large cell	18	5		
		LMP, IHC		18	2	50–90%	
Lee *et al.*(1991)	Taiwan	EBV DNA, SB		6	4		Paediatric series
Hamilton-Dutoit & Pallesen (1992)	Denmark	LMP-1, IHC	Pleomorphic	50	7	< 50%	
			Angioimmunoblastic	4	1		
			Anaplastic large cell	19	0		
			Other	9	0		
Kanavaros *et al.* (1992)	Netherlands	EBV DNA, PCR, ISH	Anaplastic large cell	3	2	50%	
		LMP, IHC		11	1	Variable	
Ott *et al.* (1992)	Germany	*Bam*HI W, SB	Pleomorphic	14	5	Variable	EBV mono- or biclonal
			Angioimmunoblastic	14	8		
			Anaplastic large cell	8	3		
			Other	10	2		
Weiss *et al.* (1992b)	USA	EBER-1, ISH	Angioimmunoblastic	23	19	Variable	Mostly B cells
Korbjuhn *et al.* (1993)	Germany	EBER, ISH	Pleomorphic	81	38	1–100%	
Tsang *et al.* (1994)	Hong Kong	EBER-1/2, ISH	Pleomorphic	7	3	≥ 90%	
			Angioimmunoblastic	6	0		Rare EBER-positive cells in 4 cases
			Other	2	0		
Zhou *et al.* (1994)	China	EBER ISH	Pleomorphic	27	18	≥ 50%	
			Angioimmunoblastic	6	4		
			Anaplastic large cell	2	0		
			Other	4	2		
		LMP-1, IHC	Pleomorphic	26	13		
			Angioimmunoblastic	6	4		
			Anaplastic large cell	2	0		
			Other	4	2		

Table 13 (contd)

Reference	Study area	Detection method	Histological type	No. of cases	No. with EBV	Tumour cells with EBV	Comments
Lopategui *et al.* (1995)	USA and Hong Kong	EBER-1, ISH	Anaplastic large cell	15	3	25–90%	
		LMP-1, IHC		15	0		
d'Amore *et al.* (1996)	Denmark	EBER-1/2, ISH	Pleomorphic	67	24	< 10–> 50%	
			Angioimmunoblastic	13	11		B cells also positive
			Anaplastic large cell	9	0		
			Other	21	0		
		LMP-1, IHC	Pleomorphic	24	8		
			Angioimmunoblastic	11	2		
			Other	5	2		
Hirose *et al.* (1996)	Japan	EBER, ISH	Pleomorphic	10	4	Few to extensive	
			Angioimmunoblastic	9	7		B cells also positive; EBV mono- or biclonal
			Other	4	4		
		LMP-1, IHC	Pleomorphic	10	3		
			Angioimmunoblastic	9	1		
			Other	4	0		

All in non-immunocompromised individuals

*Bam*HI W, DNA restriction fragment; EBER, EBV-encoded RNA; IHC, immunohistochemistry; ISH, in-situ hybridization; LMP, latent membrane protein; PCR, polymerase chain reaction; SB, Southern blot

United Kingdom and d'Amore *et al.* (1996) in Denmark found no EBV in 25 and 13 cases, respectively.

2.2.2.3 *Cohort studies*

The association between abnormal EBV seroreactivity and the risk for subsequent non-Hodgkin's lymphoma has been examined in two studies. Mueller *et al.* (1991) performed a nested case–control study based on sera from four banks established between 1964 and 1974 containing specimens from over 240 000 people in Norway and the United States, 104 of whom had developed non-Hodgkin's lymphoma an average of 63 months after their serum had been collected. They were compared with 259 controls matched for age, sex, ethnic group and date of serum collection. Immunofluorescence assays showed that elevated levels of IgG (titre, ≥ 1:320) and IgM (titre, ≥ 1:5) antibodies against EBV VCA were associated with relative risks for non-Hodgkin's lymphoma of 2.5 (95% CI, 1.1–5.7) for IgG and 3.2 (95% CI, 1.3–7.5) for IgM. Low titres (≤ 1:5) of antibody to EBNA were associated with a decreased risk for lymphoma, although the effect was significant only for cases that developed within five years of serum collection. No differences were reported in subgroup analyses of follicular versus diffuse, large-cell versus mixed- and small-cell, or low- versus intermediate- versus high-grade lymphomas. [The Working Group noted that no T versus B immunophenotyping or tissue analyses for EBV markers were reported, and that the relative risks may have been attenuated by the inclusion of all types of non-Hodgkin's lymphoma.]

Lehtinen *et al.* (1993) performed a nested case–control study of 39 000 Finnish adults from whom serum was collected between 1968 and 1972. Eleven subjects who developed non-Hodgkin's lymphoma 1–12 years after serum collection were compared with 22 controls matched for age, sex, municipality and date of phlebotomy. Enzyme immunoassays showed no significant differences in antibodies to VCA, EA or EBNA. [The Working Group noted that immunofluorescence is the standard method for detecting EBV antibodies and the relevance of the authors' enzyme immunoassay is uncertain.]

2.2.3 *Human immunodeficiency virus as a cofactor*

HIV and HHV8 also play a role in the pathogenesis of EBV-associated non-Hodgkin's lymphoma. The monograph on HHV8 in this volume covers its interaction with EBV; this section deals only with HIV.

Lymphomas in HIV-infected patients are nearly always of B-cell origin. Under the Working Formulation classification (Rosenberg, 1982), they include tumours of the small non-cleaved cell (diffuse large B cell in the REAL classification), large cell diffuse and immunoblastic types; anaplastic large cell and unclassified high-grade types are also observed. Like other immune-deficient conditions, HIV infection is associated in particular with primary central nervous system lymphoma. The association of EBV with HIV-related lymphomas varies.

2.2.3.1 *Primary central nervous system lymphomas*

Unlike central nervous system lymphomas in immunocompetent individuals, those found in association with HIV infection nearly always contain detectable EBV. In nine reported series, EBV was detected in all cases in five studies (MacMahon *et al.*, 1991; Chang *et al.*, 1993a; Cinque *et al.*, 1993; Bashir *et al.*, 1994; Arribas *et al.*, 1995) and in the majority of cases in the remaining four studies (Bashir *et al.*, 1990; DeAngelis *et al.*, 1992; Morgello, 1992; Bergmann *et al.*, 1995; Table 14). In-situ hybridization has been used to show that most or all of the tumour cells contained EBV RNA (MacMahon *et al.*, 1991; Chang *et al.*, 1993a; Cinque *et al.*, 1993; Bashir *et al.*, 1994). LMP expression is more variable; nine of 21 tumours reported by MacMahon *et al.* (1991) and 13 of 19 reported by Bergmann *et al.* (1995/96) were shown by immunohistochemistry to contain LMP.

Table 14. Presence of EBV in HIV-associated primary central nervous system non-Hodgkin's lymphoma tissue

Reference	Study area	Detection method	No. of cases	No. with EBV	Tumour cells with EBV
Bashir *et al.* (1990)	USA	*Bam*HI V, ISH	5	4	Variable
MacMahon *et al.* (1991)	USA	EBER-1, ISH	21	21	Uniform
		LMP, IHC	21	9	Many cells
DeAngelis *et al.* (1992)	USA	*Bam*HI W, PCR	13	11	
Morgello (1992)	USA	EBNA-1, PCR	12	6	
Chang *et al.* (1993a)	USA	EBER-1, ISH	5	5	Uniform
Cinque *et al.* (1993)	Sweden and Italy	EBER, ISH	16	16	40–90%
Bashir *et al.* (1994)	USA	EBER-1, ISH	5	5	Most
Arribas *et al.* (1995)	USA	LMP, IHC	6	6	Variable
Bergmann *et al.* (1995/96)	Germany	EBER, ISH	19	15	
		LMP, IHC	19	13	

*Bam*HI V and *Bam*HI W, DNA restriction fragments; EBER, EBV-encoded RNA; EBNA, EBV nuclear antigen; IHC, immunohistochemistry; ISH, in-situ hybridization; LMP, latent membrane protein; PCR, polymerase chain reaction

2.2.3.2 *Systemic non-Hodgkin's lymphomas*

EBV is found in a large fraction of systemic tumours. In 13 series of 10 or more patients, the frequency of EBV positivity ranged from 38 to 79% (Subar *et al.*, 1988; Borisch-Chappuis *et al.*, 1990a,b; Boyle *et al.*, 1991; Guarner *et al.*, 1991; Borisch *et al.*, 1992a; Ballerini *et al.*, 1993; Carbone *et al.*, 1993a,b; Hamilton-Dutoit *et al.*, 1993a; Shibata *et al.*, 1993; Raphael *et al.*, 1994; Bacchi *et al.*, 1996b; Carbone *et al.*, 1996; Table 15). In many of the tumours, the majority of the cells contained EBV (Borisch-Chappuis *et al.*, 1990a,b; Guarner *et al.*, 1991), although in several studies the fraction of EBV positivity was somewhat variable (Borisch *et al.*, 1992a; Carbone *et al.*, 1993a;

Table 15. Presence of EBV in HIV-associated systemic non-Hodgkin's lymphoma tissue

Reference	Study area	Detection method	Histological type	No. of cases	No. with EBV	Tumour cells with EBV	Comments
Subar *et al.* (1988)	USA	EBV DNA, SB	Diffuse large cell	4	1		
			Immunoblastic	2	2		
			Small non-cleaved cell	10	3		
		EBNA, IFA	Diffuse large cell	3	1	80%	
			Immunoblastic	1	1		
			Small non-cleaved cell	4	2		
Borisch-Chappuis *et al.* (1990a,b)	Germany	*Bam*HI W, ISH	Diffuse large cell	1	1	> 50%	
			Immunoblastic	2	1		EBV-positive tumour, T immunophenotype
			Small non-cleaved cell	9	4		
			Anaplastic large cell	1	0		
			Other high grade	1	1		
Boiocchi *et al.* (1990)	Italy	*Bam*HI W, SB	Immunoblastic	2	1		EBV monoclonal
			Small non-cleaved cell	3	0		
Boyle *et al.* (1991)	Australia	EBNA-1, PCR	Immunoblastic	12	6		
			Small non-cleaved cell	7	3		
			Other high grade	1	1		
Guarner *et al.* (1991)	USA	EBV DNA, ISH	Diffuse large cell	4	2	> 80%	
			Immunoblastic	2	1		
			Small non-cleaved cell	6	6		
			Other	2	0		
MacMahon *et al.* (1991)	USA	EBER-1, ISH	NR	7	3		
Neri *et al.* (1991)	USA	*Bam*HI	Diffuse large cell	1	1		EBV monoclonal in 10/10
			Immunoblastic	1	1		
			Small non-cleaved cell	5	5		
			Other	3	3		

Table 15 (contd)

Reference	Study area	Detection method	Histological type	No. of cases	No. with EBV	Tumour cells with EBV	Comments
Borisch *et al.* (1992a)	Switzerland	EBER-1, ISH	Diffuse large cell	3	3	Variable	
			Immunoblastic	8	8		
			Small non-cleaved cell	2	0		
			Anaplastic large cell	1	0		
		LMP, IHC	Diffuse large cell	3	1		
			Immunoblastic	8	3		
			Small non-cleaved cell	2	0		
			Anaplastic large cell	1	0		
Ballerini *et al.* (1993)	USA	EBV DNA, SB and EBNA-1, IHC	Diffuse large cell	4	1		EBV monoclonal
			Immunoblastic	4	4		
			Small non-cleaved cell	16	5		
Carbone *et al.* (1993a)	Italy	EBV DNA, ISH	Immunoblastic	4	1	Variable	
			Small non-cleaved cell	9	4		
			Anaplastic large cell	9	7		
Carbone *et al.* (1993b)	Italy	EBER-1/2 or *Bam*HI W, ISH	Diffuse large cell	6	1		
			Immunoblastic	7	3		
			Small non-cleaved cell	15	6		
			Anaplastic large cell	12	10		
		LMP, IHC	Diffuse large cell	6	0		
			Immunoblastic	7	3		
			Small non-cleaved cell	15	0		
			Anaplastic large cell	12	9		
Hamilton-Dutoit *et al.* (1993a)	France and Denmark	EBER-1, ISH or *Bam*HI W, SB LMP-1, IHC	Immunoblastic	30	22	30–90%	
			Small non-cleaved cell	19	11		
			Immunoblastic	30	16		
			Small non-cleaved cell	19	3		

Table 15 (contd)

Reference	Study area	Detection method	Histological type	No. of cases	No. with EBV	Tumour cells with EBV	Comments
Shibata *et al.* (1993)	USA	EBNA-1, PCR and EBER-1, ISH	Diffuse large cell	11	6		EBV monoclonal
			Immunoblastic	20	17		
			Small non-cleaved cell	28	16		
Raphael *et al.* (1994)	France	*Bam*HI W, SB or EBER, ISH	Diffuse large cell	3	0		
			Immunoblastic	9	8		
			Small non-cleaved cell	16	8		
			Anaplastic large cell	1	0		
			Other high grade	3	1		
Bacchi *et al.* (1996b)	Brazil	EBER-1, ISH	Diffuse large cell	11	6	40–100%	1 EBV-negative tumour T immunophenotype
			Immunoblastic	4	3		
			Small non-cleaved cell	5	2		
Carbone *et al.* (1996)	Italy	EBER-1/2, ISH	Small non-cleaved cell	11	4	25–75%	EBV monoclonal
			Anaplastic large cell	5	4		
		LMP, IHC	Small non-cleaved cell	10	0		
			Anaplastic large cell	5	3		

*Bam*HI V and *Bam*HI W, DNA restriction fragments; EBER, EBV-encoded RNA; EBNA, EBV nuclear antigen; IFA, immunofluorescence assay; IHC, immunohistochemistry; ISH, in-situ hybridization; LMP, latent membrane protein; PCR, polymerase chain reaction; SB, Southern blot; NR, not reported

Hamilton-Dutoit *et al.*, 1993a; Bacchi *et al.*, 1996b; Carbone *et al.*, 1996). Immunoblastic lymphomas (classified as diffuse large-cell lymphomas in the REAL classification) tend to be associated with more advanced immunosuppression, and these tumours more commonly contain EBV than other diffuse large-cell and small non-cleaved-cell lymphomas (Ballerini *et al.*, 1993; Hamilton-Dutoit *et al.*, 1993a; Raphael *et al.*, 1994); Carbone *et al.* (1993a,b; 1996) also noted a higher frequency of EBV positivity in anaplastic large-cell tumours. EBV monoclonality has been consistently observed by terminal-repeat sequence analysis (Boiocchi *et al.*, 1990; Neri *et al.*, 1991; Ballerini *et al.*, 1993; Shibata *et al.*, 1993; Carbone *et al.*, 1996).

2.2.4 *Congenital immunodeficiency syndromes*

Patients with X-linked lymphoproliferative syndrome (see section 4.2) are at increased risk for non-Hodgkin's lymphoma, which occurs in up to 25% of affected individuals (Purtilo, 1981; Sullivan & Woda, 1989). Wiskott-Aldrich syndrome, ataxia telangiectasia and other primary immunodeficiency syndromes are also associated with greatly increased risks for lymphoma (Filipovich *et al.*, 1992; Schuster *et al.*, 1995).

Patients with X-linked lymphoproliferative syndrome have a specific defect in their control of infection with EBV. EBV was found in all of 82 lymphomas associated with this condition (Sullivan & Woda, 1989). Several case reports of lymphomas associated with Wiskott-Aldrich syndrome (Okano *et al.*, 1984; Nakhleh *et al.*, 1991; Nakanishi *et al.*, 1993), ataxia telangiectasia (Saemundson *et al.*, 1981; Mselati *et al.*, 1983) and severe combined immunodeficiency (Garcia *et al.*, 1987) have confirmed the near universal presence of EBV in lymphomas associated with congenital immunodeficiency, although one EBV-negative case has been reported (Okano *et al.*, 1984; Table 16).

Table 16. Prevalence of EBV-associated lymphomas arising in children with primary immunodeficiency

Reference	Study area	Detection method	Primary immunodeficiency	No. of cases	No. with EBV
Sullivan & Woda (1989)	USA and Europe	Immunofluorescence	X-linked lymphoproliferative	82	82
Okano *et al.* (1984)	Japan	Immunofluorescence and EBV DNA	Wiskott-Aldrich	2	1
Nakanishi *et al.* (1993)	Japan	EBV DNA	Wiskott-Aldrich	1	1
Nakhleh *et al.* (1991)	USA	EBV DNA, in-situ hybridization	Wiskott-Aldrich	1	1
Saemundsen *et al.* (1981)	Turkey	EBV DNA	Ataxia telangiectasia	1	1
Mselati *et al.* (1983)	France	Serology	Ataxia telangiectasia	2	2
Garcia *et al.* (1987)	USA	EBV DNA	Severe combined	1	1

2.3 Hodgkin's disease

2.3.1 *Pathology and clinical features*

Histologically, Hodgkin's disease is characterized by mononuclear Hodgkin cells and their multinucleated variants, the Reed-Sternberg cells, together abbreviated as HRS cells. HRS cells are embedded in a background of abundant reactive cells, including lymphocytes, plasma cells, histiocytes and eosinophils (Lukes & Butler, 1966). Typically, HRS cells account for only a small proportion of cells in an affected lymph node, rarely amounting to more than 2% of the total cell population. The Rye classification distinguishes four major types of Hodgkin's disease: nodular lymphocyte predominant, nodular sclerosis, mixed cellularity and lymphocyte-depleted (Lukes & Butler, 1966; Herbst & Niedobitek, 1993; Harris *et al.*, 1994). It is now accepted that lymphocyte-depleted Hodgkin's disease represents a separate tumour entity probably derived from germinal-centre B cells, and this is considered separately from the other three 'classical' forms of Hodgkin's disease. Lymphocyte-depleted Hodgkin's disease is encountered only rarely at primary diagnosis of Hodgkin's disease. It is usually seen as recurrent Hodgkin's disease in patients in whom nodular sclerosis or mixed cellularity Hodgkin's disease had been diagnosed previously. Moreover, many cases diagnosed as lymphocyte-depleted Hodgkin's disease are now reported as CD30-positive anaplastic large-cell lymphomas. There is thus increasing evidence to suggest that Hodgkin's disease is not a single entity but rather a heterogeneous group of diseases. This prompted inclusion of Hodgkin's disease in the Revised European–American Lymphoma (REAL) classification (Harris *et al.*, 1994).

The nature and clonal origin of HRS cells are a matter of controversy: most of the constituent cells of the lymph node have been proposed as HRS precursor cells at some stage (Herbst *et al.*, 1996a). HRS cells are characterized immunophenotypically by the expression of lymphocyte-activation antigens, such as CD25, CD30 and CD70 (Herbst *et al.*, 1993; Harris *et al.*, 1994). In a proportion of cases of Hodgkin's disease, HRS cells express B-cell antigens, and in a number of cases antigens characteristic of T cells can be demonstrated (Kadin *et al.*, 1988; Schmid *et al.*, 1991; Herbst *et al.*, 1996a); however, in most cases, no lineage-specific antigens are seen (Herbst *et al.*, 1993). Most recent studies indicate that HRS cells in many (but not all) cases are derived from B cells (Schmid *et al.*, 1991; Küppers *et al.*, 1994), and a germinal-centre origin has been proposed (Kanzler *et al.*, 1996).

The clinical presentation of Hodgkin's disease varies in different geographical locations. In this section, the clinical features seen in the western world are described briefly. Hodgkin's disease usually arises as a unifocal lesion in cervical lymph nodes. Contiguous spread of the tumour to adjacent lymph nodes gives rise to palpably enlarged nodes. With spread of the tumour through lymphatic channels, other organs are involved, the preferential sites of involvement including the spleen and distant lymph nodes. Subsequently, as the disease becomes more aggressive, other organs are involved, including the liver and the kidneys (Kaplan, 1980).

The presence of Hodgkin's disease at each of 17 sites of potential nodal involvement was determined in 719 patients in the United States. The predominant sites of lymph

node involvement were mediastinal (59%), left neck (58%) and right neck (55%), and at least one of these sites was involved in 92% of the patients (Mauch *et al.*, 1993). The spleen was involved in 27% of patients; the epitrochlear, popliteal and mesenteric lymph nodes were not commonly involved. Abdominal lymph node involvement without splenic involvement was rare, and the risk for abdominal lymph node involvement increased with increasing splenic size.

Bone-marrow involvement in Hodgkin's disease is indicative of extensive tumour infiltration and is associated with systemic symptoms including leukopenia, anaemia, thrombocytopenia and elevated levels of alkaline phosphatases. Other organs occasionally involved in Hodgkin's disease include the skin, subcutaneous tissue and breast. Involvement of the central nervous system is relatively rare, except through invasion and extension of the epidural space for enlarged para-aortic nodes. Hilar and mediastinal adenopathy may predispose to pleural involvement, with pleural effusions and pericardial involvement (DeVita *et al.*, 1993).

2.3.2 *Epidemiology*

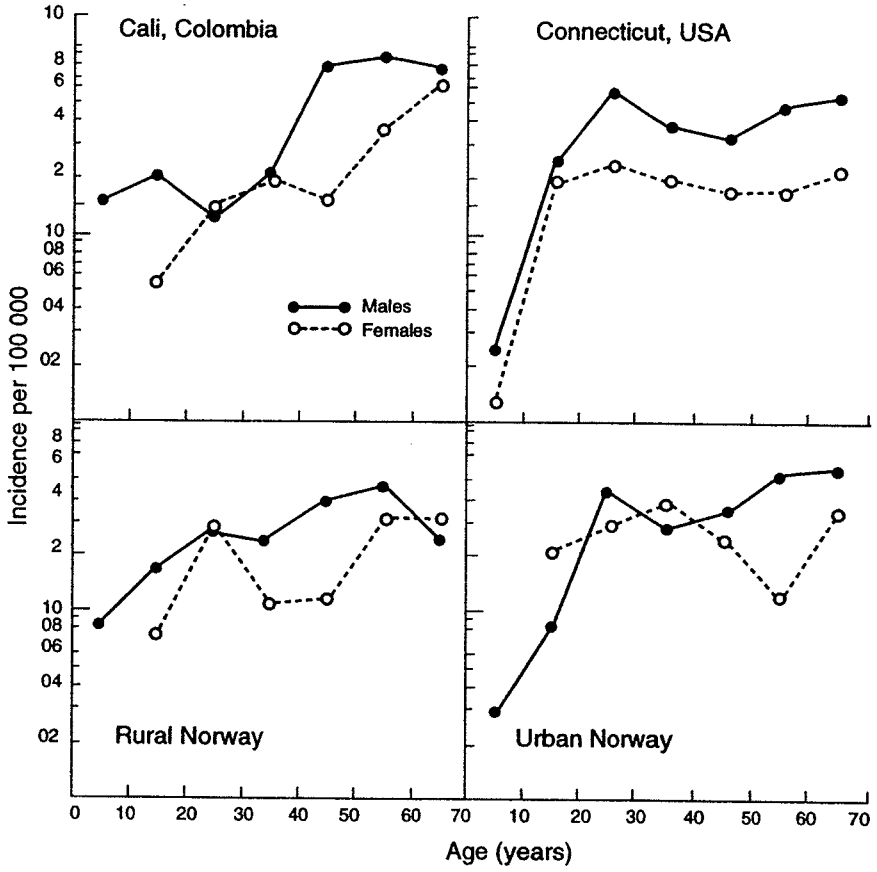
2.3.2.1 *Descriptive epidemiology*

The distinguishing epidemiological feature of Hodgkin's disease characteristically seen in most western populations such as the United States is the bimodal age–incidence curve (Figure 8). In such populations, very few cases occur among children; a rapid increase in incidence among teenagers peaks at about age 25; the incidence then decreases to a plateau through middle age, after which the rates increase with age to a second peak. There is an excess among males, which is pronounced at older ages. MacMahon (1957) proposed that the bimodality results from the overlap of two disease distributions with peaks at different ages. He further suggested that Hodgkin's disease in young adults is caused by a biological agent of low infectivity, while the cause among the elderly is probably similar to those of other lymphomas (MacMahon, 1966).

In 1971, Correa and O'Conor noted a different age pattern among poor populations, with an initial peak in childhood only among boys, relatively low rates among young adults, followed by a late peak among those of advanced age. They further described an intermediate pattern, contrasting data for rural and urban Norwegians in the 1960s (see Figure 8). The shift from a 'developing' to an 'intermediate' pattern in parallel with economic development has been noted by others (Glaser, 1990; Hartge *et al.*, 1994). An intermediate pattern of the occurrence of Hodgkin's disease was seen in African Americans in the late 1970s (Olisa *et al.*, 1976; Cozen *et al.*, 1992), which is less evident in recent data (Parkin *et al.*, 1997).

Currently, essentially all of the majority populations in Europe and North America have a well-defined 'developed' pattern of incidence of Hodgkin's disease. The peak of occurrence in young adulthood varies within this set of countries, being high in Canada, France, Switzerland and the United States and lower in southern Europe. The pattern within eastern European countries is variable. In contrast, the pattern in Asia and Africa is generally 'intermediate' or 'developing' (Parkin *et al.*, 1997).

Figure 8. Age-specific incidence rates of Hodgkin's disease per 100 000 population of each sex in (a) Cali, Colombia (1962–66), (b) Connecticut, United States (1960–62), (c) rural Norway (1964–66) and (d) urban Norway (1964–66)



From Correa and O'Conor (1971)

Alexander *et al.* (1991a,b) used a large, population-based registry of leukaemia and lymphoma covering about half of the United Kingdom during a five-year period to evaluate the characteristics of over 1800 cases of Hodgkin's disease by area-based indices of socioeconomic status and population density. They reported that significantly more of the 486 cases diagnosed in people under the age of 25 occurred in areas of higher socioeconomic status (relative risk [RR], 1.2), and there was a significant trend to increased incidence in areas closer to 'built-up areas' (mutually adjusted). For cases among people aged less than 35 years at the time of diagnosis, there was a significantly positive association with social class, while a negative association was found for cases grouped by ages 35–49 and 50–79, the trend for the latter being significant.

Descriptive studies have shown that the association between higher social class and Hodgkin's disease in young adults is specific for the nodular sclerosis subtype. Henderson *et al.* (1979) computed the incidence rates of Hodgkin's disease of specific histological types in Los Angeles County (United States) in 1972–75 by social class. They reported that the incidence of the nodular sclerosis type was directly related to social class; there was no consistent association for the other histological types. These data were confirmed and extended through 1985 by Cozen *et al.* (1992), who also found

an increase in incidence between 1972 and 1985 only among cases of the nodular sclerosis subtype. They further reported that the risk pattern for Hodgkin's disease of mixed cellularity was quite distinct and negatively associated with social class. This latter finding fits the general observation that cases of Hodgkin's disease occurring in economically developing populations (Mueller, 1987) and among groups of lower social class in developed populations (Hu *et al.*, 1988) are predominantly of the mixed cellularity and lymphocyte depletion subtypes.

These data are consistent with the rates in the United States in 1969–80 (Glaser, 1987): thus, the incidence rates for young adults were positively correlated with community-level indicators of social class and the incidence of the nodular sclerosis subtype increased in parallel with regional indices of social class.

There is a consistent body of evidence that the risk for Hodgkin's disease occurring from early childhood through middle age is associated with factors in the childhood environment that influence the age at infection with a virus such as EBV. The pattern of association with these factors varies with the age at diagnosis. The data have been reviewed (Jarrett *et al.*, 1996; Mueller, 1996).

Because Hodgkin's disease in childhood occurs primarily in developing countries, the children at risk for the disease would appear to be of lower social class and thus to be infected earlier. This has been a consistent finding in the few case–control studies that have been reported (Sobrinho-Simões & Areias, 1978; Gutensohn & Shapiro, 1982; Bogger-Goren *et al.*, 1983). A shift occurs in young adults (generally defined as between 15 and 40 years of age), who have been found quite consistently to be of higher social class than expected, as measured by occupation or educational attainment (Cohen *et al.*, 1964; Gutensohn & Cole, 1977; Abramson *et al.*, 1978; Serraino *et al.*, 1991). In addition, there is an inverse association between risk for Hodgkin's disease and number of siblings and also with birth order (Gutensohn & Cole, 1977, 1981; Bernard *et al.*, 1987; Bonelli *et al.*, 1990). The young adult patients also lived in less crowded conditions during childhood. Among the oldest patients, however, no consistent association with social class was seen (Abramson *et al.*, 1978; Gutensohn, 1982).

As is generally recognized for most haematopoietic malignancies, Jews are at somewhat higher risk for Hodgkin's disease than non-Jews (MacMahon, 1960). In a study of cases occurring in the 1950s in Brooklyn, New York, United States, MacMahon (1957) reported that older, but not younger, Jews were at increased risk. In a population-based case–control study conducted in Boston–Worcester (United States) in the 1970s, however, Jewish people of all ages in the population were at particularly high risk (Gutensohn & Cole, 1981). Bernard *et al.* (1984, 1987) also noted an excess of Jewish cases in their population-based studies in Yorkshire, United Kingdom. Similarly, an increased incidence among Jews was documented in Los Angeles County, United States (Cozen *et al.*, 1992).

In summary, there is evidence that the risk for Hodgkin's disease in young adulthood through middle age is associated with higher education, higher social class, fewer siblings, less crowded housing and early birth rank. All of these factors lead to susceptibility to late infections with the common childhood infections. As in the model of

paralytic polio, such late infections tend to be more severe than those in younger children; however, social class does not appear to predict the occurrence of Hodgkin's disease in the late decades of life, when primary infection with EBV is unlikely.

2.3.2.2 *Association with EBV*

(*a*) *Case reports and case series*

There have been numerous case reports of Hodgkin's disease developing in close association with serologically documented primary infection with EBV (Kaplan, 1980) but with no direct evidence of the presence of the virus in the tumour itself.

(i) *Infectious mononucleosis*

Poppema *et al.* (1985) described a case of mixed cellularity Hodgkin's disease that developed after heterophil-negative infectious mononucleosis. The patient had been followed clinically by multiple node biopsies and serology during this period and was described as having a clinical and serological picture consistent with chronic EBV infection. The investigators demonstrated the presence of EBNA in HRS cells from involved lymph nodes.

(ii) *Serology*

Few data are available on the relationship between EBV serology and EBV status. Ohshima *et al.* (1990) reported a single case of an eight-year-old boy with EBV-positive Hodgkin's disease, as determined by Southern blot and PCR. His titres were 1:1280 anti-VCA IgG, 1:10 anti-VCA IgA, 1:10 anti-VCA IgM, 1:160 anti-EA and 1:40 anti-EBNA, consistent with an active EBV infection. In an overlapping series of 107 cases, Brousset *et al.* (1991) and Delsol *et al.* (1992) concluded that there was no association between the presence of EBV and a serological pattern of reactivation, which they defined as > 1:640 anti-VCA, > 1:40 anti-EA and > 1:160 anti-EBNA; however, only one of 35 EBV-negative and none of 16 EBV-positive cases had this rather extreme pattern. Levine *et al.* (1994) assessed the relationship of the EBV status of 39 cases with serological results published previously. No differences in anti-VCA or anti-EA titres were seen between EBV-positive and EBV-negative cases. When the sera of 19 of these cases were subsequently tested for anti-EBNA-1 and anti-EBNA-2, none of the 5 EBV-positive cases showed elevated titres (> 1:320) of EBNA-1, whereas 5 of 14 EBV-negative cases did so. Conversely, 2 of the 5 positive cases and 2 of the 14 negative cases had elevated titres (> 1:80) of EBNA-2. Neither of these differences was statistically significant (Mueller, 1997).

In preliminary reports from two groups, specimens from 54 patients with Hodgkin's disease who had reported a history of infectious mononucleosis were tested for the presence of the EBERs. They were found in only one (Mueller, 1997).

(iii) *Viral nucleic acid and protein*

With the advent of highly sensitive molecular probes, dozens of case series have now been published on the detection of genomic segments, transcripts and viral products of EBV in a substantial proportion of biopsy samples from patients with Hodgkin's disease.

Many of these reports (restricted to HIV-1-negative cases) are summarized in Table 17. Weiss *et al.* (1987, 1988) and Staal *et al.* (1989) first reported the detection of monoclonal EBV genome in Hodgkin's disease tissue, Staal *et al.* noting that the number of viral episomes per cell was low. Weiss *et al.* (1989a), Uccini *et al.* (1989) and Anagnostopoulos *et al.* (1989) all reported detection of the viral genome within HRS cells (and their variants) themselves. In 1990, Wu *et al.* reported detection of RNA EBER transcripts in HRS cells. Pallesen *et al.* (1991a) and Herbst *et al.* (1991a) reported that the EBV in Hodgkin's disease has a restricted latent phenotype of LMP-1 expression without detectable EBNA-2, as in nasopharyngeal carcinoma. These findings have been replicated in dozens of populations by a large number of laboratories.

EBV nucleic acid has also been found in a fraction of small lymphocytes in both EBV genome-positive and -negative tissue from patients with Hodgkin's disease (Masih *et al.*, 1991; Weiss *et al.*, 1991; Herbst *et al.*, 1992; Khan *et al.*, 1992; Ambinder *et al.*, 1993; Bhagat *et al.*, 1993; Chang *et al.*, 1993b; Jiwa *et al.*, 1993b), and at a low frequency in normal lymph nodes (Niedobitek *et al.*, 1992a).

Consistency of viral marker status of individual patients: EBV status is consistent among anatomically distinct sites involved at diagnosis. Vasef *et al.* (1995) evaluated 14 cases with two to five involved sites for the concordance of EBV status among patients. All of the biopsy samples from eight patients were EBV genome-negative and all those from six patients were EBV genome-positive. In five of the latter cases, the investigators analysed the 3′ end of the *LMP-1* gene at all sites of disease by PCR. In three patients, all of the sites had a 30-base pair deletion; in the other two cases, there was discordance in the presence of this deletion, some sites having the germ-line configuration.

EBV status appears to be stable if not identical over time. Delsol *et al.* (1992) reported that EBV status was consistent in subsequent biopsy samples at relapse (range, 14–126 months) in 12 cases, of which seven were initially EBV-positive. Two EBV-positive cases showed substantial reduction or loss of staining for LMP-1 in their later biopsies. Coates *et al.* (1991a) found that sequential biopsy samples from three EBV-positive patients (age range, 2–10 years) contained EBV at about the same level as in the initial sample. Boiocchi *et al.* (1993a) reported the same clonal EBV genome in each of two patients from whom two or three biopsy samples had been taken over a period of up to 11 months. Brousset *et al.* (1994) evaluated 12 cases of relapsed Hodgkin's disease for consistency of EBV status: five showed no EBV at either initial diagnosis or relapse, while seven that were initially EBV-positive remained positive at relapse. For two of the latter, additional assays — including terminal repeat polymorphism by Southern blot and LMP-1 polymorphism sequencing by PCR — were carried out on tissue taken at both diagnosis and relapse. In one case, the EBV genome appeared to be identical in sequential biopsy samples taken nine years apart. In the other case, a monoclonal episome was detected by Southern blot in the initial sample but not in that taken at relapse eight years later. The authors noted that the latter finding may be due to a low viral load that cannot be detected by Southern blot. Both samples from this patient contained an identical mutation in EBV *LMP-1*.

Table 17. Detection of the EBV genome or gene products in tissues from HIV-1-negative cases of Hodgkin's disease

Reference	No. of cases	No. with EBV genome or gene products	Method of detection	Comments
Weiss *et al.* (1987, 1988)	21	4	SB, slot–blot	More mixed cellularity than nodular sclerosis types positive; no cytomegalovirus; overlaps with next study
Weiss *et al.* (1989a)	16	3	SB, slot–blot, ISH	
Staal *et al.* (1989)	28	8	SB	More mixed cellularity than nodular sclerosis types positive
Uccini *et al.* (1989)	32	6	SB, ISH	
Boiocchi *et al.* (1989)	17	7	SB	
Herbst *et al.* (1989)	39	5	SB	Overlaps with next study
Anagnostopoulos *et al.* (1989)	42	7	SB, ISH	
Uhara *et al.* (1990)	31	8	PCR, ISH	More mixed cellularity than nodular sclerosis types positive
Bignon *et al.* (1990)	16	8	PCR	Mostly young adults
Wu *et al.* (1990)	8	6	ISH (EBER)	EBV-positive cases from Staal *et al.* (1989)
Herbst *et al.* (1990)	198	114	PCR, ISH	No variation by age or sex
Libetta *et al.* (1990)	34	15	SB	No variation by age
Uccini *et al.* (1990)	20	3	SB, ISH	None contained VCA or EA; all positive cases were of mixed cellularity type
Ohshima *et al.* (1990)	7	2	SB, PCR	Serology available for one case: high titre of anti-VCA and EA IgG, positive for anti-VCA IgM and IgA
Pallesen *et al.* (1991a)	84	40	mAb to LMP-1 and EBNA-2	Positive only for LMP-1; more mixed cellularity than nodular sclerosis types positive
Gledhill *et al.* (1991)	35	11	PCR	8 of 8 analysed were of EBV type 1; no association with histology and little with age

Table 17 (contd)

Reference	No. of cases	No. with EBV genome or gene products	Method of detection	Comments
Brousset *et al.* (1991)	54	16	ISH ('mRNA')	More mixed cellularity than nodular sclerosis types positive; no correlation with serology (one case seronegative); results later interpreted as detection of EBV DNA (Delsol *et al.*, 1992)
Herbst *et al.* (1991a)	47	32 with DNA 18 with LMP	PCR, mAb to LMP-1, EBNA-2 and gp350/250	All LMP-1-positive positive by PCR; no variation by histology
Knecht *et al.* (1991)	48	38	PCR (semi-quantitative)	About two-thirds of cases had numerous HRS; no association with histology or proportion of HRS
Masih *et al.* (1991)	52	30	PCR, slot–blot, SB	More mixed cellularity than nodular sclerosis types positive, 43% of controls with hyperplastic lymph nodes were EBV-positive; two of six tested were clonal
Pallesen *et al.* (1991c)	96	47	mAb to LMP-1, ZEBRA, EA, VCA, MA	Includes cases from Pallesen *et al.* (1991a); no cytomegalovirus; three positive for LMP-1 also positive for ZEBRA; none positive for EA, VCA or MA
Vestlev *et al.* (1992)	66	27	Follow-up of cases from above	LMP-1-positive cases more likely to be of mixed cellularity type, male, less likely to have mediastinal involvement; LMP-1 status not associated with prognosis
Jarrett *et al.* (1991)	95	43	SB	48 cases selected for age and histological type; includes cases from Gledhill *et al.* (1991); positivity highest among children and older adults; 30 of 30 samples tested were of EBV type 1
Weiss *et al.* (1991)	36	14	PCR, ISH (EBER-1)	More mixed cellularity than nodular sclerosis types positive; some background B and T cells positive

Table 17 (contd)

Reference	No. of cases	No. with EBV genome or gene products	Method of detection	Comments
Coates *et al.* (1991a)	55	9	ISH	More cases of nodular sclerosis (7/24) than of mixed cellularity (2/16) positive; no difference in mean age of EBV-positive and EBV-negative cases; variation in amount of virus between cases; 3 patients ≤ 10 years with sequential biopsies positive at same level in all specimens; in 8 other cases, EBV found only in non-neoplastic cells
Brocksmith *et al.* (1991)	57	33	SB, PCR	More mixed cellularity than nodular sclerosis types positive; no variation with age
Delsol *et al.* (1992)	107	37	ISH, mAb to LMP-1, EBNA-2	Overlaps with Brousset *et al.* (1991a); more mixed cellularity types positive; all 12 cases tested at diagnosis and relapse remained concordant for EBV; one LMP-1-positive case became LMP-negative and one LMP-1-positive case became intermediate; no correlation with serology or short-term prognosis; all 13 positive cases EBNA-2-negative
Khan *et al.* (1992)	33	12	ISH (EBER)	In six cases, EBER localized to non-neoplastic small lymphocytes only
Herbst *et al.* (1992)	46	26	ISH (EBER-1/2); mAb to LMP-1	18 positive for LMP-1 (all EBER-positive); EBER-positive small lymphocytes found in 39 cases at low levels, 3 at high levels
Fellbaum *et al.* (1992)	187	66	PCR	More mixed cellularity types positive; no association with survival
Murray *et al.* (1992b)	46	22	mAb to LMP-1	Positivity and proportion of LMP-positive HRS cells increased with 'histological grade'
Ambinder *et al.* (1993)	36	20	ISH (EBER-1); mAb to LMP1	All cases < 15 years; 11 of 11 cases from Honduras and 9 of 25 from USA positive; no cytomegalovirus; EBV-positive small lymphocytes also seen in 9 positive cases; more mixed cellularity than nodular sclerosis types positive in US series

Table 17 (contd)

Reference	No. of cases	No. with EBV genome or gene products	Method of detection	Comments
Boyle *et al.* (1993)	12	3	PCR, ISH	Positivity associated with younger age
Brousset *et al.* (1993)	35	3	ISH (ZEBRA, EBER-1/2), mAb to LMP-1, SB	All patients had relapsed; all consistent in EBV status at sequential biopsies; in 2 cases analysed, EBV appeared to be identical in both samples
Chang *et al.* (1993b)	32	30	ISH (EBER-1), mAb to LMP-1, MA	Patients from Peru; positivity by age: 19 of 19 ≤ 15 years, 5/6 15–39, 6/7 older; some small lymphocytes positive
Carbone *et al.* (1993c)	39	15	ISH, mAb to LMP-1, vimentin	More mixed cellularity than nodular sclerosis types positive; vimentin found in 24 cases localized to HRS cells, including all 11 LMP-1-positive cases
Deacon *et al.* (1993)	23	16	ISH (EBER-1/2), mAb to LMP-1	Transcription analysis demonstrated EBV latency pattern found in nasopharyngeal carcinoma
Jiwa *et al.* (1993b)	33	19	PCR, ISH (EBER-1/2), mAb to LMP, bcl-2, c-myc	Almost all cases nodular sclerosis type; some small lymphocytes also positive; 20 of 29 cases expressed bcl-2 and 30 of 32 expressed c-myc in HRS cells independently of EBV status
Niedobitek *et al.* (1993a)	116	33	ISH (EBER), mAb to p53	More mixed cellularity than nodular sclerosis types positive; 37 positive for p53 in HRS cells independently of histology, less frequent in EBV-positive (21%) than EBV-negative (36%) [$p = 0.12$]
Lin *et al.* (1993a)	23	16	PCR (EBER) (multiple gene loci)	2 of 10 reactive hyperplasia nodes were EBV-positive
Bhagat *et al.* (1993)	11	4	ISH (EBER-1), mAb to bcl-2	Most cases nodular sclerosis: 3 of 7 with t(14;8) and 5 of 6 without t(14;18) were bcl-2-positive; bcl-2 antibody reacted with HRS cells but also in majority of small lymphocytes; EBV-positivity not correlated with presence of t(14;18) or bcl-2

Table 17 (contd)

Reference	No. of cases	No. with EBV genome or gene products	Method of detection	Comments
Khan *et al.* (1993)	77	25	ISH (EBER), mAb to LMP-1	Cases selected on basis of histology and age; high positivity associated with mixed cellularity type (68%); some increase with age; some small lymphocytes positive; no cytomegalovirus or HHV6
Kanavaros *et al.* (1994)	22	12	ISH (EBER), mAb to LMP-1	Paediatric cases from Greece; more mixed cellularity than nodular sclerosis types positive
Gulley *et al.* (1994)	125	58	SB, ISH (EBER)	79 cases from USA, 31 from Mexico and 15 from Costa Rica; more mixed cellularity than nodular sclerosis types positive; Hispanic ethnicity associated with positivity (RR = 4.3)
Poppema & Visser (1994)	72	19	ISH (EBER-1/2), mAb to LMP-1, HLA-A2	Cases from Canada; more mixed cellularity types positive; no association with HLA-A2
Zarate-Osorno *et al.* (1994)	27	18	ISH (EBER-1)	Cases from Mexico; more mixed cellularity types positive
Quintanilla-Martínez *et al.* (1995)	50	35	mAb to LMP-1	Cases from Mexico; more mixed cellularity than nodular sclerosis types positive
Preciado *et al.* (1995)	41	22	SB; ISH (EBER), mAb to EBNA-2, LMP-1	Paediatric cases from Argentina; more mixed cellularity than nodular sclerosis types and more younger children (< 7) positive
Chan *et al.* (1995b)	23	15	ISH (EBER-1), mAb to BHLF1	Cases from Hong Kong; more mixed cellularity than nodular sclerosis types positive for EBER; none positive for BHLF1; least positivity among persons aged 15–49 years
Li *et al.* (1995b)	40	17	ISH (EBER-1), mAb to LMP-1	Cases from Japan; more mixed cellularity than nodular sclerosis types positive; positive cases more likely to have some positive small lymphocytes in background

Table 17 (contd)

Reference	No. of cases	No. with EBV genome or gene products	Method of detection	Comments
Vasef *et al.* (1995)	14	6	ISH (EBER-1, LMP-1)	Patients had two to five separate involved sites; all biopsy samples consistent for EBV status; three positive cases had identical LMP-1 genes; 2 patients had discordant LMP-1 genes at different sites
Leoncini *et al.* (1996)				
Kenya	92	85	ISH (EBER)	More mixed cellularity types positive in both series; positivity among cases of nodular sclerosis in Kenya higher than that in cases in Italy. In Kenya, more children ≤ 15 years (41 of 42) positive than adults > 15 years (44 of 50)
Italy	65	31		
Weinreb *et al.* (1996a)	101	85	ISH (EBER-1/2), mAb to LMP-1	Cases from Kenya. Positivity varied by age: 100% in 53 paediatric cases, 67% in 48 adult cases
Tomita *et al.* (1996)	50	32	PCR, SB, ISH (EBER)	Cases from Japan; more mixed cellularity than other types, more older (> 40) than younger (< 40 years) and more males positive
Weinreb *et al.* (1996b)	277	196	ISH (EBER-1/2), mAb to LMP-1	Children in 10 countries; positivity 50–100%, more mixed cellularity type and more males positive [HIV status unknown]
Huh *et al.* (1996)	87	60	ISH, ISH (EBER, BHLF), mAb to LMP-1	Cases from Republic of Korea; positivity increased with stage; more mixed cellularity than nodular sclerosis positive; only 1 case positive for *Bam*HI H left-frame transcript

Adapted from Mueller (1996)

HRS, Hodgkin and Reed-Sternberg; ISH, in-situ hybridization (for EBV genome probe, unless otherwise specified); SB, Southern blot; PCR, polymerase chain reaction; LMP-1, latent membrane protein 1; EBNA, EBV nuclear antigen; EBER, EBV-encoded RNA; mAb, monoclonal antibody; EA, early antigen; ZEBRA, Z EBV replication activator; gp350/250, envelope glycoprotein; VCA, viral capsid antigen; MA, membrane antigen

Relationship to clinical features: Few clinical or other molecular features appear to be related to EBV status. O'Grady *et al.* (1994) reported that EBV positivity in stage-I disease is associated with cervical neck node presentation, irrespective of histological subtype. This could be related to primary EBV infection in the oropharynx and would appear to strengthen the association between Hodgkin's disease and previous infectious mononucleosis. Similarly, Kapadia *et al.* (1995) reported a relatively high rate of EBV positivity in 8 of 12 cases of Hodgkin's disease occurring in Waldeyer's tonsillar ring. Primary Hodgkin's disease of the tonsils is rare, however, and recent studies have suggested that HRS cells in cases of Hodgkin's disease arising in association with infectious mononucleosis are predominantly EBV-negative (Mueller, 1997). EBV genome status has not been found to be an independent predictor of prognosis (Delsol *et al.*, 1992; Fellbaum *et al.*, 1992; Vestlev *et al.*, 1992).

Relationship to histology, age and ethnicity: Several factors appear to be predictive of EBV status. Viewing the data on the two most common subtypes overall (Table 17), cases of the mixed cellularity subtype generally occur at higher rates than those of nodular sclerosis. Given the difference in the age and sex distribution of these two subtypes of Hodgkin's disease (Medeiros & Greiner, 1995), the presence of EBV should have a U-shaped relationship with age, with the highest rates at the extremes of age and the lowest in young adulthood. The general consensus is that EBV infection of HRS cells is at best rare in cases of the nodular lymphocyte-predominant type, consistent with the notion that these are an entity distinct from the 'classical' forms of Hodgkin's disease (Pallesen *et al.*, 1991c; Shibata *et al.*, 1991a; Alkan *et al.*, 1995). Few cases of lymphocyte-depletion Hodgkin's disease have been reported in the literature, and their association with EBV is highly variable, consistent with the origin of such cases from Hodgkin's disease of either nodular sclerosis or mixed cellularity type and with the proposed overlap with CD30-positive anaplastic large-cell lymphomas (Zhou *et al.*, 1993; Bai *et al.*, 1994; Quintanilla-Martínez *et al.*, 1995; Herbst *et al.*, 1996a).

In studies of cases in developing countries, where patients generally present with more advanced disease, the rate of positivity is notably high, particularly among children. Gulley *et al.* (1994) analysed 125 cases from Costa Rica, Mexico and the United States by multivariate analysis and found that Hispanic ethnicity *per se* was an independent predictor of the presence of the EBV genome, with a RR of 4.3. Murray *et al.* (1992b) noted that the proportion of HRS cells containing LMP-1 in EBV-positive specimens increased in parallel with a less favourable histological subtype: 41% in one of 12 cases of lymphocyte-predominant disease, 49–78% in 12 of 24 cases of the nodular sclerosis type, 63–81% in six of seven cases of the mixed cellularity type and 89–97% in all three cases of lymphocyte-depletion Hodgkin's disease.

Glaser *et al.* (1997) analysed the data from a number of investigators and previously unpublished data by multivariate analysis, with a total series of 1546 HIV-1-negative cases of Hodgkin's disease, in order to identify characteristics that differentiate EBV-positive and EBV-negative cases. Cases of mixed-cell disease were significantly more likely to contain EBV than those of nodular sclerosis in all age groups: < 15 years, RR, 7.3; 15–49 years, RR, 13; ≥ 50 years, RR, 4.9. Patients under 15 years of age in developing countries were significantly more likely to have EBV than those from more deve-

loped countries [RR, 6.0]; similar results were not found for older individuals. Men aged 15–49 years, but not those in other age groups, were significantly more likely to have tumours containing the EBV genome (RR, 2.5). Overall, it appears that the presence of EBV is related to a less favourable host response.

HIV infection: Essentially all HIV-1-infected patients with Hodgkin's disease have a higher rate of EBV positivity: 8 of 10 (Moran *et al.*, 1992), 11 of 11 (Hamilton-Dutoit *et al.*, 1993b) and 14 of 18 (Tirelli *et al.*, 1995). In general, these patients present with advanced Hodgkin's disease and have a poor prognosis. [HIV-1-infected patients may be at increased risk for Hodgkin's disease (IARC, 1996).]

(*b*) *Case–control studies*

(i) *Infectious mononucleosis*

The association between history of infectious mononucleosis and Hodgkin's disease has been evaluated in several case–control studies (Table 18). A weak positive association is usually seen, particularly in young adults and patients with the nodular sclerosis subtype.

(ii) *Serology*

In case–control studies of more than 2000 patients with Hodgkin's disease of all ages, the proportion who had IgG antibodies to VCA, indicative of prior infection, was similar to that of controls (Table 19); however, the cases consistently had higher mean antibody titres than controls, except in two studies involving paediatric patients (Lange *et al.*, 1978; Shope *et al.*, 1982). Similarly, in most studies, the cases had a higher prevalence of antibodies (as well as higher titres) against the EA complex of EBV, indicative of active viral replication (Table 20). Evans and Gutensohn (1984) found that subjects with a history of infectious mononucleosis had higher GMTs against VCA and against both EA(D) and EA(R) than did subjects who did not have such a history. This was true among both cases as a group and among the sibling controls as a group, although only sera in which antibody was present at the lowest dilution were included in calculating GMTs.

In eight studies, antibodies against EBNA were also evaluated (Table 21). Of these, three showed no higher levels in diagnosed cases than in controls. Lennette *et al.* (1993) and Merk *et al.* (1995) also tested for antibodies against specific components of the EBNAs, using recombinant proteins as targets. In a study of 20 Hodgkin's disease patients and 74 grouped controls (Lennette *et al.*, 1993), the cases had higher (non-significant) GMTs against EBNA-2A and EBNA-3C but not against EBNA-1 or EBNA-2B. In a later study of 61 untreated cases and 109 healthy EBV antibody-positive controls (Merk *et al.*, 1995) tested with the same assays, the cases had elevated titres against EBNA-1, EBNA-2A and EBNA-3C but not against EBNA-2B.

Chen *et al.* (1992b) developed an assay to test for immune response to recombinant full-length LMP-1. They found that 16 of 27 patients with Hodgkin's disease but only two of 26 EBV-seropositive controls and five of 22 patients with nasopharyngeal carcinoma had IgG antibodies against LMP-1. Lennette *et al.* (1995) developed a monoclonal,

Table 18. Results of case–control studies of the association between a history of infectious mononucleosis and the risk for Hodgkin's disease

Reference	Study population	No. of cases	No. of controls	Relative risk	Factors controlled
Henderson *et al.* (1979)	Population-based: Los Angeles County (USA), incident cases, 1972–73; neighbourhood controls	212	212	1.3 Nodular sclerosis, 1.5	Age, ethnicity, sex, neighbourhood
Gutensohn & Cole (1981)	Population-based: Eastern Massachusetts (USA), incident cases, 15–39 years, 1973–77; population controls	225	447	1.8 $p < 0.05$	Age, sex, family size, birth order, housing density in childhood
Gutensohn (1982)	As above, cases 40–54 years; population controls	53	106	1.3	Age, sex, family size, religion
Evans & Gutensohn (1984)	As above, cases 15–54 years; sibling controls	262	250	1.5	Family
Bernard *et al.* (1987)	Population-based: Yorkshire Health District (UK), incident cases, 1979–84; hospital controls [subjects appear to be ≥ 15 years]	248	489	1.0 Males 15–35 years, 4.9 $p = 0.04$	Sex, age, health district
Serraino *et al.* (1991)	Hospital-based: Pordenone (Italy), 1985–90, cases 15–77 years	152	613	8.2 Nodular sclerosis, 13.1 $p < 0.05$	Sex, education

Table 19. Results of case–control studies of the association between antibody titres against EBV capsid antigen and risk for Hodgkin's disease

Reference	Cases				Controls				RR
	No.	Prevalence	GMT	High titre[a]	No.	Prevalence	GMT	High titre[a]	
Goldman & Aisenberg (1970)	57	0.58	–	0.12	54	0.61	–	–	–
Johansson *et al.* (1970)	60	0.95	100	0.47	47	0.89	43	0.17	4.3
Levine *et al.* (1971)	63	–	367	0.35[b]	85	–	91	0.05	11
de Schryver *et al.* (1972)	17	–	60	0.24	63	–	23	0.17	1.5
Henle & Henle (1973b)	489	0.89	105	0.40	294	0.84	55	0.14	4.1
Henderson *et al.* (1973)	142	0.94	92	0.47	142	0.93	53	0.25	2.6
Langenhuysen *et al.* (1974)[c]	25	0.92	1580	0.88[d]	25	0.92	585	0.60	4.9
Hirshaut *et al.* (1974)	51	0.82	141	0.16[d]	45	0.89	–	0.09	1.9
Rocchi *et al.* (1975)	100	0.98	177	0.68	100	0.91	34	0.12	16
Gotlieb-Stematsky *et al.* (1975)	67	0.91	67	0.46	186	0.66	9	0.01	79
Hilgers & Hilgers (1976)	43	0.95	2420	–	43	0.95	401	–	1.9
Hesse *et al.* (1977)[e]	185	0.95	272	0.54	185	0.95	141	0.30	12
Evans *et al.* (1978)	67	1.00	146	0.31[e]	162	–	51	0.04	12
Lange *et al.* (1978)[f]	27	0.63	116[c]	–	71	0.61	101	–	–
ten Napel *et al.* (1980)[c]	15	0.94	3180	–	17	1.00	924	–	–
Mochanko *et al.* (1979)	37	1.00	69	0.27	40	0.83	44	0.07	4.6
Evans *et al.* (1980)	70	0.97	110	0.35[e]	70	0.84	30	0.03	17
Shope *et al.* (1982)[f]	15	0.73	103	0.20	24	0.58	73	0.25	0.8
Evans & Gutensohn (1984)	304	0.86	176	0.39[e]	276	0.87[g]	58	0.14	4.0
Lennette *et al.* (1993)	23	1.00	1359	–	75	1.00	303	–	–

Table 19 (contd)

Reference	Cases				Controls				RR
	No.	Prevalence	GMT	High titre[a]	No.	Prevalence	GMT	High titre[a]	
Merk *et al.* (1995)[c]	61	1.00	–	0.72[h]	109	1.00	–	0.14	16
Lenette *et al.* (1995)	113	–	878	–	30	–	199	–	–

From Evans and Gutensohn (1984), except for the last three references
RR, relative risk; GMT, geometric mean titre
[a] ≥ 1:160, unless otherwise noted
[b] > 1:640
[c] Sera collected before initiation of treatment; all other studies involve treated patients
[d] ≥ 1:640
[e] ≥ 1:320
[f] Children only
[g] Siblings of cases
[h] ≥ 1:1280

enhanced, indirect immunofluorescence assay for detecting antibodies against LMP 2A/2B. None of 113 Hodgkin's disease patients but 64% of patients with nasopharyngeal carcinoma had these antibodies.

Table 20. Results of case–control studies of the association between prevalence of antibodies against the early antigen complex of the EBV and risk for Hodgkin's disease

Reference	Cases				Controls				RR
	No.	Prevalence[a]	High titre[b]	GMT	No.	Prevalence	High titre	GMT	
de Schryver *et al.* (1972)	15	0.27	0.07	–	0	–	–	–	–
Henle & Henle (1973b)	458	0.30[c]	–	30	1718	0.03[c]	–	20	14
Rocchi *et al.* (1975)	100	0.50[c]	0.28	34	100	0.02	0	5	49
Gotlieb-Stematsky *et al.* (1975)	63	0.27	–	–	101	0	–	–	∞
Hilgers & Hilgers (1976)	43	0.91	–	52	43	0.81	–	9	2.4
Hesse *et al.* (1977)	176	0.84	0.26[d]	63	176	0.82	0.27	55	1.2
Evans *et al.* (1978)	42	0.21	–	–	11	0.09	–	–	2.7
Lange *et al.* (1978)[e,f]	17	0.53[c]	–	–	43	0.09	–	–	11
Evans & Gutensohn (1984)[g]	304	0.46	0.30[c]	13	276[h]	0.23	0.11	8	2.8
Lennette *et al.* (1993)[g]	23	0.39	–	20	75	0.04	–	5	15
Merk *et al.* (1995)[e]	61	0.25	0.11[i]	–	109	0.21	0.09	–	1.3

From Evans and Gutensohn (1984), except the last two references
RR, relative risk; GMT, geometric mean titre
[a] ≥ 1:5
[b] ≥ 1:40
[c] ≥ 1:10
[d] ≥ 1:160
[e] Sera collected before initiation of treatment
[f] Children only
[g] EA(D) only
[h] Siblings of cases
[i] ≥ 1:40

Table 21. Results of case–control studies of the association between proportion of high titres against the nuclear antigen complex of the EBV and risk for Hodgkin's disease

Reference	Cases			Controls			RR
	No.	High titre (%)	GMT	No.	High titre (%)	GMT	
Rocchi *et al.* (1975)	100	34[a]	18.9	100	0	6.2	∞
Hilgers & Hilgers (1976)	20[b]	–	186.0	20	–	169.0	–
	23[c]	–	128.0	23	–	198.0	–
Lange *et al.* (1978)[d]	28	–	13.0[e]	43[f]	–	42.8	–
Mochanko *et al.* (1979)	37	49[g]	58.2	40	14	36.2	[1.8]
Shope *et al.* (1982)[d]	15	13[g]	–	24	0	–	∞
Wutzler *et al.* (1983)	57	–	8.0	57	–	7.0	–
Lennette *et al.* (1993)	20	–	197.0	73	–	245.0	–
Merk *et al.* (1995)[h]	61	26[i]	–	109	17	–	[1.7]

Anticomplement immunofluorescence test (Reedman & Klein, 1973) used in all studies; numbers in square brackets were calculated by the Working Group.
[a] ≥ 1:40
[b] Mixed cellularity type
[c] Nodular sclerosis type
[d] Children only
[e] Before therapy
[f] Children and adults
[g] ≥ 1:80
[h] Untreated cases
[i] ≥ 1:1280

(c) *Cohort studies*

(i) *Infectious mononucleosis*

The risk for Hodgkin's disease after a diagnosis of infectious mononucleosis has been evaluated in six cohort studies (Table 22) involving nearly 42 000 young adults with serologically confirmed infectious mononucleosis. The expected number of cases of Hodgkin's disease was based on data for the general population in each study. Overall, there was about a threefold excess of Hodgkin's disease.

Miller and Beebe (1973) identified 2437 men in whom infectious mononucleosis had been diagnosed in 1944 while they were in the service of the United States Army. This cohort was traced for death through 1965. Diagnoses of Hodgkin's disease were based on information on death certificates. The two cases identified both died with Hodgkin's disease 20 years after the diagnosis of infectious mononucleosis.

Table 22. Results of cohort studies of persons with a history of infectious mononucleosis (IM) and subsequent Hodgkin's disease (HD)

Reference	Study population	No. of cases of IM	Follow-up period	No. of cases of HD observed	No. of cases of HD expected[a]	RR
Miller & Beebe (1973)	US Army during Second World War	2 437	1946–65	2	[1]	[2.0]
Connelly & Christine (1974)	Connecticut, USA	4 529	1948–68	5	[1]	[5.0]
Rosdahl *et al.* (1974)	Denmark	17 073	1940–70	17	6	2.8
Carter *et al.* (1977)	University students, USA	2 282	1949–74	3[b]	1.3	[2.3]
Muñoz *et al.* (1978)	Scotland and Sweden	9 454	1959–73	7	1.8	3.9
Kvåle *et al.* (1979)	Norway	5 840	1961–75	3[b]	[1]	[3.0]

RR, relative risk; numbers in square brackets calculated by the Working Group

[a] Expected number based on data for general population, except when taken from original paper

[b] After 12 months

Connelly and Christine (1974) identified 4529 residents of Connecticut (United States) in whom infectious mononucleosis was diagnosed between 1948 and 1964, from the records of the State Department of Health. This cohort was followed through 1968, with matching to the Connecticut Tumor Registry. Five cases (four female, one male) of Hodgkin's disease were diagnosed 3–10 years (median, 8 years) after the diagnosis of infectious mononucleosis.

Rosdahl *et al.* (1974) identified 17 073 Danes in whom infectious mononucleosis had been diagnosed at the Statens Seruminstitut in Copenhagen between 1940 and 1969. The records of the Danish Cancer Registry were screened for cancers diagnosed after an interval of 12 months to mid-1970. Seventeen of these people (one woman and 16 men) subsequently developed Hodgkin's disease between one and seven years (median, three years) after the diagnosis of infectious mononucleosis.

Carter *et al.* (1977) identified 2282 former university students in the United States in whom infectious mononucleosis was diagnosed between 1949 and 1969. Of these, three men had a subsequent diagnosis of Hodgkin's disease three, four and seven years after their infectious mononucleosis.

Muñoz *et al.* (1978) identified a cohort of 9454 persons in whom infectious mononucleosis had been diagnosed from laboratory records in either the city hospital in Aberdeen, Scotland, in 1959–1971 or at four public health laboratories in Sweden in 1952–70. The cohort was matched against local cancer registries for cancer occurring two years later than the diagnosis of mononucleosis. The seven cases (five female, two

male) of Hodgkin's disease were diagnosed 2–12 years (median, 2 years) after infectious mononucleosis.

Kvåle *et al.* (1979) identified 5840 persons with infectious mononucleosis diagnosed in nine laboratories in Norway in 1961–72 and matched them against the records of the Cancer Registry of Norway. Three male cases were diagnosed at least 12 months (one, two and four years) after diagnosis of mononucleosis.

(ii) *Serology*

Two case–control studies nested within cohorts included reports on EBV serology preceding a diagnosis of Hodgkin's disease. The first, by Mueller *et al.* (1989), consolidated the resources of five serum banks containing specimens from over 240 000 persons and identified 43 patients from whom blood had been drawn and stored for an average of 50.5 months before diagnosis of Hodgkin's disease. These were compared with 96 matched controls from whom blood had been drawn at the same time. All of the blood specimens were assayed for IgG, IgA and IgM antibodies against VCA and IgG antibodies against EA(D) and EA(R) and EBNA. As had been reported in the case–control studies, the cases had elevated IgG titres against VCA (RR, 2.6; 90% CI, 1.1–6.1), EA(D) (RR, 2.6; 90% CI, 1.1–6.1) and EA(R) (RR, 1.9; 90% CI, 0.90-4.0), with adjustment for IgM antibodies. In addition, a greater proportion of the cases than controls had elevated titres of IgA against VCA (RR, 3.7; 90% CI, 1.4–9.3; adjusted for IgM), and substantially fewer had IgM antibodies against VCA (RR, 0.22; 90% CI, 0.04–1.3). When all of the antibodies were controlled for simultaneously, the most significant findings were the prevalence of high titres of EBNA (RR, 6.7; 1.8–25) and an inverse association for IgM antibodies (RR, 0.07; 90% CI, 0.01–0.53). This finding of altered EBV antibody patterns before the diagnosis of Hodgkin's disease was generally stronger in blood specimens drawn at least three years before diagnosis than in those tested closer to the time of diagnosis and did not vary appreciably between the sexes, by age or by histological type.

Lehtinen *et al.* (1993) conducted a similar study in a cohort of 39 000 healthy Finnish adults who were followed-up for 12 years. Of these, six were subsequently found to have Hodgkin's disease. Although the data were not shown, the authors reported that the risk for Hodgkin's disease was associated with increased antibody responses to EBNA and to EA, consistent with the results of the previous study.

2.4 Nasopharyngeal carcinoma

2.4.1 *Clinical features and histopathology*

2.4.1.1 *Clinical features*

Nasopharyngeal carcinoma commonly arises from the fossa of Rosenmüller, a region of the nasopharynx rich in lymphoreticular tissue, and eustachian cushions, regardless of race or geographical area. Nasopharyngeal carcinoma may also arise in the roof of the nasopharynx and very rarely in the anterior and inferior walls of the nasopharynx (Simons & Shanmugaratnam, 1982).

Clinically, nasopharyngeal carcinoma may metastasize, particularly to cervical lymph nodes, and as a result is frequently identified only after dissemination. In several series, more than 50% of patients presented with cervical lymph node metastases (Ho, 1971; Sugano *et al.*, 1978; Levine & Connelly, 1985; Pathmanathan, 1997). The jugulodigastric lymph nodes are the most commonly affected cervical nodes (Stanley, 1997) and are found to be enlarged in 50–70% of patients. The presenting symptoms may thus be bilateral cervical node enlargement and also blood-stained nasal discharge, nasal obstruction, blood-stained sputum, tinnitus and hearing loss due to tumour involvement of the eustachian tube. Cranial nerves may be involved singly or in different combinations early or late in the disease; however, cranial nerve palsies, particularly involving cranial nerves III, IV, V and VI, IX and X are often seen. Intracranial extension of the tumour into the base of the skull may give rise to frequent headaches, which are occasionally unilateral, central or retro-orbital in distribution. The neurological signs and symptoms reflect the severity and extent of tumour invasion.

2.4.1.2 *Histopathology*

The microscopic appearance of nasopharyngeal carcinoma or of a metastatic nasopharyngeal carcinoma in lymph node material may be diagnostic and provide confirmation of a clinical diagnosis. When a biopsy sample from an early lesion shows no carcinoma cells, particularly in a patient with suggestive serological results (see below), additional curettings of the nasopharynx should be done and the tissue examined.

The WHO histopathological classification of nasopharyngeal carcinoma (Shanmugaratnam & Sobin, 1991) has been adopted by several investigators. It comprises (1) keratinizing squamous-cell carcinoma, (2) differentiated non-keratinizing carcinoma and (3) undifferentiated carcinoma. Keratinizing squamous-cell carcinomas are further divided into well, moderately or poorly differentiated squamous-cell carcinomas, depending on the presence of intercellular bridges and/or evidence of keratinization (pearl formation). Differentiated non-keratinizing carcinomas have organized cellular patterns but show no evidence of keratinization. The cells have vesicular nuclei with prominent nucleoli, well-defined cell margins, and show a stratified or pavemented pattern. A plexiform pattern may be seen, but the syncytial pattern of undifferentiated carcinomas is not apparent. Undifferentiated carcinomas include lymphoepitheliomas. They are composed of malignant epithelial cells arranged in a syncytial fashion with heavy infiltration of non-malignant lymphocytes. The malignant epithelial cells have vesicular nuclei and prominent nucleoli, and the cell margins are indistinct, consistent with the types described by Schminke (1921) and Regaud (1921). Occasionally, spindle-shaped tumour cells with hyperchromatic nuclei may be present.

2.4.2 *Epidemiology*

Nasopharyngeal carcinoma is a disease with a remarkable racial and geographical distribution. It constitutes 75–95% of all malignant tumours occurring in the nasopharynx in low-risk populations and virtually all of those in high-risk populations (Ho, 1971; Sugano *et al.*, 1978; Levine & Connelly, 1985).

2.4.2.1 *Descriptive epidemiology*

(*a*) *International patterns*

Nasopharyngeal carcinoma is a rare malignancy in most parts of the world, where the age-standardized incidence rate for people of either sex is generally less than 1 per 100 000 persons per year (Parkin *et al.*, 1997). Table 23 lists the handful of populations that are known to deviate from this low-risk pattern, together with the age-standardized (world population) incidence rates for men and women separately. The overall incidence of nasopharyngeal carcinoma is elevated in China, with substantial variation between regions. In general, the incidence increases from the north to the south. Whereas the rates in Chinese men in the northernmost provinces are about 2–3/100 000 person-years, those residing in the southernmost province of Guangdong have rates of 25–40/100 000 person-years (National Cancer Control Office, Nanjing Institute of Geography, 1979; Yu *et al.*, 1981; Parkin *et al.*, 1997). High rates approaching those observed in southern China are seen among Inuits and other natives of the Arctic region (Albeck *et al.*, 1992; Nutting *et al.*, 1993). Intermediate to moderately high rates of nasopharyngeal carcinoma (3–10/100 000 person-years) are seen among many indigenous peoples of Southeast Asia, including Thais, Vietnamese, Malays and Filipinos (Parkin *et al.*, 1997). In Sabah, Malaysia, rates similar to those observed among the Inuits have been reported for the native Kadazans (Rothwell, 1979). Intermediate to moderately high rates of nasopharyngeal carcinoma are also observed among Arabs in Kuwait and Algeria (Parkin *et al.*, 1997). Reviews of hospital series indicate that the rates of nasopharyngeal carcinoma are increased in the mainly Arab populations of Tunisia, Morocco, the Sudan and Saudi Arabia (Muir, 1971; Cammoun *et al.*, 1974; Hidayatalla *et al.*, 1983; Al-Idrissi, 1990). Migrants to Israel from North Africa (Morocco, Algeria and Tunisia) have intermediate rates of nasopharyngeal carcinoma which persist in their offspring born in Israel (Parkin & Iscovich, 1997).

The proportions of the histological types of nasopharyngeal carcinoma appear to vary with geographical location and race. In the low-risk areas of Japan, type-1 nasopharyngeal carcinoma accounts for 12% of cases, whereas type 3 predominates in high-risk areas of Taiwan (Sugano *et al.*, 1978). In sub-Saharan Africa, which is a relatively low-risk area for nasopharyngeal carcinoma, type-3 nasopharyngeal carcinoma is also the predominant histopathological type (Cammoun *et al.*, 1974). Further details on the pathology of nasopharyngeal carcinomas are given in the proceedings edited by Muir and Shanmugaratnam (1967) and de Thé and Ito (1978).

(*b*) *Migration*

Most Chinese living outside China originate from the provinces of Guangdong and Fujian in the south-east (Ho, 1959), a region with a high incidence of nasopharyngeal carcinoma (National Cancer Control Office, Nanjing Institute of Geography, 1979). Southern Chinese migrants, irrespective of the country to which they migrate, continue to have high rates of nasopharyngeal carcinoma (Worth & Valentine, 1967; King & Haenszel, 1973; Armstrong *et al.*, 1979; Gallagher & Elwood, 1979; Lee *et al.*, 1988;

Table 23. Populations at increased risk for nasopharyngeal cancer

Population	Age-standardized (world) incidence[a]		Reference
	Male	Female	
Chinese, Hong Kong	24.3	9.5	Parkin *et al.* (1997)
Chinese, Taipei	8.1	3.2	Chen *et al.* (1988a)
Chinese, Shanghai	4.5	1.8	Parkin *et al.* (1997)
Chinese, Tianjin	1.8	0.6	Parkin *et al*. (1997)
Inuits, Greenland	12.7	9.2	Albeck *et al.* (1992)
Inuits, Athabascans, Aleuts, Alaska	11.9	5.6	Nutting *et al.* (1993)
Thais, Chiang Mai	2.6	1.5	Parkin *et al.* (1997)
Vietnamese, Hanoi	10.3	4.8	Parkin *et al.* (1997)
Malays, Singapore	6.5	2.0	Parkin *et al.* (1997)
Filipinos, Manila	7.6	3.7	Parkin *et al.* (1997)
Kadazans, Sabah	15.9	8.7	Rothwell (1979)
Kuwaitis, Kuwait	2.3	0.6	Parkin *et al.* (1997)
Algerians, Sétif	8.0	2.7	Parkin *et al.* (1997)
Israeli Jews born in Morocco, Algeria or Tunisia	2.8	1.3	Steinitz *et al.* (1989)

[a] Per 100 000 person-years

Parkin *et al.*, 1997), although succeeding generations of such immigrants in countries such as the United States (King & Haenszel, 1973; Buell, 1974; Yu *et al.*, 1981) and Australia (Worth & Valentine, 1967) show continually declining rates. In contrast, Singapore-born Chinese do not have lower rates of nasopharyngeal carcinoma than Chinese born in China (Lee *et al.*, 1988), perhaps because Chinese in Southeast Asia generally adhere to their traditional culture and customs while those in western countries gradually adopt the occidental way of life.

There have been reports that low-risk racial groups born and raised in high-risk areas have an increased risk for nasopharyngeal carcinoma. Buell (1973) reviewed deaths due to nasopharyngeal carcinoma in California, United States, and observed that white men born in the Philippine Islands or China had a higher risk than other white men, and Jews in Israel who were born in North Africa had higher rates of nasopharyngeal carcinoma than other Jews (Steinitz *et al.*, 1989), the increase persisting in their children (Parkin & Iscovich, 1997). Men of French origin who were born in North Africa had a significantly higher rate of nasopharyngeal carcinoma than French men born in France (Jeannel *et al.*, 1993).

(*c*) *Sex and age*

In virtually all of the populations studied, the rates are higher among males than females. In most populations, the male:female ratio is 2–3:1 (Parkin *et al.*, 1997). The

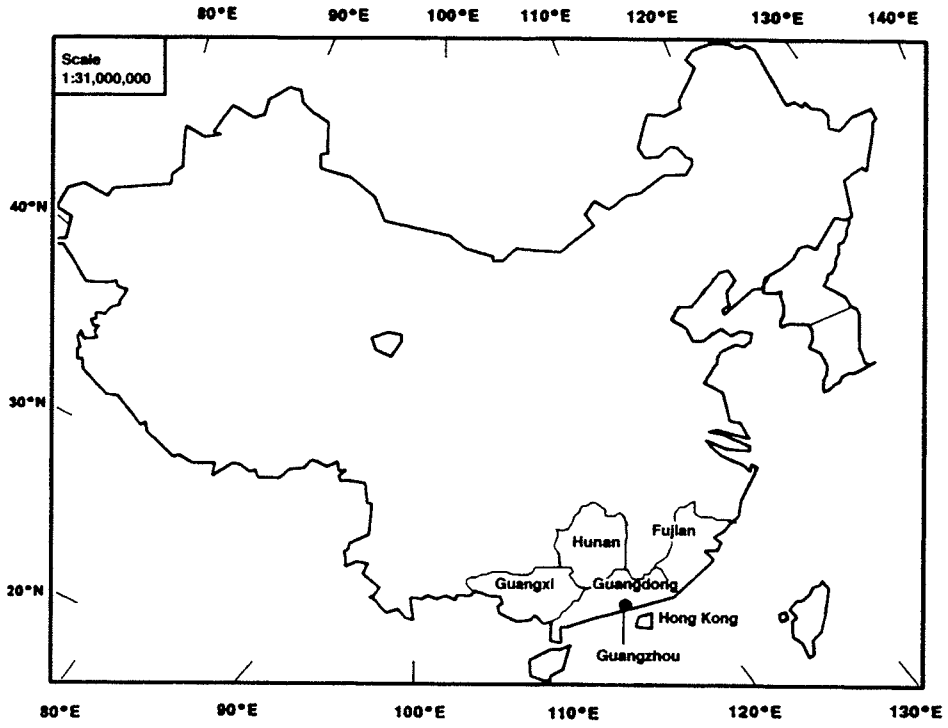
age distribution, however, shows interesting differences by population. In high-risk southern Chinese, the incidence in people of each sex increases steadily with age, reaching a peak at 45–54, and shows a definite decline at older ages (Armstrong *et al.*, 1979; Yu *et al.*, 1981; Parkin *et al.*, 1997). The rates in China as a whole show no such decline after the age of 55 years; and the increase continues to at least 70–74 (National Cancer Control Office, Nanjing Institute of Geography, 1979). The distribution of age-specific rates in the low-risk populations studied, at least after age 20 years, is similar to the distribution of the overall rates in China (Balakrishnan, 1975; Burt *et al.*, 1992; Parkin *et al.*, 1997).

A peak in incidence is observed in adolescents of each sex in a number of populations at low to moderate risk for nasopharyngeal carcinoma. In the United States, a minor peak in the age group 10–19 years is seen in blacks (Burt *et al.*, 1992; Parkin *et al.*, 1997). In Sabah, Malaysia, Kadazan men have a secondary peak between the ages of 15 and 24 years (no data were available for Kadazan women; Rothwell, 1979). In India, the age distribution of 666 consecutive cases of nasopharyngeal carcinoma showed a peak at age 13–22 years, regardless of sex (Balakrishnan, 1975). When Balakrishnan (1975) pooled the incidence rates of nasopharyngeal cancer in 48 population groups published by Doll *et al.* (1970), he found a definite mode in young people of each sex between the ages of 10 and 19 years, which was not seen when similarly pooled data for cancers of the nose and nasal sinuses were plotted against age.

(*d*) *Race and ethnicity*

The high risk for nasopharyngeal carcinoma among Chinese is mainly confined to those residing in the southern provinces of Guangdong, Guangxi, Hunan and Fujian (see Figure 9; National Cancer Control Office, Nanjing Institute of Geography, 1979). The several distinct racial and ethnic groups that reside in this high-risk region have different rates of nasopharyngeal carcinoma. The highest rates are observed among the Tankas, a sub-ethnic group of Cantonese who inhabit the Pearl River Delta basin in central Guangdong. One feature that distinguishes the Tankas from other Cantonese is that they are seafaring people (either fishermen or sea transporters), who live on houseboats moored along the banks of the many branches of the Pearl River. The rates of nasopharyngeal carcinoma among the Tankas are twice those among land-dwelling Cantonese (Ho, 1978; Li *et al.*, 1985). In turn, the land-dwelling Cantonese (who comprise 98–99% of Cantonese) have a twofold higher rate of nasopharyngeal carcinoma than the Hakka and Chiu-Chau dialect groups, who live in north-east Guangdong (Yu *et al.*, 1981; Li *et al.*, 1985). The people of Fujian Province are culturally similar to the Chiu-Chau people in Guangdong Province, and so are their rates of nasopharyngeal carcinoma (National Cancer Control Office, 1980). It is interesting to note that the Hakkas (who rarely intermarry with other dialect groups) originated from northern China more than 500 years ago (Ho, 1959), but their rates resemble those of their Chiu-Chau neighbours and not those of their low-risk ancestors in the north. Even after they migrate to other parts of Southeast Asia, the Cantonese continue to have a twofold higher risk for nasopharyngeal carcinoma than the Hakkas, Chiu-Chaus and Fujianese (Armstrong *et al.*, 1979; Lee *et al.*, 1988).

Figure 9. Map of China, showing provinces where the risk for nasopharyngeal carcinoma is high



From National Cancer Control Office, Nanjing Institute of Geography (1979)

Two distinct racial groups inhabit the Autonomous Region of Guangxi. While the Zhuang people in western Guangxi have a rate of nasopharyngeal carcinoma that is one-fifth of that in Cantonese, the Han (the predominant race in China) people of eastern Guangxi, who are ethnically close to the Cantonese in Guangdong, have similar rates. The areas of Hunan Province that border both Guangxi and Guangdong to the north, not surprisingly have high rates of nasopharyngeal carcinoma. In addition, the Tujia and Miao minorities who inhabit the mountainous region of western Hunan have rates of nasopharyngeal carcinoma that approach those of the Cantonese (National Cancer Control Office, 1980).

In summary, the geographical variation in the incidence of nasopharyngeal carcinoma within southern China closely parallels the distribution of racial and ethnic groups inhabiting the region. The relatively high rates observed among the Hakkas who originated from low-risk northern China argues against genetic predisposition as a major cause of the varying risk patterns among these population groups. As these ethnic groups differ, however, in their customs and food habits, environmental factors inherent in their cultures may be responsible for the differences in susceptibility to nasopharyngeal carcinoma.

In Malaysia and Singapore, large numbers of Chinese and Indians live alongside the native Malays. As mentioned earlier, the Chinese and Malays have high rates; however, Indians in Southeast Asia have very low rates of nasopharyngeal carcinoma, comparable to those seen in whites in the United States (Armstrong *et al.*, 1979; Parkin *et al.*, 1997).

Different rates of nasopharyngeal carcinoma have also been reported among different ethnic groups inhabiting different parts of sub-Saharan Africa (Clifford, 1965; Schmauz & Templeton, 1972; Hidayatalla *et al.*, 1983).

(*e*) *Socioeconomic status*

Among the high-risk southern Chinese, individuals in the lower social strata have higher rates of nasopharyngeal carcinoma than those in the higher social strata (Geser *et al.*, 1978; Armstrong *et al.*, 1978; Yu *et al.*, 1981). Similar information for low-risk populations is scanty. Among white men living in rural or semi-rural counties in the United States, an inverse association is seen between years of schooling and the rate of mortality from nasopharyngeal carcinoma, while no clear trend is apparent among those living in predominantly urban counties (Hoover *et al.*, 1975).

(*f*) *Urbanization*

No difference in the risk for nasopharyngeal carcinoma has been noted between urban and rural residents in south China, or among Chinese in the Malaysian state of Selangor (Armstrong *et al.*, 1979). In metropolitan Hong Kong, local-born Cantonese have rates similar to those of people born and raised in the rural regions of Guangdong Province (Yu *et al.*, 1981). Urban residents in some low-risk populations, however, seem to have higher rates of nasopharyngeal carcinoma than their rural counterparts. In the United States, the mortality rate from this cancer in counties that are 100% urban is about twice that in counties that are 100% rural (Hoover *et al.*, 1975). In a comparison of the rates in urban and rural residents in 11 populations in Australia, Europe and Japan, seven populations had similar rates, while the urban rates were 1.3–2 times higher than those in rural areas in four populations (Muir *et al.*, 1987).

(*g*) *Time trends*

Early records showed that nasopharyngeal carcinoma was common among southern Chinese well over 50 years ago (Ho, 1978). Examinations of cancer registries in Malaysia between 1968 and 1977 and in Singapore between 1968 and 1987 showed little change in incidence in these southern Chinese populations (Armstrong *et al.*, 1979; Lee *et al.*, 1988, 1992). Similar data in Hong Kong, however, indicated a monotonic decline in the rates of nasopharyngeal carcinoma in people of each sex between 1974 and 1992 (the latest year for which complete incidence data were available). The male and female rates in 1974–77 were 32.9 and 14.4, respectively, while the corresponding figures in 1988–92 were 24.3 and 9.5, respectively (see Table 24). This decrease in the incidence of nasopharyngeal carcinoma in Hong Kong Chinese parallels a reduction in the use of Chinese-style salted fish (a human carcinogen, see IARC, 1993, and section 2.4.3.1(*a*)) since the mid- to late 1940s, as Hong Kong developed economically (Geser *et al.*, 1978; Yu *et al.*, 1986).

Data from the Connecticut Tumor Registry (United States) between 1935 and 1974 show relatively stable rates over the 40-year period (Levine *et al.*, 1980b). Similarly, an analysis of data collected from the nationwide Surveillance, Epidemiology and End

Table 24. Time trends in average annual incidence rates of nasopharyngeal carcinoma (per 100 000) in Hong Kong

Period	Average annual incidence	
	Males	Females
1974–77	32.9	14.4
1978–82	30.0	12.9
1983–87	28.5	11.2
1988–92	24.3	9.5

Based on data from *Cancer Incidence in Five Continents* (Waterhouse *et al.*, 1982; Muir *et al.*, 1987; Parkin *et al.*, 1992, 1997)

Results programme in the United States during 1973–86 showed no evidence of a change in incidence rates during the 14-year period (Burt *et al.*, 1992). A review of data on Canadian Inuits over a 25-year period (1949–74) also indicated no appreciable variation in rates over time (Schaefer *et al.*, 1975). In contrast, the rates for Chinese Americans have been declining steadily since 1950, such that the mortality rate from this cancer in men was halved between 1950–54 and 1970–79, from 12 to 6 per 100 000 person-years (Fraumeni & Mason, 1974; Levine *et al.*, 1987). This trend is likely to be the result of increased representation of local-born Chinese in the age groups at high risk for nasopharyngeal carcinoma as this lower-risk population ages, and increased migration from Taiwan and other intermediate- to low-risk regions in China since the 1950s. Prior to that time, virtually all Chinese Americans originated from Guangdong Province (Chinn *et al.*, 1969; Lai, 1988).

(*h*) *Correlation with age-specific prevalence of EBV infection*

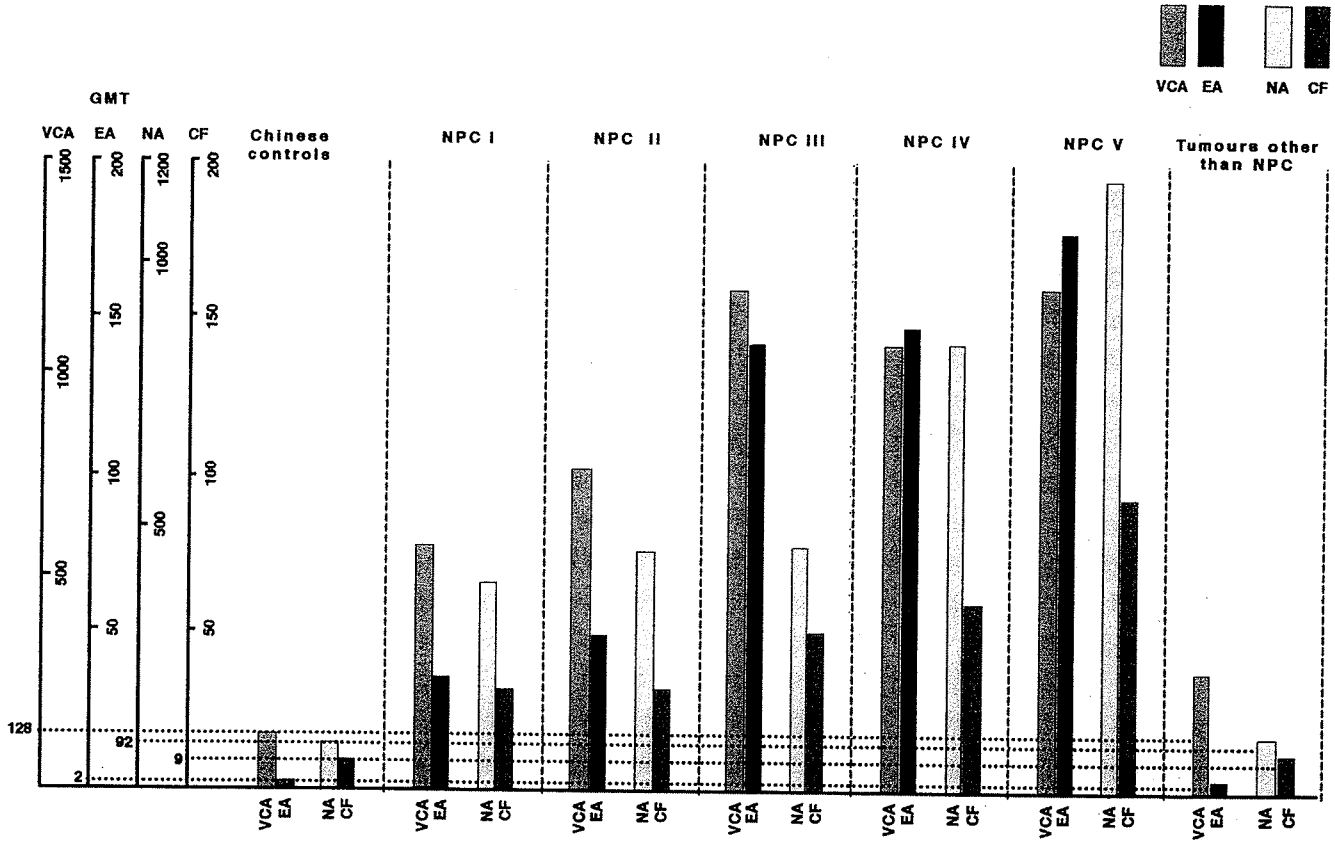
Throughout China, there is little variation in the prevalence of infection or the age at primary infection with EBV (Zeng, 1985), although a more than 20-fold difference in risk exists within the country. Virtually all Chinese children are infected by the age of three to five, and no difference in serological profile has been observed between Chinese populations with drastically different rates of incidence of nasopharyngeal carcinoma (Gu & Zeng, 1978; Zeng, 1985). de Thé *et al.* (1975) compared the age-specific prevalence of antibodies to EBV VCA in three populations at low risk for nasopharyngeal carcinoma (Singapore Indian, Ugandan and French Caucasian) with that in Singapore Chinese, who have the highest incidence of nasopharyngeal carcinoma in the world (Parkin *et al.*, 1997). In comparison with Singapore Chinese, the age at primary infection with EBV is earlier in Ugandans and Singapore Indians and later in French Caucasians; yet the high risk for nasopharyngeal carcinoma is unique to Singapore Chinese.

2.4.2.2 *Case series*

(*a*) *Antibodies in sera and throat washings*

The association between nasopharyngeal carcinoma of the undifferentiated type and EBV was first revealed by Old *et al.* (1966). As seen in Figure 10, titres of IgG antibodies to VCA, EA and soluble complement-fixing antigens increase with clinical stage of disease (Henle *et al.*, 1970; de Thé *et al.*, 1975). IgA antibodies to VCA and EA were later found to be an outstanding feature of nasopharyngeal carcinoma (Wara *et al.*, 1975; Henle & Henle, 1976b; Ho *et al.*, 1976; Desgranges *et al.*, 1977; Pearson *et al.*, 1978b).

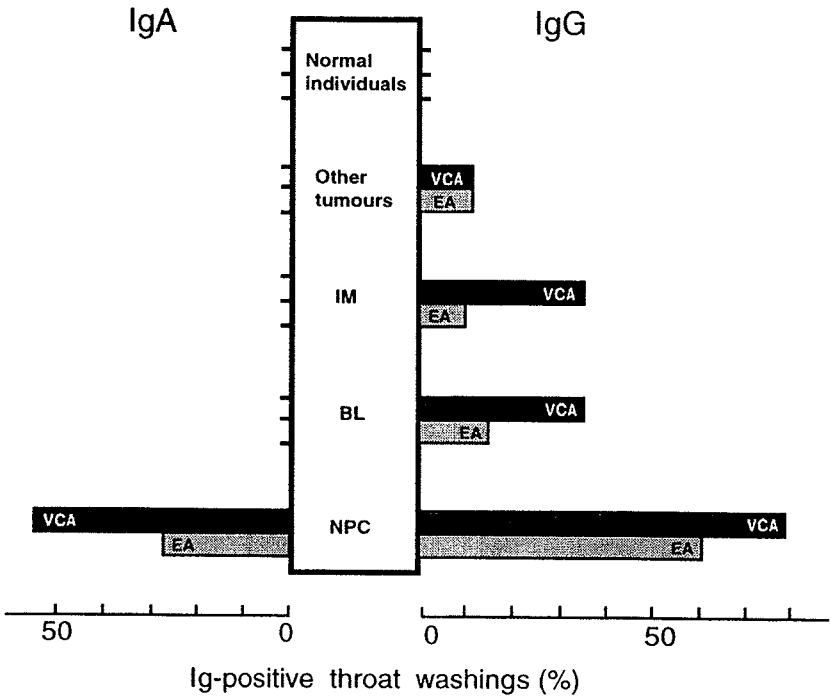
Figure 10. Titres of IgG antibodies to EBV in Chinese nasopharyngeal carcinoma (NPC) patients at different stages of the disease and, for comparison, in normal Chinese controls and Chinese patients with other tumours



From de Thé *et al.* (1975b)
GMT, geometric mean titre; VCA, viral capsid antigen; EA, early antigen; NA, nuclear antigen; CF, complement-fixing antigens

Figure 11 shows the prevalence of IgG and IgA antibodies to VCA and EA in throat washings from patients with nasopharyngeal carcinoma and with other cancers. The titres of both complement fixing antibodies to a soluble EBNA (Sohier & de Thé, 1971) and antibodies to EBNA have been found to be elevated in nasopharyngeal carcinoma patients (de Thé *et al.*, 1973; Baskies *et al.*, 1979).

Figure 11. Titres of IgA and IgG antibodies to EBV in throat washings from patients with nasopharyngeal carcinoma (NPC), Burkitt's lymphoma (BL), infectious mononucleosis (IM) or other tumours and from normal individuals



From Desgranges and de Thé (1978)
VCA, viral capsid antigen; EA, early antigen

Several studies have indicated that the EBV serological profile also differs between patients with different histological types of nasopharyngeal carcinoma. In one of the first studies of these histological subsets, patients with squamous-cell carcinoma were found to have lower EBNA titres but similar titres of IgG to VCA and EA (Shanmugaratnam *et al.*, 1979). Krueger *et al.* (1981) showed elevated titres of IgA to VCA and of IgG to EA and VCA in patients with type-2 or type-3 nasopharyngeal carcinoma, while two patients with type-1 squamous-cell carcinomas had normal titres of IgG to VCA. In a study of Malaysian patients of Chinese, Malay and other ethnic origins, patients with any form of nasopharyngeal carcinoma had elevated IgA titres to VCA (Sam *et al.*, 1989). EBV serology is useful in the identification of patients with occult nasopharyngeal carcinoma (Ho *et al.*, 1976), as shown by elevated titres of anti-IgA VCA; however, histopathological characterization of the tumour is essential for diagnosis, prognosis and treatment.

(*b*) *Nucleic acid markers in carcinoma cells*

Nearly all cases of types-2 and -3 nasopharyngeal carcinoma had detectable EBV DNA sequences, as ascertained by DNA/DNA or c-RNA/DNA hybridization and DNA/-DNA reassociation kinetics in biopsy samples (zur Hausen *et al.*, 1970; Wolf *et al.*,

1973; Nonoyama & Pagano, 1973; Desgranges *et al.*, 1975; Pagano *et al.*, 1975). EBV EBNA was detected in touch smears of nasopharyngeal carcinomas (de Thé *et al.*, 1973; Huang *et al.*, 1974; Klein *et al.*, 1974; Zeng *et al.*, 1981).

Early studies by hybridization kinetics analysis revealed that the viral DNA found in Burkitt's lymphoma is homologous to that in nasopharyngeal carcinomas and that the latter have a relatively high copy number of the EBV genome (zur Hausen *et al.*, 1970; Nonoyama & Pagano, 1973). In another study, viral DNA and EBNA were detected in malignant epithelial cells rather than in the abundant infiltrating lymphoid cells (Wolf *et al.*, 1973). All samples of type-3 nasopharyngeal carcinoma from endemic areas and from areas of intermediate or low incidence contained EBV DNA (Desgranges *et al.*, 1975; Pagano *et al.*, 1975; Raab-Traub *et al.*, 1987). The detection of EBV DNA and EBERs has been useful in identifying carcinomas that have metastasized to lymph nodes when the primary tumour has not been identified (Ohshima *et al.*, 1991; Chao *et al.*, 1996). Type-1 nasopharyngeal carcinoma has been associated with a low copy number of EBV (Raab-Traub *et al.*, 1987): in some studies, EBV was not found in more than half of the cases of type 1 nasopharyngeal carcinoma from nonendemic areas, whereas cases from endemic areas all contained EBV (Pathmanathan *et al.*, 1995a).

Clonal EBV infection was demonstrated in biopsy samples from nasopharyngeal carcinomas in China and the United States (Raab-Traub & Flynn, 1986; Raab-Traub *et al.*, 1987; Pathmanathan *et al.*, 1995b), and also in dysplastic lesions of the nasopharynx in individuals with elevated IgA titres in China, suggesting involvement of EBV prior to the carcinomatous state (Pathmanathan *et al.*, 1995b).

Viral replication is minimal in tumour cells, but the virus can be isolated afer grafting into nude mice (Trumper *et al.*, 1977).

(*c*) *Viral gene expression in tumour specimens*

Transcriptional expression of EBV latent genes in nasopharyngeal carcinoma cells has been studied by northern hybridization (Raab-Traub *et al.*, 1983; Gilligan *et al.*, 1990b, 1991; Brooks *et al.*, 1992; Busson *et al.*, 1992a; Chen *et al.*, 1992c; Karran *et al.*, 1992), by RT-PCR and, in some cases, by in-situ hybridization (Wu *et al.*, 1991; Cochet *et al.*, 1993).

BARFO, *LMP-2*, *EBER* and *EBNA-1* are always expressed in nasopharyngeal carcinoma cells (Fåhraeus *et al.*, 1988; Young *et al.*, 1988; Brooks *et al.*, 1992; Gilligan *et al.*, 1991; Sbih-Lammali *et al.*, 1996), whereas LMP-1 is expressed in a variable proportion of tumour cells (Fåhraeus *et al.*, 1988; Young *et al.*, 1988). The early genes *BALF 5*, *BZLF*, *BMFRI*, *EA(D)* and *BHRF* (*EA(R)*) are occasionally detected in a few cells. Early proteins such as ribonucleotide reductase, BZLF1, BMRF1 (EA(D)) and BHRF-1 (EA(R)) can be detected with monoclonal antibodies (Luka *et al.*, 1988; Lung *et al.*, 1989; Cochet *et al.*, 1993). The presence of EA(D), encoded by *BMRF1*, was not confirmed (Young *et al.*, 1989a), and the site of EA production, which is at the origin of the strong humoral response to that antigen by nasopharyngeal carcinoma patients, remains an open question.

2.4.2.3 *Case–control studies*

(*a*) *Based on pre-diagnostic serological tests*

Lanier *et al.* (1980a) compared the results of serological tests on seven native Alaskan patients with histologically confirmed nasopharyngeal carcinoma and eight controls matched for sex, age, race and residence for whom frozen sera could be found. Several sera were collected for each subject. The oldest samples were collected 22–120 months before diagnosis of nasopharyngeal carcinoma in cases and 3–90 months after the date of diagnosis of the cases in controls. IgA antibodies to VCA were detected in one case 22 months before and three months after diagnosis but in none of the controls. Two patients who showed no IgA antibodies to VCA on two occasions before diagnosis had raised titres at the time of diagnosis and in subsequent tests. All of the cases and controls had detectable titres of anti-EBNA: GMT, [60] in cases, [21] in controls; median titre, [80] in cases, [20] in controls.

(*b*) *Based on serological tests at time of diagnosis*

These studies are summarized in Table 25.

Patients with advanced nasopharyngeal carcinoma, whether Cantonese Chinese in Hong Kong, Maghrebian Tunisians or Caucasians in France, had higher IgG and IgA titres to VCA and EA than patients with other tumours or than normal subjects (de Thé *et al.*, 1978b).

Pearson *et al.* (1983b) compared the IgG and IgA anti-VCA antibody patterns of sera from 124 consecutive cases of nasopharyngeal carcinoma in the United States and in 278 sera obtained from relatives of the cases or of patients with benign diseases or cancer of the head and neck other than nasopharyngeal carcinoma. All cases and 90% of controls had detectable levels of IgG to VCA; IgA antibodies to VCA were detected in 69% of the cases (84% of undifferentiated carcinomas) and 9% of the controls, and IgG antibodies to EA were found in 76% of cases (86% of undifferentiated carcinomas) and 29% of controls.

Two case–control studies in Chinese populations examined the association between the prevalence of anti-VCA IgA antibodies in nasopharyngeal carcinomas and in population controls. Zheng *et al.* (1994a) reported an odds ratio associated with infection of 55 (95% CI, 11–280) adjusted for sex and age and foods associated with nasopharyngeal carcinoma. Chen *et al.* (1987) also found a significant increase in the odds ratio with increasing anti-VCA IgA titres. The odds ratio for positive versus negative subjects, adjusted for sex, age, marital status and consumption of foods associated with nasopharyngeal carcinoma, was 39 (statistically significant).

2.4.2.4 *Cohort studies*

Chan *et al.* (1991) identified seven cases of undifferentiated or poorly differentiated nasopharyngeal carcinoma in four Asians and three Caucasians (six male and one

Table 25. Case–control studies of nasopharyngeal carcinoma and EBV serology at time of diagnosis

Reference and region	Ethnicity	No of cases/No. of controls	Morphology	EBV marker	Measure	Results	Comments
de Thé *et al.* (1978b) Hong Kong, France, Tunisia	Chinese, Causasian, North African	132/150 selected cases of advanced nasopharyngeal carcinoma; controls in same age range	[92%] undifferentiated or poorly differentiated	IgG/VCA IgG/EA	Geometric mean titre	Significantly higher in cases	
Pearson *et al.* (1983b) Northern USA	Mainly Caucasian	124 /278 Controls: age-matched relatives, head-and-neck tumours other than nasopharyngeal carcinoma, benign disorders of head and neck	Undifferentiated, 85 Non-keratinizing, 11 Squamous, 26	IgG/VCA IgA/VCA IgG/EA	Positive versus negative Positive versus negative Positive versus negative Positive for both IgG EA and IgA VCA	Positive: 100% cases, 90% controls 69% cases (84% undifferentiated), 9% controls 76% cases (86% undifferentiated), 29% controls 62% of undifferentiated and non-keratinizing, 7% of squamous, 1% of normal	Crude odds ratio [23], 95% CI [13–40] [32] [18–57]
Chen *et al.* (1988b) Taiwan	Chinese	205/205 neighbourhood controls matched on sex and age	Histologically verified	IgA/VCA Anti-EBV DNase	Positive versus negative < 2 2 ≥ 4	Odds ratio, 39, $p < 0.01$ 1 12 62, $p < 0.01$	Adjusted for matching variables and dietary factors
Zheng *et al.* (1994a) China	Chinese	203/163 (blood available), neighbouhood controls matched on sex and age	Histologically confirmed but type not reported	IgA/VCA	Positive versus negative	Odds ratio, 55 (95% CI, 11–280)	Adjusted for matching variables and some dietary factors

Numbers in square brackets were calculated by the Working Group.
VCA, viral capsid antigen; EA, early antigen; CI, confidence interval

female) that developed in a cohort of over 240 000 individuals in Norway and the United States and for whom sera had been stored in four banks. Two controls per case were selected from the cohort and matched on bank, storage duration, age, sex and race. The intervals between serum collection and diagnosis of nasopharyngeal carcinoma were 26, 76, 122, 124, 134, 153 and 154 months. The GMTs of IgA antibodies to VCA, anti-EA(D), anti-EA(R) and anti-EBNA were similar in cases and controls. The odds ratio associated with high titres of IgA to VCA was 1.0 (95% CI, 0.1–11) and that for high anti-EBNA titres was 0.7 (95% CI, 0.1–5.3).

2.4.2.5 *Mass serological surveys*

These studies are summarized in Table 26.

Two screening surveys for EBV positivity in sera were conducted in Guangxi Autonomous Region, China. In the first, 148 029 subjects living in rural areas of Zangwu County were screened for IgA antibodies to VCA. Among the first 56 584 examined, 117 (0.2%) had positive results. These people then underwent thorough clinical investigation, which led to the diagnosis of 18 cases of nasopharyngeal carcinoma; two additional cases were diagnosed in the 10 months after the intervention (Zeng *et al.*, 1980). The final number of antibody-positive subjects in the cohort was 3533 (prevalence, 2.4%), and 55 nasopharyngeal carcinomas were found in this group (Zeng *et al.*, 1983a). Thus, the prevalence of IgA antibodies to VCA in the 91 445 subjects recruited after the interim report (Zeng *et al.*, 1980) was 3.7%. In the second survey, individuals aged 40 years or more and resident in Wuzhou City in Zangwu County were examined. Among 20 726 people screened, 5.5% had IgA antibodies to VCA; 18 cases of nasopharyngeal carcinoma (1.6%) were detected among the latter (Zeng *et al.*, 1985; Zeng, 1987). Similar prevalences of antibody-positive subjects and nasopharyngeal carcinoma cases had been reported in a preliminary analysis of the first 12 932 individuals screened (Zeng *et al.*, 1982). Thirty-five additional cases of nasopharyngeal carcinoma were identified retrospectively in the cohort of antibody-positive individuals during the 10 years after the survey; however, the number of person-years of observation was not available. Four nasopharyngeal carcinomas were seen in subjects who had no IgA antibodies to VCA (Zeng *et al.*, 1993).

In a third survey, conducted in Taiwan (Chen *et al.*, 1989), anti-EBV DNase activity was found in 1250 of 22 596 (5.5%) Government employees and 1176 of 9869 (11.9%) residents of a high-risk area. When those with antibodies were referred for clinical investigation, three and 11 nasopharyngeal carcinoma cases were detected among those who complied with referral in the two groups, corresponding to prevalences of 0.6 and 1.3%, respectively. EBV-negative individuals were not screened for nasopharyngeal carcinoma, and no long-term follow up of the cohort has been performed.

Table 26. Detection of EBV in mass serological surveys and association with nasopharyngeal carcinoma

Reference and region	No. screened	EBV detection	Presence of antibodies		Cases of nasopharyngeal carcinoma in EBV-positive cases		Incidence in EBV-positive cases
			No.	%	No.	%	
Zeng *et al.* (1979, 1980, 1983a); rural Zangwu County, China	Interim analysis: 56 584 Final cohort: 148 029	IgA VCA	117 3 533	0.2 2.4	18 55	15.4 1.6	Two additional cases in 10-month follow-up
Zeng *et al.* (1982, 1985); Zeng (1987); Wuzhou City, Zangwu County	Interim analysis: 12 932 Final cohort: 20 726	IgA VCA	680 1 136	5.3 5.5	13 18	5.3 1.6	35 additional cases in 10-year follow-up (Zeng *et al.*, 1993)
Chen *et al.* (1989); Taiwan		Anti-EBV DNAse					
Government employees	22 596		1 250	5.5	3/477[a]	0.6	477 who complied with referral
High-risk area	9 869		1 176	11.9	11/829[a]	1.3	829 who complied with referral

[a] Number of people attending a specific clinic at the National Taiwan University Hospital

2.4.3 *Cofactors*

2.4.3.1 *Dietary factors*

(*a*) *Cantonese-style salted fish*

The higher rates of nasopharyngeal carcinoma among the boat-dwelling Tankas than among the land-dwelling Cantonese in Hong Kong was first noted by Ho (1967), who also observed that salted fish is the principal source of supplementary food in the diet of these people, which consists mainly of rice. He subsequently suggested that Cantonese-style salted fish be investigated as a 'possible etiological factor' for nasopharyngeal carcinoma (Ho, 1971). He made two additional observations: the salting process is inefficient and the product, aged for several days to weeks, becomes partially putrefied, liberating a pungent odour that is offensive to those who were not raised in the southern Chinese culture (Ho, 1972). Later, he recognized that salted fish mixed with soft rice is a popular weaning food in southern China (Ho, 1976).

Eleven case–control studies have been conducted in various Chinese populations with distinct risks for nasopharyngeal carcinoma to investigate the possible association with the consumption of salted fish. Five were conducted in high-risk Cantonese populations (Geser *et al.*, 1978; Henderson & Louie, 1978; Yu *et al.*, 1986, 1989a; Zheng *et al.*, 1994a), three were carried out in populations with about half the incidence rate of Cantonese (Chen *et al.*, 1988b; Yu *et al.*, 1988; Zheng *et al.*, 1994b), one study was conducted in the relatively low-risk population of Tianjin in northern China (Ning *et al.*, 1990), and two studies were conducted in various Chinese populations in Southeast Asia (Armstrong *et al.*, 1983; Lee *et al.*, 1994b). One epidemiological study addressed the relationship between consumption of Cantonese-style salted fish and nasopharyngeal carcinoma risk in a non-Chinese population (Sriamporn *et al.*, 1992).

Henderson and Louie (1978) in the United States interviewed 74 Chinese patients with nasopharyngeal carcinoma and 110 Chinese control subjects. The patients represented incident cases identified from the population-based cancer registries of Los Angeles County and the San Francisco Bay area. The controls were clinic or hospital patients who were frequency matched to the cases on race, age, sex and socioeconomic status; the subjects were all asked about how often they ate salted fish. A statistically significant association with risk for nasopharyngeal carcinoma was seen (p for linear trend = 0.02), and the odds ratio for consumption two or more times per week relative to no consumption was 3.1.

Geser *et al.* (1978) conducted a hospital-based case–control study in Hong Kong involving 150 patients with nasopharyngeal carcinoma and 150 age- and sex-matched hospital controls. Salted fish intake was not determined for the study subjects, but older women in the households of the patients and controls were interviewed, when possible, about weaning practices. This information was collected from 108 mothers of nasopharyngeal carcinoma patients and 103 mothers of controls. The only food given to babies during and after weaning for which a statistically different prevalence was found between case and control households was salted fish (odds ratio, 2.6; $p < 0.01$).

In Hong Kong, Yu *et al.* (1986) interviewed 250 patients with nasopharyngeal carcinoma under the age of 35 and an equal number of age- and sex-matched friends about their dietary habits three years before diagnosis of the index case and at 10 years of age. Furthermore, 182 mothers of cases and 155 mothers of control subjects were interviewed about the dietary habits of the subjects during weaning, between the ages of one and two years and at the age of 10 years. All of the cases were histologically confirmed at the four hospitals in Hong Kong in which over 90% of new nasopharyngeal carcinoma cases are diagnosed. Salted fish consumption at all periods was significantly related to the risk for this tumour, and increasing frequency of intake was consistently associated with increasing risk. The association with salted fish was stronger when exposure occurred during childhood as compared with adulthood (i.e. three years before interview). Exposure during weaning was associated with an odds ratio of 7.5 (95% CI, 3.9–15); the odds ratio for weekly or more versus less than monthly consumption at age 10 was 38 (95% CI, 14–100). No other dietary or environmental factor studied was significantly associated with nasopharyngeal carcinoma after adjustment for salted fish intake, and the odds ratios for salted fish consumption remained highly significant after all other potential risk factors had been taken into account. It was estimated that over 90% of the cases of nasopharyngeal carcinoma in young people in Hong Kong could be attributed to childhood consumption of Cantonese-style salted fish.

Yu *et al.* (1989a) interviewed 306 patients with histologically confirmed nasopharyngeal carcinoma who lived in Guangzhou, China, and were under the age of 50 years and an equal number of controls matched for age, sex and neighbourhood. Subjects were asked about their dietary pattern three years before diagnosis of the index case and at the age of 10. The mothers of 110 cases under the age of 45 years and of 139 of their matched controls were interviewed about their own consumption of salted fish during the index pregnancy and during lactation and about the subjects' intake of salted fish during weaning, between the ages of one and two years, and at the age of 10. Exposure to salted fish, whether in adulthood or in childhood, was significantly associated with an increased risk for nasopharyngeal carcinoma. The association was strongest for exposure during weaning (odds ratio, 2.1; 95% CI, 1.2–3.6); exposures at all other times were no longer significantly related to the risk for nasopharyngeal carcinoma after adjustment for exposure during weaning. Childhood exposure to salted fish remained a highly significant risk factor after adjustment for other dietary and non-dietary risk factors identified in this study.

Zheng *et al.* (1994a) conducted a hospital-based case–control study in Guangzhou, China, comprising 205 histologically confirmed cases of nasopharyngeal carcinoma under the age of 55 years and an equal number of friends who were individually matched to the cases by age and sex. Subjects were asked about their dietary habits during the past seven years and at the age of 10. Furthermore, the mothers of 151 cases and 195 controls were interviewed about the subjects' dietary habits during the first three years of life and at the age of 10. Salted fish consumption during all of the periods studied was significantly associated with risk for nasopharyngeal carcinoma. The association was strongest for consumption during the first three years of life ($p < 0.001$): the odds ratio for weekly or daily intake relative to less than monthly was 13 (95% CI, 5.2–21). The results

were unchanged after adjustment for the presence of IgA antibodies against EBV VCA. It was estimated that salted fish intake could explain 73% of the cases of nasopharyngeal carcinoma occurring in this Cantonese population.

In a case–control study in Taipei, Taiwan, involving 205 histologically confirmed cases from a single hospital and 205 neighbourhood controls individually matched to the cases by age and sex, Chen *et al.* (1988b) collected information on the frequency of intake of a number of preserved foods before the age of 20, by interview. Individuals who ate salted fish 10 or more times per month had an odds ratio of 1.5 relative to less than monthly intake, which was not statistically significant. Childhood exposure to salted fish was not examined in this study.

Yu *et al.* (1988) interviewed 128 mothers of 231 eligible patients with nasopharyngeal carcinoma under the age of 45 in Yulin Prefecture, Guangxi Autonomous Region, China, in order to examine the relationship between exposures during childhood and subsequent development of nasopharyngeal carcinoma. They also interviewed 174 mothers of 231 individually matched population controls. The mothers were asked about their consumption of salted fish during the index pregnancy and lactation and about the subjects' intake of salted fish during weaning, between the ages of one and two and at the age of 10. Salted fish was not a common weaning food in this population: only 6.3% of controls were exposed. Nonetheless, exposure to salted fish during this early period of life was associated with a 2.6-fold increase in the risk for nasopharyngeal carcinoma (95% CI, 1.2–5.6). The mother's consumption of salted fish during the pregnancy and lactation was also significantly related to risk (p for linear trend = 0.003 and 0.01, respectively). Exposure at the age of 10 was less strongly related, the odds ratios for monthly and weekly intake as compared to less than monthly consumption being 1.5 in both instances and not statistically significant.

Zheng *et al.* (1994b) conducted a case–control study in Zangwu County in eastern Guangxi, China, which involved 88 incident cases of nasopharyngeal carcinoma and twice as many population controls individually matched to the cases by age, sex and neighbourhood of residence. Subjects were asked about their dietary habits during weaning, before the age of two, between the ages of two and 10 and one year before diagnosis of nasopharyngeal carcinoma. Salted fish intake at any of the three times during childhood was significantly related to the risk for nasopharyngeal carcinoma (during weaning, odds ratio, 2.4; $p = 0.01$). Consumption one year before diagnosis of the tumour was relatively rare (2.3% in cases and 0.6% in controls) and was not significantly related to risk.

Ning *et al.* (1990) studied 100 histologically confirmed cases of nasopharyngeal carcinoma in the Han population in people under the age of 65 identified from a population-based cancer registry covering the Tianjin Metropolitan Area in northern China, and three times as many population controls individually matched to the cases by age, sex and neighbourhood of residence. Questions were asked about the frequency of intake of selected foods, including salted fish, at or before the ages of 10, 20, 30, 40 and 50 years. In this relatively low-risk population, in which salted fish is not a common dietary item, any exposure to salted fish was a significant risk factor for nasopharyngeal

carcinoma (odds ratio, 2.2; 95% CI, 1.3–3.7). Four characteristics of exposure to salted fish contributed independently to the increased risk: (1) low age at first exposure; (2) long duration of consumption; (3) frequent consumption at age 10; and (4) cooking by steaming as opposed to frying, grilling or boiling. The last factor could be determined in this population because, unlike the southern Chinse who almost always steam salted fish, consumers in Tianjin use a variety of methods. It was estimated that 40% of nasopharyngeal carcinoma cases occurring in this low-risk region could be attributed to the consumption of salted fish.

Armstrong *et al.* (1983) conducted a case–control study among Malaysian Chinese resident in 27 census districts surrounding (and including) metropolitan Kuala Lumpur, in central Selangor. The 100 cases were all histologically confirmed and identified at the only radiotherapy centre in Malaysia. The 100 control subjects were selected from the neighbourhoods in which the cases lived and were individually matched to the index case by sex and age. Subjects were asked about their intake of salted fish during childhood, during adolescence and at the time of interview. Since there was evidence that the diet of many of the patients had changed since manifestation of clinical cancer, the investigators discarded the information on intake at the time of interview which, for a number of the cases, was several years after the diagnosis. Salted fish intake during childhood and adolescence were both significantly related to the risk for nasopharyngeal carcinoma, with a stronger association for exposure during childhood. The odds ratio was 3.0 ($p = 0.04$) for any consumption during childhood and 17 (95% CI, 2.7–110) for daily consumption.

In Singapore, Lee *et al.* (1994b) compared 200 Chinese patients in whom nasopharyngeal carcinoma had been diagnosed consecutively at a major general hospital; the 406 controls were patients at the same hospital who were frequency matched to the cases by ethnicity, age and sex. Information on dietary habits one year previously was collected from all cases and controls, and information on diet during infancy and at the age of 10 was collected from the mothers of cases or controls, if they were available for interview, or from the subjects themselves. A total of 64 mothers of cases and 103 mothers of controls were interviewed, for a participation rate of 28%. The results for diet during infancy were considered by the authors to be inconclusive, and the Working Group considered that the information on diet at the age of 10 is also uninterpretable because information was obtained from both subjects and mothers and is likely to be of variable reliability. No significant relationship was found between salted fish intake one year before diagnosis and the risk for nasopharyngeal carcinoma (p for linear trend = 0.6), although the highest frequency category was associated with an excess fourfold risk (odds ratio, 4.4; 95% CI, 0.7–26).

Two types of salted fish are eaten by the population in northeast Thailand: home-made salted freshwater fish and Cantonese-style salted marine fish available on markets. Sriamporn *et al.* (1992) examined current consumption of both kinds of salted fish in a hospital-based case–control study involving 120 histologically confirmed cases of nasopharyngeal carcinoma and an equal number of hospital controls individually matched to the cases by age and sex. Intake of Thai-style salted fish was not related to the risk for nasopharyngeal carcinoma, but consumption of Chinese-style salted fish showed a clear,

statistically significant dose–response relationship with risk, weekly consumers having an odds ratio of 2.5 (95% CI, 1.2–5.2) relative to non-consumers.

In summary, the epidemiological data strongly support the hypothesis that Cantonese-style salted fish is a nasopharyngeal carcinogen in humans. The studies suggest that age at exposure is an important co-determinant of risk, earlier age at exposure being associated with a higher risk for disease. Experimental data to support the carcinogenicity of Cantonese-style salted fish is summarized in section 4.5.2.

(*b*) *Other types of salted fish*

There is preliminary evidence that early exposure to other types of salted fish may be responsible for at least some of the increase in risk for nasopharyngeal carcinoma in the native peoples of Southeast Asia and of the Arctic region. Armstrong and Armstrong (1983) studied 13 Malay cases of nasopharyngeal carcinoma and 50 Malay population controls of comparable age and sex distribution in a small case–control study conducted in Selangor, Malaysia. Five cases and four controls had eaten salted fish daily during childhood. The excess was not statistically significant.

In the Philippines, West *et al.* (1993) studied 104 predominantly Filipino cases of nasopharyngeal carcinoma from a single hospital, 104 hospital controls from the same hospital and 101 community controls, who were matched to the cases by age and sex; the hospital controls were also matched to the cases on type of hospital ward (private versus public), and the community controls were further matched to the cases on neighbourhood of residence. The two sets of controls were combined in the analysis. Individuals with the highest frequency of current intake of salted fish had a nonsignificantly increased risk for nasopharyngeal carcinoma relative to those with the lowest frequency (crude rate, 60%; adjusted rate, 30%). Childhood consumption of salted fish was not investigated in this study.

Lanier *et al.* (1980b) interviewed 13 nasopharyngeal carcinoma patients and 13 controls in Alaska who were individually matched to the cases by age, sex, race (Inuit, Indian, Aleut) and village of residence. The same questionnaire was administered to 17 patients with other head-and-neck tumours and their similarly matched control subjects. More nasopharyngeal carcinoma patients than controls had eaten salted fish as children, while no such association was observed for the patients with other head-and-neck cancers.

(*c*) *Other preserved foods*

Early exposure to other preserved foods has also been shown to be related to the risk for nasopharyngeal carcinoma in Chinese populations and was addressed in some of the studies described in section 2.4.3.1(a). In Yulin, China, where salted fish is a relatively rare food item, a variety of preserved foods other than salted fish eaten during childhood have significant, independent effects on the risk (Yu *et al.*, 1988). These include salted ducks' eggs (odds ratio, 5.0; $p = 0.03$), salted mustard greens (odds ratio, 5.4; $p = 0.03$) and *chung choi* (a salted root; odds ratio, 2.0; $p = 0.003$) eaten during weaning, and dried fish (p for linear trend = 0.002), fermented black-bean paste (p for linear trend = 0.009) and fermented soya-bean paste (p for linear trend = 0.007) eaten between the ages of one

and two. In the Cantonese population of Guangzhou, China, childhood exposure to mouldy bean curd (p for linear trend = 0.07), salted shrimp paste (p for linear trend = 0.04), *chan pai mui* (p for linear trend = 0.01) and *gar ink gee* (p for linear trend = 0.03) (the last two are preserved plums) was independently related to the risk for nasopharyngeal carcinoma after adjustment for intake of salted fish (Yu *et al.*, 1989a). In Taiwan, exposure before the age of 20 to fermented bean products (odds ratio for > 10 times/month versus less than once a month, 1.8; $p < 0.05$) and smoked meat (odds ratio for > 1/month versus less frequently, 3.3; adjusted for all other risk factors; $p < 0.05$) were independently associated with the risk for nasopharyngeal carcinoma after adjustment for IgA antibody titres against EBV VCA (Chen *et al.*, 1988b). In Tianjin, China, exposure to salted shrimp paste at the age of 10 years was related to risk independently of salted fish intake (odds ratio for weekly/daily versus monthly frequency, 3.2; $p = 0.007$) (Ning *et al.*, 1990). In Singapore, Lee *et al.* (1994b) noted that consumption of five preserved foods one year before cancer diagnosis was significantly associated with an increased risk. These were *belachan* (salted shrimp paste; p for linear trend = 0.04), salted soya beans (fermented soya-bean paste; p for linear trend = 0.002), tinned pickled vegetables (p for linear trend = 0.004), *szechuan chye* (a salted tuber; p for linear trend = 0.008) and salted mustard greens (*kiam chye*; p for linear trend = 0.007). The effects of salted soya beans, *szechuan chye* and *kiam chye* remained statistically significant in a multivariate model that included other risk factors for nasopharyngeal carcinoma. While the levels of childhood exposure to most of these foods were low in the series studied, *chung choi* was given at weaning in 60% of cases in Yulin (Yu *et al.*, 1988), salted shrimp paste was consumed at the age of 10 by 28% of cases in Tianjin (Ning *et al.*, 1990), and 63% of the cases in Taiwan consumed fermented bean products before the age of 20 (Chen *et al.*, 1988b).

Jeannel *et al.* (1990) conducted a case–control study among Tunisians, who are at intermediate risk for nasopharyngeal carcinoma. Eighty histologically confirmed incident cases identified at the only cancer hospital in Tunisia and 160 population controls individually matched to the cases by age, sex and neighbourhood of residence were interviewed about dietary habits in the year preceding the cancer diagnosis and during childhood. The intake of several preserved food products during childhood and/or adulthood was found to be significantly associated with the risk for nasopharyngeal carcinoma after adjustment for socioeconomic status. They were *touklia* (a stewing mixture of red and black peppers, paprika, caraway seed and/or coriander seed, salt and olive or soya-bean oil), *quaddid* (dried mutton preserved in olive oil), pickled vegetables, pickled olives and *harissa* (a mixture of red pepper, garlic, caraway seed, salt and olive oil). After adjustment for each other and for other potential confounders, only childhood exposure to *touklia*, *quaddid* and *harissa* were significant risk factors for nasopharyngeal carcinoma in Tunisia. The odds ratio for childhood consumption of *touklia* was 8.6 (95% CI, 1.7–44), that for consumption of *quaddid* more than once a month was 1.9 (95% CI, 1.0–3.7) and that for consumption of *harissa* more than once a month was 4.2 (95% CI, 1.1–17).

Studies of the presence of carcinogens, genotoxins, and EBV-activating substances in samples of foods associated with nasopharyngeal carcinoma are described in section 4.5.2.

(*d*) *Deficits of fresh vegetables and fruit*

In an uncontrolled study of 24 patients under the age of 25 with nasopharyngeal carcinoma in Hong Kong, Anderson *et al.* (1978) noted that 'all families felt that vegetables and fruits were bad for babies, and the children had been fed accordingly.' Similar findings were reported in several case–control studies conducted among Chinese. In both Hong Kong (Yu *et al.*, 1986; Ning *et al.*, 1990) and Guangzhou (Yu *et al.*, 1989a), patients with nasopharyngeal carcinoma had a lower intake of fresh vegetables and fruits during weaning, between the ages of one and two and at the age of 10 than controls, and many of the differences were statistically significant. Whereas in Hong Kong the protective effects of fresh vegetables and fruit were no longer significant after adjustment for salted fish intake, in Guangzhou the effects were not due to different consumption of salted fish and other preserved foods by cases and controls (Yu *et al.*, 1989a). In Tianjin (Ning *et al.*, 1990), fewer patients had eaten carrots at the age of 10 than controls. Increased frequency of consumption of garlic, the only other specific vegetable on the questionnaire, also resulted in decreased risks for nasopharyngeal carcinoma, although the effect was not statistically significant. In contrast, Yu *et al.* (1988) did not find a negative association between childhood exposure to fresh vegetables and fruit and the risk for nasopharyngeal carcinoma in Yulin, and Jeannel *et al.* (1990) reported no association between fruit or vegetable intake and nasopharyngeal carcinoma risk in Tunisia.

Clifford (1972) measured serum carotene levels in 17 male African patients with nasopharyngeal carcinoma and 53 male controls and reported a significantly lower level in the cases. In Singapore, Lee *et al.* (1994b) found that the dietary levels of vitamin C, vitamin E and β-carotene one year before diagnosis of nasopharyngeal carcinoma were lower among cases than controls and significantly so for vitamins C and E.

2.4.3.2 *Other environmental factors*

(*a*) *Fumes, smoke and dust*

Dobson (1924) proposed that exposure to smoke from wood fires inside chimneyless houses was the cause of nasopharyngeal carcinoma in southern Chinese, and Clifford (1972) also noted the presence of wood fires in chimneyless houses among tribal groups in Kenya with moderately elevated rates of nasopharyngeal carcinoma.

The relationship between domestic exposure to smoke and risk for nasopharyngeal carcinoma among southern Chinese has been investigated in several case–control studies. Zheng *et al.* (1994b) reported an odds ratio of 5.4 [95% CI, 1.5–20] for use of wood fuel during the year before cancer diagnosis in a case–control study in eastern Guangxi involving 88 cases of nasopharyngeal carcinoma and 176 controls matched for age, sex and neighbourhood. In contrast, Yu *et al.* (1988) observed no association between use of wood fuel and risk in a county adjacent to that studied by Zheng *et al.* (1994b). When lifetime histories of cooking fuels used were collected from each study subject in case–control studies in Hong Kong (Yu *et al.*, 1986) and Guangzhou (Yu *et al.*, 1990), China, no significant association was found with smoke from burning wood.

The role of occupational exposure to smoke, dust and fumes has been examined. A case–control study in Los Angeles and San Francisco (United States) involved 156 histologically confirmed cases of nasopharyngeal carcinoma and 267 hospital or clinic controls matched to the cases by race (whites, blacks, Hispanics, Chinese and other Asians), sex and age (Henderson *et al.*, 1976). Cases were identified through the population-based cancer registries in both metropolitan areas. All interviews were administered in person, and a lifetime occupational history was obtained, including information on usual exposure to fumes, dust, smoke, chemicals or heat in each job. Occupational exposures to fumes (odds ratio, 2.0; $p = 0.006$), dust (odds ratio, 1.5; $p = 0.07$), smoke (odds ratio, 3.0; $p = 0.008$), chemicals (odds ratio, 2.4; $p = 0.006$) and heat (odds ratio, 1.6; $p = 0.05$) were all positively related to the risk for nasopharyngeal carcinoma, but only exposure to smoke showed a clear duration–response relationship (odds ratio for 10 years or less, 1.5; odds ratio for more than 10 years, 7.5; $p < 0.05$).

In the study of Armstrong *et al.* (1983), described on p. 182, a complete occupational history was obtained from each subject by personal interview. Subjects were specifically asked if they had had regular exposure to a specific dust, smoke or chemical fume. Occupational exposure to smoke (odds ratio, 6.0; $p = 0.006$) and dust (odds ratio, 4.0; $p < 0.001$), irrespective of type, was significantly associated with the risk for nasopharyngeal carcinoma, and a clear duration–response relationship was seen for men and women which was independent of salted fish intake.

In the study of Chen *et al.* (1988b), described on p. 181, subjects were asked whether they had been exposed occupationally to dust or smoke. Exposure to smoke (odds ratio, 1.7; $p < 0.05$) was significantly associated with risk for nasopharyngeal carcinoma in univariate but not in a multivariate analysis that included other risk factors. Exposure to dust was not related to increased risk (odds ratio, 1.1).

In Guangzhou, China, Yu *et al.* (1990) interviewed 306 patients under the age of 50 with histologically confirmed nasopharyngeal carcinoma and an equal number of controls matched for age, sex and neighbourhood to obtain a complete occupational history from each respondent. For each job held for six months or longer, the subjects were asked to indicate whether they had been exposed to dust, smoke or chemical fumes and to name the substances involved. To avoid recall bias, a specialist who was unaware of the case or control status of the subjects assessed these exposures on the basis of job title, activity and industry. Occupational exposure to dust was not related to increased risk, on the basis of the subjects' responses (odds ratio, 1.2) or the expert judgement (odds ratio, 0.9); however, exposure to smoke was significantly associated with the risk for nasopharyngeal carcinoma (subjects: odds ratio, 2.1; 95% CI, 1.3–3.5; expert: odds ratio, 1.6; 95% CI, 1.1–2.5). A clear duration–response relationship was seen in both assessments. Exposure to chemical fumes was significantly related to risk, depending on the duration of exposure (odds ratio, 1.7; 95% CI, 1.1–2.4) when based on the subjects' recall but not when based on the expert judgement (odds ratio, 1.0; 95% CI, 0.7–1.4).

In the study of West *et al.* (1993), described on p. 183, lifetime occupational histories were recorded after interview with each subject. An industrial hygienist who was unaware of the case–control status of the subjects then classified each job as either likely

or unlikely to involve exposure to dust, solvents or exhaust fumes. The investigators found all three exposures to be significantly associated with risk for nasopharyngeal carcinoma. People first exposed to dust 35 or more years before cancer diagnosis had an odds ratio of 4.7 (95% CI, 1.8–13) relative to no exposure; the comparable odds ratios were 2.6 (95% CI, 1.1–6.3) for exposure to solvents and 2.8 (95% CI, 1.1–7.0) for exposure to exhaust fumes. As the three exposures were highly correlated, a combined exposure index was used in the final multivariate model. The index maintained an independent effect on risk after adjustment for other factors.

A number of epidemiological studies have suggested that woodworkers are at increased risk for nasopharyngeal carcinoma. Besides wood dust (see IARC, 1995a), these workers are exposed to various chemicals that are applied to the wood, including pesticides, phenols, chlorophenols and asbestos. Demers *et al.* (1995) conducted a pooled analysis of five cohort studies of mortality among furniture, plywood and wood-model workers in the United Kingdom and the United States. A total of 28 704 subjects, 7665 of whom died, were studied. A significant excess of nasopharyngeal cancer was noted: nine deaths were observed when 3.8 were expected on the basis of general population rates (standardized mortality ratio (SMR), 2.4; 95% CI, 1.1-4.5). This excess risk was seen among both furniture and plywood workers, and in those with either low or high probability of exposure to wood dust.

Kawachi *et al.* (1989) used data from the New Zealand Cancer Registry on male patients aged 20 years or more in whom nasopharyngeal carcinoma was diagnosed between 1980 and 1984, to identify possible cancers related to wood-working, since the current or most recent occupation of the patient at the time of cancer diagnosis is recorded in the Registry. Twenty-four cancer sites were examined, compared with all other cancer patients and for each site. Five cases of nasopharyngeal cancer were found among woodworkers (three carpenters and two forestry/logging workers), which represented a nonsignificant excess relative to controls (odds ratio, 2.5; 95% CI, 0.9–6.6).

(*b*) *Formaldehyde*

The possible role of formaldehyde (see IARC, 1995b) in development of nasopharyngeal carcinoma has been examined in three large-scale historical cohort studies of exposed workers (Blair *et al.*, 1986, 1987; Stayner *et al.*, 1988; Gardner *et al.*, 1993) and four case–control studies in diverse populations (Olsen *et al.*, 1984; Vaughan *et al.*, 1986a,b; Roush *et al.*, 1987; West *et al.*, 1993).

Blair *et al.* (1986) evaluated the mortality of 26 561 workers employed in 10 facilities where formaldehyde was produced or used in the United States. About 600 000 person–years of follow-up were available. There were seven deaths from cancer of the nasopharynx in the cohort, when 2.2 were expected on the basis of the mortality rates of the general population ($p < 0.05$). The risk increased with increasing cumulative exposure to formaldehyde among people who were also exposed to particulates (Blair *et al.*, 1987). On the basis of five cases, the SMRs were 190 for cumulative exposure of < 0.5 ppm–years, 400 for 0.5–< 5.5 ppm–years and 750 for ≥ 5.5 ppm–years (this trend was not statistically significant). A closer examination of the data revealed that three of the five deaths from nasopharyngeal carcinoma had occurred in people who had worked at the

plants for less than a year, and that the deaths at one plant out of the 10 studied were responsible for the observed excess (Collins *et al.*, 1988).

Gardner *et al.* (1993) studied the mortality experience of workers in the United Kingdom exposed to formaldehyde in six factories where this chemical was manufactured or used. The cohort consisted of 7660 men who were first employed between 1920 and 1964 and 6357 men first employed between 1965 and 1982. The cohort was followed-up from 1 January 1941 to 31 December 1989. There were no cases of nasopharyngeal cancer, while 1.3 were expected.

In Denmark, Olsen *et al.* (1984) compared the occupational exposures of 266 patients with nasopharyngeal carcinoma with those of 2465 controls with other cancers. Exposure to 12 specific substances or procedures, including formaldehyde, was assessed by industrial hygienists who were unaware of the case or control status of the cancer patients. No significant association was found between exposure to formaldehyde and risk for nasopharyngeal carcinoma, in either men (odds ratio, 0.7; 95% CI, 0.3–1.7) or women (odds ratio, 2.6; 95% CI, 0.3–21.9).

Vaughan *et al.* (1986a,b) conducted a case–control study of 27 cases of nasopharyngeal carcinoma and 552 population controls identified through the random digit dialling method in western Washington State (United States). Occupational exposures and other information were obtained by telephone interviews; however, one-half of the interviews were conducted with next-of-kin. A job–exposure matrix was used to determine if study subjects had had occupational exposure to formaldehyde. A modest, statistically non-significant association was found between duration of exposure to formaldehyde and risk for nasopharyngeal carcinoma. The odds ratios for 1–9 and ≥ 10 years of exposure were 1.2 (95% CI, 0.5–3.1) and 1.6 (95% CI, 0.4–5.8), respectively, relative to no exposure. A history of living in a mobile home (which is believed to be associated with exposure to formaldehyde from the particle-board and plywood commonly used) was positively and significantly related to increased risk, with odds ratios of 2.1 (95% CI, 0.7–6.6) for 1–9 years of living in a mobile home and 5.5 (95% CI, 1.6–19) for ≥ 10 years, relative to no residence in a mobile home. The odds ratio for those with a history of exposure to formaldehyde on the job and living in a mobile home, relative to those with neither exposure, was 6.7 (95% CI, 1.2–39).

Roush *et al.* (1987) compared 173 deaths from nasopharyngeal carcinoma with 605 control subjects selected from the Connecticut (United States) death certificate files. City directories and death certificates were used to reconstruct the occupational status of each study subject 1, 10, 20, 25, 30, 40 and 50 years before death. An industrial hygienist who was unaware of the case or control status then assigned each combination of job, industry and year to give two exposure scores: four degrees of probability of exposure (unexposed, possibly exposed, probably exposed, definitely exposed) and three levels of exposure (zero, low, high). The odds ratio for probable exposure at some level for most of a working life and probable exposure to high levels ≥ 20 years before death was 2.3 (95% CI, 0.9–6.0).

In the study of West *et al.* (1993), described on p. 183, early occupational exposure to formaldehyde was significantly related to the risk for nasopharyngeal carcinoma. First

exposure before the age of 25 was associated with an odds ratio of 2.7 (95% CI, 1.1–6.6) relative to no exposure, and the excess risk persisted after adjustment for other risk factors for nasopharyngeal carcinoma.

(*c*) *Tobacco*

The relationship between cigarette smoking (see IARC, 1986) and nasopharyngeal carcinoma has been examined in a number of case–control studies and one cohort study in diverse populations. Although the results of earlier studies, many of which were based on either small sample sizes, very young cases or hospital controls, are equivocal, more recent data from better designed studies are consistent in showing that cigarette smoking is a risk factor for nasopharyngeal carcinoma, regardless of race. Three- to fivefold increased risks were observed among the heaviest smokers in China, the Philippines and the United States (Yu *et al.*, 1990; Nam *et al.*, 1992; Chow *et al.*, 1993; West *et al.*, 1993; Zhu *et al.*, 1995).

In the study of Yu *et al.* (1990), described on p. 186, subjects and their spouses were asked about lifetime use of cigarettes and water pipes. A moderate but statistically significant association was found between tobacco use and nasopharyngeal carcinoma risk. Use of a water pipe and cigarettes were both related to increased risk. Most of the water-pipe users also were cigarette smokers. A lifetime exposure of 30 or more pack–years (assuming that 2.5 g of tobacco, equivalent to 2.5 cigarettes, were consumed each time a subject smoked a water pipe) resulted in a 3.7-fold increased risk for nasopharyngeal carcinoma relative to non-users, after adjustment for all other risk factors (95% CI, 1.2–12).

Nam *et al.* (1992) conducted a case–control study of white cases of nasopharyngeal carcinoma identified from the National Mortality Followback Survey, which provides information from death certificates on people who died at the age of 25 years or more in the United States in 1986. White control subjects were selected from the same database and were matched to the cases by sex and age. Those who had died from causes known to be related to cigarette or alcohol use were excluded from the pool of potential controls. Thus, 204 cases of nasopharyngeal carcinoma and 408 controls were included in the study. Proxy interviews were conducted by using a structured questionnaire, mainly with spouses. Cigarette use was related to the risk for nasopharyngeal carcinoma in both men and women, and the risk increased with increasing duration of use and with increasing number of cigarettes smoked per day. For the heaviest smokers (those with ≥ 60 pack–years of cumulative exposure), the odds ratio was 3.1 (95% CI, 1.6–6.1) in men and 4.9 (95% CI, 1.2–20.9) in women.

Chow *et al.* (1993) used information collected in 1954 and 1957 on 248 046 United States veterans and their mortality through 30 September 1980 to examine the relationship between tobacco use and the development of nasopharyngeal carcinoma. Forty-eight deaths from this cause were studied. Current smokers had a 3.9-fold increased risk for nasopharyngeal carcinoma (95% CI, 1.5–10), and the risk increased with the number of cigarettes smoked per day. There was no excess risk among people who smoked only cigars or pipes.

In the study of West *et al.* (1993), described on p. 183, no association between cigarette smoking and nasopharyngeal carcinoma was found in comparison with hospital controls, but a significant association between heavy use of cigarettes and risk was observed when the cases were compared with community controls. After adjustment for all other risk factors, the odds ratio for smokers of ≥ 31 years was 4.9 (95% CI, 1.6–15).

Zhu *et al.* (1995) carried out a case–control study in eight areas of the United States covered by population-based cancer registries. The cases were in men aged 15–39 in 1968 in whom nasopharyngeal cancer had been diagnosed in 1984–88. The controls were men selected from the general populations of the eight study areas by random-digit dialling and matched to the cases by age. A total of 113 cases and 1910 controls were included; 62% of the cases were white (including Hispanics), 11% were black and 27% were Asian. Most of the subjects were interviewed directly by telephone. Cigarette smoking was found to be related to the risk for nasopharyngeal carcinoma, and the risk increased monotonically with increasing number of cigarettes smoked per day and with increasing number of pack–years of cumulative exposure. The odds ratio for individuals with ≥ 45 pack–years of exposure was 3.9 (95% CI, 2.0–7.8) relative to nonsmokers.

Vaughan *et al.* (1996) conducted a case–control study in five locations in the United States covered by population-based cancer registries. Telephone interviews were completed with 231 cases and with 246 controls matched to the cases on age, sex and race. Most of the cases occurred in non-Hispanic whites (77%) or African Americans (10%). A statistically significant dose–response relationship was observed between cigarette smoking and risk for nasopharyngeal carcinoma, which was confined to differentiated squamous-cell carcinomas and those classified as epithelial tumours not otherwise specified; no association was observed between cigarette smoking and the 54 cases of undifferentiated non-keratinizing carcinoma.

The possible role of passive smoking in the development of nasopharyngeal carcinoma has been examined in two case–control studies, with inconclusive results. In the study of Yu *et al.* (1988), described on p. 181, mothers were asked about the smoking habits of all household members around the time of birth of the index subject and when he or she was 10 years old. The fathers of more cases than controls had smoked when the subjects were born (odds ratio, 1.5; $p = 0.05$), and the risk increased with increasing number of cigarettes smoked by the father (p for linear trend = 0.04). Very few mothers had smoked (5.5% of case mothers and 5.2% of control mothers), and the odds ratio was 1.0. More cases than controls had been exposed to tobacco smoke from other household members, but the difference was not statistically significant. Overall, the presence of a smoker in the household around the time of birth was associated with an increased risk for nasopharyngeal carcinoma (odds ratio, 2.0; $p = 0.004$). Exposure at birth and at the age of 10 was highly correlated, with a slightly stronger association for exposure at birth; there was no significant residual effect for exposure at the age of 10 after adjustment for exposure at birth. Adjustment for personal cigarette smoking did not affect these findings.

In the study of Yu *et al.* (1990), described on p. 186, in which a significantly positive association with active smoking was observed, the same set of questions as used in the

study described above was given to the mothers of cases and controls. No associations were observed. Having a spouse who smoked was also not related to the risk for nasopharyngeal carcinoma (odds ratio, 1.2; 95% CI, 0.6–2.4). The odds ratio for any domestic exposure to passive smoking was 0.9 (95% CI, 0.6–1.4). The results were unchanged when the analysis was restricted to non-tobacco users.

(*d*) *Alcohol*

The possible association between alcohol intake and the development of nasopharyngeal carcinoma has been investigated in a number of case–control studies among Chinese in and outside of China and among whites in the United States (Lin *et al.*, 1973; Henderson *et al.*, 1976; Geser *et al.*, 1978; Shanmugaratnam *et al.*, 1978; Armstrong *et al.*, 1983; Mabuchi *et al.*, 1985; Chen *et al.*, 1988b; Ning *et al.*, 1990; Nam *et al.*, 1992; Vaughan *et al.*, 1996). Only the studies of Nam *et al.* (1992) and Vaughan *et al.* (1996) found an association. Nam *et al.* (1992) relied on surrogate interviews for information on exposure, as all of the cases and controls were identified from death certificates. The 80% excess risk noted in that study after control for level of cigarette smoking could have been the result of residual confounding. Vaughan *et al.* (1996) reported a significant association between heavy alcohol use (21 or more drinks per week) and risk for nasopharyngeal carcinoma after adjustment for cigarette use. The association was confined to differentiated squamous-cell carcinoma and epithelial tumours not otherwise specified.

(*e*) *Herbal drugs*

A number of Chinese herbs have been shown to induce EBV antigens in human lymphoblastoid cell lines carrying the EBV genome (Hirayama & Ito, 1981; Zeng *et al.*, 1983b; Zeng, 1987), raising the possibility that exposure to these products may affect the risk for nasopharyngeal carcinoma. The geographical distribution of *Croton tiglium*, the seeds of which are used in Chinese herbs, has been noted to loosely parallel that of nasopharyngeal carcinoma within China (Hirayama & Ito, 1981). In fact, croton seeds are rarely used in herbal mixtures due to their extreme potency. In the studies of Yu *et al.* (1986, 1988, 1990), questions on lifetime use of croton seeds were asked, but none of the subjects reported exposure to this herb. Yu *et al.* (1990) also examined lifetime use of *Phyllanthus emblica* and *Croton crassifolius*, two EBV-inducing herbs that are commonly prescribed in Guangzhou (according to Government sales figures); no relationship with nasopharyngeal carcinoma was observed.

Use of herbal drugs in general was reported to be associated with nasopharyngeal carcinoma in three case–control studies (Lin *et al.*, 1973; Hildesheim *et al.*, 1992; Zheng *et al.*, 1994b). Lin *et al.* (1973) interviewed 343 patients with nasopharyngeal carcinoma identified in four counties in Taiwan and 1017 population controls matched to the cases by age, sex and neighbourhood of residence. Use of herbal drugs was associated with the risk for nasopharyngeal carcinoma, occasional users having an odds ratio of 1.7 and frequent users an odds ratio of 3.5 ($p < 0.001$), relative to non-users. The authors acknowledged that usage may have been related to the clinical symptoms of the tumour

and/or recall bias. [Use of herbal drugs is a part of the traditional life style that is an established risk factor for nasopharyngeal carcinoma.]

In the Philippines, Hildesheim *et al.* (1992) studied 104 predominantly Filipino cases of nasopharyngeal carcinoma (see West *et al.*, 1993, p. 183). Information on the use of herbal medicines was collected from study subjects at personal interviews. Since the prevalence of use was similar in the two groups of control subjects, they were combined for the analysis. Patients with nasopharyngeal carcinoma were significantly more likely than controls to have used herbal medicines (odds ratio, 2.5; 95% CI, 1.4–4.5), and the result was unchanged after adjustment for potential confounders. [The same methodological concerns outlined at the end of the last paragraph apply to this study.]

In the study of Zheng *et al.* (1994b), described on p. 181, subjects were asked about their use of herbal drugs during the year before cancer diagnosis and during childhood. Recent use was strongly associated with the risk for nasopharyngeal carcinoma (odds ratio, 4.5; $p = 0.006$), and the risk was reduced when childhood use was considered (odds ratio, 1.8; $p = 0.07$).

In contrast, three case–control studies conducted in southern China of the frequency of use of the most popular herbal tea in the region, 'cooling soup', during adulthood (three years before cancer diagnosis) or childhood (at the age of 10) showed no association between exposure and risk (Yu *et al.*, 1986, 1988, 1989a). Herbal medicines are almost never given to Chinese infants.

(f) *Incense and anti-mosquito coils*

Domestic exposure to burning incense and anti-mosquito coils has been postulated as a risk factor for nasopharyngeal carcinoma and has been investigated in a number of case–control studies conducted in various southern Chinese populations. In Taiwan (Lin *et al.*, 1973; Chen *et al.*, 1988b), Hong Kong (Yu *et al.*, 1986), Guangxi, Yulin prefecture (Yu *et al.*, 1988) and Guangzhou (Yu *et al.*, 1990), such exposures were not associated with the risk for nasopharyngeal carcinoma. Yu *et al.* (1986, 1988, 1990) examined the frequencies of the two exposures at birth, at the age of 10 and three years before cancer diagnosis, while Lin *et al.* (1973a) and Chen *et al.* (1988b) compared any with no exposure to the two types of smoke. In Singapore, two case–control studies of incense burning and risk for nasopharyngeal carcinoma showed no association (Shanmugaratnam *et al.*, 1978; Lee *et al.*, 1994b).

The study of Shanmugaratnam *et al.* (1978) was a hospital-based study with two sets of hospital controls: non-nasopharyngeal carcinoma patients from the same clinics and wards where the cases were recruited and medical, surgical and orthopaedic patients from a different Government hospital. The two sets of controls were not comparable to the cases with regard to socioeconomic status; the cases were less educated than the controls with ear-nose-and-throat conditions and were more educated than the 'other hospital' controls. Anti-mosquito coil burning was associated with increased odds ratios of 1.3–1.4 ($p < 0.05$) in comparison with both control groups when users were compared with non-users and those of unknown status. The frequencies of the groups with no use, any use and unknown status were not given.

In the study of West *et al.* (1993), described on p. 183, use of mosquito coils in the year before cancer diagnosis was investigated. Daily users of anti-mosquito coils had a sixfold increased risk for nasopharyngeal carcinoma relative to non-users (odds ratio, 5.9; 95% CI, 1.7–20).

(*g*) *Chinese nasal oil*

The use of Chinese nasal oil, the main ingredients of which are camphor and menthol, has been postulated as a risk factor for nasopharyngeal carcinoma. Yu *et al.* (1986, 1990) noted greater recent (three years before) use among cases than controls, which was related to the clinical symptoms of nasopharyngeal carcinoma. The more relevant exposure during childhood was examined in three case–control studies (Yu *et al.*, 1986, 1988, 1990). Although in two studies (Yu *et al.*, 1988, 1990) the mothers of more cases than controls reported use during the subjects' childhood, most of the exposures were infrequent and/or unsubstantiated by a medical condition that would suggest intense, sustained use. Several other case–control studies of Chinese have shown greater recent use in cases than controls, but none could rule out the possibility that the oil was used to treat symptoms of nasopharyngeal carcinoma (Lin *et al.*, 1973; Shanmugaratnam *et al.*, 1978; Lee *et al.*, 1994b).

2.4.3.3 *Host factors*

Case–control studies have established several associations between HLA locus A and B antigens and risk for nasopharyngeal carcinoma in southern Chinese. The presence of both A2 and BW46 antigens was associated with a twofold increased risk for nasopharyngeal carcinoma among Chinese in Singapore, Malaysia, Hong Kong and Guangzhou (Simons *et al.*, 1976, 1977, 1978, 1980; Chan *et al.*, 1983a). Interestingly, the frequency of the A2-BW46 phenotype is twice as common among Cantonese than in the Chiu Chau/Fujianese dialect group, parallelling the twofold difference in nasopharyngeal carcinoma incidence between these two ethnic groups (Simons *et al.*, 1976). Other HLA antigens that show an association with nasopharyngeal carcinoma in southern Chinese are B16 (RR, 6.0), B17 (RR, 2.1–2.3), especially in combination with B17 (RR, 2.4–2.5), A11 (RR, 0.5) and B13 (RR, 0.5) (Simons *et al.*, 1978; Chan *et al.*, 1981, 1983a; Wu *et al.*, 1989). Preliminary HLA locus DR typing in this high-risk population has shown significant differences in antigen frequencies between cases and controls (Chan *et al.*, 1981, 1983b; Wu *et al.*, 1989). A linkage study of affected pairs of siblings in southern Chinese in China, Hong Kong and Singapore suggests that a gene (or genes) closely linked to the HLA locus is associated with a 20-fold increased risk for nasopharyngeal carcinoma (Lu *et al.*, 1990). A recent study in Singapore Chinese showed that allele DRB10803 is associated with a 2.8-fold increase (95% CI, 1.0–7.5). In the same study, the investigators found a 3.5-fold increased risk (95% CI, 1.6–7.6) among individuals with allele 4 of the D6S1624 microsatellite locus located on chromosome 6 (Ooi *et al.*, 1997).

An association between HLA profile and nasopharyngeal carcinoma risk has also been reported in non-Chinese populations. B46 has been associated with the occurrence of this tumour in Thais (Chan *et al.*, 1986), and antigen B17 has been found to be posi-

tively associated with the risk of Malays (Chan *et al.*, 1985) and Australian whites (Simons & Shanmugaratnam, 1982). Other antigens shown to be associated with nasopharyngeal carcinoma in selected populations are: A29 in Kenyans and Tanzanians, B18 in Malays, A3 in Australian whites, B5 in Germans and A2 in whites in the United States (Hall *et al.*, 1982; Simons & Shanmugaratnam, 1982; Chan *et al.*, 1985; Burt *et al.*, 1994). In contrast to southern Chinese, white Americans, Europeans, Tunisians and Malays rarely have BW46, and no association with nasopharyngeal carcinoma was seen in these populations (Betuel *et al.*, 1975; Chan *et al.*, 1979b, 1985; Beigel *et al.*, 1983; Moore *et al.*, 1983).

2.4.3.4 *Familial aggregation*

Multiple cases of nasopharyngeal carcinoma occurring in first-degree relatives have been documented in diverse populations, ranging from high-risk southern Chinese and Alaskan and Greenland natives, low- to intermediate-risk Africans, to low-risk Caucasians (Stinson, 1940; Bell & Maguda, 1970; Nevo *et al.*, 1971; Ho, 1972; Williams & de Thé, 1974; Brown *et al.*, 1976; Jonas *et al.*, 1976; Lanier *et al.*, 1979; Gajwani *et al.*, 1980; Fischer *et al.*, 1984; Yu *et al.*, 1986; Schimke *et al.*, 1987; Yu *et al.*, 1990; Albeck *et al.*, 1993). Familial aggregation can be the result of shared genes, shared environments or both. Among the high-risk southern Chinese, a potent environmental factor that is strongly correlated within families — dietary exposures at weaning — has been identified, and consistent associations with certain HLA antigens imply the presence of disease susceptibility genes. Genetic studies in non-Chinese populations also suggest the involvement of hereditary factors in the development of nasopharyngeal carcinoma, and analytical studies have implicated environmental factors. Familial clustering of nasopharyngeal carcinoma is therefore likely to be a product of genetic constitution and environmental exposures.

2.5 Comparison of characteristics of Burkitt's lymphoma, Hodgkin's disease and nasopharyngeal carcinoma

Table 27 gives a comparison of the epidemiology, virological markers and sites of African Burkitt's lymphoma, Hodgkin's disease and nasopharyngeal carcinoma in areas of high risk for those tumours.

2.6 Other malignancies

2.6.1 *Lymphoepithelial carcinomas outside the nasopharynx*

The detection of EBV in virtually all undifferentiated nasopharyngeal carcinomas prompted studies into the possible association of the virus with other lymphoepithelial carcinomas. These studies concentrated initially on carcinomas morphologically similar to type-3 nasopharyngeal carcinoma, which can be distinguished from gastric carcinoma with lymphoid stroma, which may account for up to 50% of gastric carcinomas (Matsunou *et al.*, 1996). Lymphoepithelial carcinomas of the stomach are a relatively rare subtype of gastric neoplasm (Rowlands *et al.*, 1993).

Table 27. Characteristics of Burkitt's lymphoma, Hodgkin's disease and nasopharyngeal carcinoma in high-risk regions

Characteristic	African Burkitt's lymphoma	Hodgkin's disease	Nasopharyngeal carcinoma
Epidemiology			
Age	< 15 years (mode, 8–10)	Bimodal (1st mode, 15–50 years; 2nd mode, > 50 years)	> 15 years (mode, 45–54)
Sex	Males > females	Males > females	Males > females
High-risk regions	Equatorial Africa	Developed countries	Southeast Asia and south China
Period of exposure to probable carcinogens	Early	Early or post-adolescence	Early and continuous
Co-factors	Holoendemic malaria	None identified	Selected preserved foods
Virological markers			
Nucleic acids, proteins			
EBV DNA	+	+ (30–50%)	+
EBER	+	+ (30–50%)	+
EBNA	+	–	+
Serology			
VCA IgA	–	–	+
IgG	+	–	–
IgM	+	+	–
Anti-EBNA	–	++	+
Reactive stroma			
T Cells	–	++	+
B Cells	–	–	–
NK and other cells	–	+	–

EBER, EBV-encoded RNA; EBNA, EBV nuclear antigen; VCA, viral capsid antigen; Ig, immunoglobulin; NK, natural killer

An association of EBV with lymphoepithelial carcinomas arising outside the nasopharynx was initially proposed on the basis of the detection of EBV genomes in DNA extracts (Table 28). Saemundsen *et al.* (1982) found EBV genomes in DNA extracted from a lymphoepithelial salivary gland carcinoma in a Greenland Inuit. This observation was subsequently confirmed by Hamilton-Dutoit *et al.* (1991a), who also localized the viral genomes to the tumour cells by DNA in-situ hybridization in 11/11 of these carcinomas. Further studies have shown the consistent association of salivary gland lymphoepithelial carcinomas with EBV (Chan *et al.*, 1994; Leung *et al.*, 1995a; Kotsianti *et al.*, 1996; Tsai *et al.*, 1996a). Moreover, the viral episomes in these tumours have been shown to be of monoclonal origin (Leung *et al.*, 1995a). An EBV-associated lymphoepithelial carcinoma in the lachrymal sac was reported in a Chinese patient (Leung *et al.*, 1996).

Using DNA in-situ hybridization, Weiss *et al.* (1989b) detected EBV DNA in all of six nasopharyngeal lymphoepitheliomas but not in lymphoepithelial carcinomas occurring in the skin, cervix uteri, tonsil, pharynx or larynx. Of 14 lymphoepithelial carcinomas outside the nasopharynx, the only one that contained EBV was a carcinoma of the lung in a Chinese woman. This observation was confirmed by subsequent reports (Table 29; Butler *et al.*, 1989; Gal *et al.*, 1991; Pittaluga *et al.*, 1993; Wöckel *et al.*, 1995). Pittaluga *et al.* (1993) were also able to show monoclonal viral episomes in some of their cases. [The Working Group noted that the cases in these studies that contained EBV were generally in Asians.]

On the basis of PCR investigations, Burke *et al.* (1990) and Min *et al.* (1991) first suggested that lymphoepithelial gastric carcinomas are associated with EBV infection. Clonal viral episomes were subsequently detected in such cases by Southern blot hybridization (Pittaluga *et al.*, 1992). Studies with in-situ hybridization have confirmed those reports by localizing the virus to the malignant epithelial cells in such cases (Shibata *et al.*, 1991b; Niedobitek *et al.*, 1992b; Oda *et al.*, 1993; Matsunou *et al.*, 1996). Several studies have indicated that about 80% of lymphoepithelial gastric carcinomas are associated with EBV (Rowlands *et al.*, 1993; Osato & Imai, 1996; see Table 30).

The association of EBV with lymphoepithelial carcinomas arising at other anatomical sites is tenuous. Nicholls *et al.* (1994) detected EBV in one of five non-keratinizing tonsillar carcinomas among Chinese patients, but Niedobitek *et al.* (1991b) found no evidence for an association with EBV in Chinese or Malays. Mori *et al.* (1994) detected EBV in a single lymphoepithelial carcinoma of the oesophagus by PCR and EBER in-situ hybridization. Morphologically similar carcinomas of the breast, so-called medullary carcinomas, have consistently been found to be EBV-negative (Niedobitek *et al.*, 1991b; Kumar & Kumar, 1994; Lespagnard *et al.*, 1995), as were lymphoepithelial carcinomas of the skin, thyroid gland, larynx, urinary bladder, uterine cervix and vulva (Weiss *et al.*, 1989b; Carr *et al.*, 1992; Weinberg *et al.*, 1993; Martinez-Leandro *et al.*, 1994; Requena *et al.*, 1994; Axelsen & Stamp, 1995; Gulley *et al.*, 1995; MacMillan *et al.*, 1996; Shek *et al.*, 1996).

Table 28. Presence of EBV in lymphoepithelial and other carcinomas

Cancer site and reference	Ethnicity	Morphology	Method of detection of EBV	EBV-positive/ total tested
Salivary gland				
Saemundsen *et al.* (1982)	Inuit	Lymphoepithelial	ISH	1/1
		Adenocarcinoma		0/1
Hamilton-Dutoit *et al.* (1991a)	Inuit	Lymphoepithelial-like	ISH	11/11
	Non-Inuit			0/2
Chan *et al.* (1994b)	Chinese	Lymphoepithelial	ISH	5/5
		Other		0/55
Kotsianti *et al.* (1996)	NR (Greek)	Lymphoepithelial-like	ISH	1/1
Tsai *et al.* (1996a)	Chinese	Lymphoepithelial	ISH	7/7
		Adenocarcinomas		0/49
Thymus				
Dimery *et al.* (1988)	NR (USA)	Thymoma (lymphoepithelioma)	Southern blot	1/1
Teoh *et al.* (1989)	Chinese	Thymomas (lymphoepithelial-like)	NR	2/13
McGuire *et al.* (1988)	Chinese	Thymomas	Southern blot	3/3
Leyvraz *et al.* (1985)	Hispanic	Lymphoepithelial-like	*Bam*HI W	1/1
Borisch *et al.* (1990)	NR (Switzerland)	Epithelial tumours	Southern blot	0/32
Niedobitek *et al.* (1991b)	NR (Germany)	3 lymphoepithelial-like + 11 other	ISH	0/14
Mann *et al.* (1992)	NR (USA)	Carcinoma	ISH	1/7
		Thymoma		0/14
Head and neck (other than nasopharyngeal carcinoma)				
Tyan *et al.* (1993)	Chinese	Various	PCR	30/44
Weiss *et al.* (1989b)	Caucasian and black	Lymphoepithelial	ISH	0/2
		Squamous-cell		0/4

Table 28 (contd)

Cancer site and reference	Ethnicity	Morphology	Method of detection of EBV	EBV-positive/ total tested
Tonsil				
Brichácek *et al.* (1984)	NR (Czechoslovakia)	Squamous-cell carcinoma	ISH	6/7
Niedobitek *et al.* (1991b)	NR (Germany)	Squamous-cell carcinoma	ISH	0/26
		Lymphoepithelial-like		0/2
Nicholls *et al.* (1994)	Chinese	Undifferentiated carcinoma	ISH	1/5
		Squamous-cell carcinoma		0/5
Weiss *et al.* (1989b)	Caucasian	Lymphoepithelial	ISH	0/3
		Squamous-cell carcinoma		0/1
Cervix				
Martínez-Leandro *et al.* (1994)	Caucasian	Lymphoepithelial-like	ISH	0/1
Niedobitek *et al.* (1991b)	NR (Germany)	Carcinoma	ISH	0/14
Leung *et al.* (1995a)	Chinese	Lymphoepithelial-like	ISH	0/1
Payne *et al.* (1995)	NR (United Kingdom)	Pre-invasive squamous lesions	ISH	0/3
Weinberg *et al.* (1993)	Black	Lymphoepithelial	PCR and ISH	0/1
Hilton *et al.* (1993)	Caucasian	Squamous-cell and adenocarcinomas	ISH	0/24
		Intraepithelial		0/10
Landers *et al.* (1993)	Caucasian	Squamous-cell carcinoma	PCR	8/18
		CIN-III	ISH	2/25
		CIN-II		2/25
		CIN-I + normal	PCR and ISH	0/50
Se Thoe *et al.* (1993)	NR (Malaysia)	Carcinoma	ISH	5/8
Weiss *et al.* (1989b)	Caucasian	Lymphoepithelial	ISH	0/1
Vagina				
Dietl *et al.* (1994)	NR (Germany)	Lymphoepithelial	ISH	0/1

Table 28 (contd)

Cancer site and reference	Ethnicity	Morphology	Method of detection of EBV	EBV-positive/ total tested
Vulva				
Axelsen & Stamp (1995)	NR (Denmark)	Lymphoepithelial	ISH	0/1
Testis				
Rajpert-de Meyts *et al.* (1994)	NR (Denmark)	Germ-cell	PCR EBER ISH	0/19
Breast				
Niedobitek *et al.* (1991b)	NR (Germany)	Medullary carcinoma	ISH	0/9
Kumar & Kumar (1994)	NR (USA)	Lymphoepithelial-like	ISH	0/1
Labrecque *et al.* (1995)	NR (United Kingdom)	Various	PCR EBER ISH DNA ISH	19/91 6/19 12/19
Lespagnard *et al.* (1995)	NR (Belgium)	Medullary carcinoma	PCR and ISH	0/10
Thyroid gland				
Shek *et al.* (1996)	Chinese	Lymphoepithelial	ISH	0/1
Parotid gland				
Huang *et al.* (1988)	Chinese	Undifferentiated	ISH	0/1
Gallo *et al.* (1995)	Caucasian	Undifferentiated	ISH	3/7
Urinary bladder				
Gulley *et al.* (1995)	NR (USA)	Lymphoepithelial	ISH	0/11
Skin				
Weiss *et al.* (1989b)	Caucasian	Lymphoepithelial	ISH	0/4
Carr *et al.* (1992)	White	Lymphoepithelial	ISH	0/1
Requena *et al.* (1994)	NR (Spain)	Lymphoepithelial	ISH	0/1

Table 28 (contd)

Cancer site and reference	Ethnicity	Morphology	Method of detection of EBV	EBV-positive/ total tested
Oesophagus				
Mori *et al.* (1994)	NR (Japan)	Lymphoepithelial-like Other	ISH and PCR	1/1 0/29
Colon and rectum				
Yuen *et al.* (1994)	Chinese	Adenocarcinoma	ISH	0/36
Larynx				
Brichácek *et al.* (1983)	NR (Czechoslovakia)	Poorly differentiated carcinoma	ISH	3/5
MacMillan *et al.* (1996)	NR (USA)	Lymphoepithelial-like	ISH	0/8
Paranasal sinus				
Leung *et al.* (1995b)	Chinese	29 carcinomas 8 keratinizing squamous-cell carcinomas 11 transitional-cell carcinomas 4 adenocarcinomas 2 mucoepithelial carcinomas 2 adenoid cystic carcinomas 2 undifferentiated carcinomas	ISH	7/29 4/8 1/11 1/4 0/2 0/2 1/2
Lopategui *et al.* (1994)	Asian NR (western)	NR NR	ISH	7/11 0/11
Lachrymal sac				
Leung *et al.* (1996)	Chinese	Undifferentiated lymphoepithelial carcinoma	ISH	1/1

NR, not reported; ISH, in-situ hybridization; PCR, polymerase chain reaction; CIN, cervical intraepithelial neoplasia; EBER, EBV-encoded RNA

Table 29. Presence of EBV in lung carcinomas

Reference	Ethnicity	Morphology	EBV detection method	EBV-positive/ total tested
Weiss *et al.* (1989b)	Asian Caucasian	Lymphoepithelial-like carcinoma	ISH	1/1 0/3
Pittaluga *et al.* (1993)	Chinese	Lymphoepithelial-like carcinoma Other carcinomas	ISH	5/5 0/132
Conway *et al.* (1996)	Caucasian, Hispanic	Adenocarcinoma Pleural mesothelioma	ISH	0/80 0/50
Ferrara & Nappi (1995)	Caucasian	Lymphoepithelial-like carcinoma	ISH	0/2
Wöckel *et al.* (1995)	Caucasian	Lymphoepithelial-like carcinoma	ISH and PCR	0/1
Butler *et al.* (1989)	Chinese Caucasian	Lymphoepithelial-like carcinoma	ISH	1/1 1/3
Gal *et al.* (1991)	Chinese	Lymnphoepithelial-like carcinoma	ISH and PCR	1/1
Wong *et al.* (1995)	Chinese	167 carcinomas	ISH	9/167 (all 9 lympho-epithelial-like)

ISH, in-situ hybridization

2.6.2 *Other carcinomas*

2.6.2.1 *Stomach*

EBV was first detected in gastric adenocarcinomas by Shibata and Weiss (1992), in 22 of 138 cases (16%) in the United States by PCR and DNA and EBER in-situ hybridization. This observation prompted several groups to study gastric adenocarcinomas in other areas. Studies in Europe showed a generally lower proportion of EBV-associated gastric adenocarcinomas, ranging between about 2 and 8% (Rowlands *et al.*, 1993; Ott *et al.*, 1994; Selves *et al.*, 1996b). Interestingly, in one study, no difference in the prevalence of EBV was found between tumours from the United Kingdom and from Japan (Rowlands *et al.*, 1993). A number of studies were carried out in Japan where the incidence of gastric carcinoma is very high. In several large studies comprising well over 2000 cases, EBV was detected in about 7% of cases (Tokunaga *et al.*, 1993; Fukayama *et al.*, 1994; Imai *et al.*, 1994a; Osato & Imai, 1996). Shin *et al.* (1996) found that 12 of 89 consecutive gastric tumours in Korean patients were EBV-positive; nine of these EBV-infected carcinomas were of the lymphoepithelial lymphoma type. These studies are summarized in Table 30.

Table 30. Presence in EBV in gastric carcinomas

Reference	Ethnicity	Morphology	EBV detection method	EBV-positive/ total tested
Burke *et al.* (1990)	Filipino	Lymphoepithelial-like	PCR	1/1
	NR (USA)	Adenocarcinoma		0/1
Min *et al.* (1991)	White	Lymphoepithelial-like	ISH and PCR	3/3
Shibata *et al.* (1991b)	Japanese	Lymphoepithelial-like	PCR	6/7
	Caucasian (USA)			1/1
Pittaluga *et al.* (1992)	Chinese	Lymphoepithelial-like	ISH	1/1
Shibata & Weiss (1992)	NR (USA)	Adenocarcinoma	ISH and PCR	22/138
Oda *et al.* (1993)	Japanese	Lymphoepithelial-like	ISH and PCR	13/14
		Other	ISH	2/8
Rowlands *et al.* (1993)	NR (UK) and Japanese	Intestinal	ISH	1/81
		Diffuse		0/44
		Mixed		2/28
		Unclassified		0/21
		Selected lympho-epithelial-like		6/6
Fukayama *et al.* (1994)	Japanese	Lymphoepithelial-like	ISH and PCR	6/6
		Other		2/66
Mori *et al.* (1994)	Japanese	Lymphoepithelial-like	ISH and PCR	2/2
		Other		0/29
Ott *et al.* (1994)	NR (Germany)	Lymphoepithelial-like	ISH	4/4
		Other		3/35
Tokunaga *et al.* (1993)	Japanese	Carcinoma	ISH	69/999
		Lymphoepithelial-like		8/9
Yuen *et al.* (1994)	Chinese	Intestinal	ISH	6/52
		Lymphoepithelial-like		1/3
		Diffuse		0/14
		Mixed		0/5
Harn *et al.* (1995)	Chinese	Lymphoepithelial-like	ISH and PCR	1/1
		Other	ISH	5/54
Blasco *et al.* (1996)	NR (Argentina)	Lymphoepithelial-like	ISH	1/1
Gulley *et al.* (1996)	NR (USA)	Carcinoma	ISH	11/95
		Other		3/4
Matsunou *et al.* (1996)	Japanese	Lymphoepithelial-like	ISH	22/26
		Other (synchronous)		4/4
Selves *et al.* (1996b)	NR (France)	Intestinal	ISH	1/22
		Lymphoepithelial-like		4/6
		Diffuse		0/21
		Mixed		0/10
Shin *et al.* (1996)	Korean	Adenocarcinoma	ISH	12/89
		Lymphoepithelial-like		9/10
		Normal gastric		0/37

Table 30 (contd)

Reference	Ethnicity	Morphology	EBV detection method	EBV-positive/ total tested
Vasef *et al.* (1996)	NR (USA) (1 patient with 2 tumours)	Lymphoepithelial-like N-Cell lymphoma in MALT	ISH and PCR	1/1 1/1

NR, not reported; ISH, in-situ hybridization; PCR, polymerase chain reaction; MALT, mucosa-associated lymphoid tissue

EBV has been detected in both intestinal and diffuse gastric adenocarcinomas. No difference in EBV positivity was found in carcinomas in different locations in the stomach (Rowlands *et al.*, 1993; Tokunaga *et al.*, 1993; Fukayama *et al.*, 1994). The frequency of EBV positivity in gastric adenocarcinomas was 9.2% in men and 3.1% in women in a study of 999 cases in Japan (Tokunaga *et al.*, 1993) (see also Table 30).

Viral genomes in EBV-associated gastric carcinomas have consistently been found to be monoclonal (Pittaluga *et al.*, 1992; Imai *et al.*, 1994a; Gulley *et al.*, 1996). While Fukayama *et al.* (1994) reported the detection of EBV in shed gastric epithelial cells by in-situ hybridization, evidence of viral infection of normal gastric mucosa was not found in other studies with similar methods (Rowlands *et al.*, 1993; Tokunaga *et al.*, 1993; Gulley *et al.*, 1996). Gulley *et al.* (1996) reported the detection of EBV in dysplastic gastric mucosa adjacent to areas of EBV-positive adenocarcinomas.

2.6.2.2 *Other sites*

Examination of adenocarcinomas and squamous-cell carcinomas arising at other anatomical sites for the presence of EBV has produced mostly negative results (see Table 28).

Squamous-cell carcinomas of the oesophagus and colorectal adenocarcinomas were shown not to contain EBER in two studies (Mori *et al.*, 1994; Yuen *et al.*, 1994). The number of cases investigated, however, was small and an association of the virus with a small proportion of such carcinomas cannot be excluded. In addition, since EBER transcription may be suppressed in well-differentiated squamous-cell tumours, this technique is inappropriate for detecting EBV in these tumours.

Brichácek *et al.* (1984) found that six of seven squamous-cell carcinomas of the tonsils contained EBV, but this finding was not confirmed in subsequent studies with EBV DNA and EBER in-situ hybridization (Niedobitek *et al.*, 1991b; Nicholls *et al.*, 1994). Brichácek *et al.* (1983) also reported the detection of EBV in a small group of poorly differentiated laryngeal carcinomas by DNA in-situ hybridization, in contrast to the absence of the virus in lymphoepithelial carcinomas at this site (MacMillan *et al.*, 1996).

In studies of carcinomas of the salivary glands (Chan *et al.*, 1994b) and lungs (Wong *et al.*, 1995; Conway *et al.*, 1996), no association with EBV was demonstrated.

Carcinomas of the paranasal sinus constitute a wide morphological spectrum of tumours including undifferentiated carcinomas, adenocarcinomas and squamous-cell carcinomas (Leung *et al.*, 1995b). The group of undifferentiated sinonasal carcinomas includes some cases with morphological features similar to those of nasopharyngeal carcinoma. In two studies, EBV was detected in at least some sinonasal carcinomas arising in Asian patients (Lopategui *et al.*, 1994; Leung *et al.*, 1995b). The EBV-positive cases included undifferentiated carcinomas, squamous-cell carcinomas and adenocarcinomas. None of the undifferentiated sinonasal carcinomas arising in western patients was EBV-positive (Lopategui *et al.*, 1994).

EBV was detected by DNA PCR in 19 of 91 cases of breast carcinoma. Of the 18 cases found to contain EBV by PCR, six were also shown to be EBV-positive by EBER in-situ hybridization, and 12 of 18 tested were EBV-positive by *Bam* HI W in-situ hybridization (Labrecque *et al.*, 1995). [The Working Group noted that it was not clear whether some cases were positive by both methods.]

Fuelled by a report suggesting that the cervix may be a site of EBV shedding (Sixbey *et al.*, 1986), several groups examined the possible association of EBV infection with cervical carcinoma. Using in-situ hybridization with biotinylated probes, Landers *et al.* (1993) found EBV DNA in 8% of grade-II cervical intraepithelial neoplasia (CIN), in 8% of CIN III and in 43% of cervical carcinomas. A similar finding was reported by Se Thoe *et al.* (1993). Other studies in which EBV DNA or EBER in-situ hybridization was used consistently failed to detect the virus in CIN lesions or in invasive cervical carcinomas (Niedobitek *et al.*, 1991b; Hilton *et al.*, 1993; Payne *et al.*, 1995).

EBV DNA was detected by Southern blot hybridization in some thymomas, i.e. benign epithelial tumours of the thymus, and in thymic carcinomas, both often showing a prominent reactive lymphoid cell infiltrate (Leyvraz *et al.*, 1985; Dimery *et al.*, 1988; Katzin *et al.*, 1988; McGuire *et al.*, 1988; Teoh *et al.*, 1989). These tumours were mainly obtained from Asian patients. Subsequent studies by PCR and in-situ hybridization of thymic epithelial tumours in western patients provided no convincing evidence of an association with EBV (Borisch *et al.*, 1990; Niedobitek *et al.*, 1991b; Mann *et al.*, 1992).

2.6.3 *Smooth-muscle tumours*

Immunosuppressed transplant recipients and HIV-infected patients are at increased risk for smooth-muscle tumours, both benign leiomyomas and malignant leiomyosarcomas (Mueller *et al.*, 1992; Lee *et al.*, 1995b). The increase is observed primarily in immunosuppressed children; cases in adults are observed rarely. The tumours that occur in this situation have frequently been found to contain EBV.

McClain *et al.* (1995) tested five leiomyosarcomas and two leiomyomas from five HIV-infected children aged less than nine years and one HIV-infected adult, aged 24, by EBER-1 in-situ hybridization. Essentially all cells in all seven tumours showed positive signals. In contrast, none of seven smooth-muscle tumours (three leiomyosarcomas and four leiomyomas) from seven HIV-negative children (under 15) were EBV-positive. EBV clonality was assessed in two of the HIV-associated cases of leiomyosarcoma: one

showed biclonality; the other consisted of two tumours at different sites, each of which contained a different clone of EBV.

Lee *et al.* (1995b) tested six smooth-muscle tumours from three children under six years of age who had undergone immunosuppression for liver allotransplantation. Most cells in all six tumours were shown to contain EBV by EBER-1 in-situ hybridization; all three tumours contained monoclonal EBV. Southern blot analysis of one tumour suggested that the EBV DNA was associated with host sequences, indicating viral integration. When tested by immunohistochemistry, all six tumours were LMP-negative but three of three tumours tested were EBNA-2-positive.

These results were corroborated by additional case reports of EBV-positive smooth muscle tumours in AIDS patients (van Hoeven *et al.*, 1993; Prévot *et al.*, 1994) and transplant recipients (Timmons *et al.*, 1995; Kingma *et al.*, 1996; Morel *et al.*, 1996), although an EBV-negative case was described in one transplant recipient (van Gelder *et al.*, 1996).

2.6.4 *Other tumours*

Conway *et al.* (1996) reported the absence of the virus in a series of mesotheliomas. When PCR and EBER in-situ hybridization were used, no evidence of EBV was found in testicular germ-cell tumours (Rajpert-de Meyts *et al.*, 1994).