# Progress in Pathology

VOLUME 7

Edited by

Nigel Kirkham and
Neil A. Shepherd

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## Progress in Pathology

Volume 7

*Progress in Pathology* reviews many aspects of pathology, describing issues of everyday diagnostic relevance and the mechanisms underlying some of these processes. Each volume in the series reviews a wide range of topics and recent advances in pathology of relevance to daily practice, keeping consultants, trainees, laboratory staff and researchers abreast of developments as well as providing candidates for the MRCPath and other examinations with answers to some of the questions they will encounter.

Highly illustrated in full colour, topics covered in this volume include:

Immunohistochemistry as a diagnostic aid in gynaecological pathology, Drug induced liver injury, Childhood lymphoma, Immune responses to tumors, Post-mortem imaging, Understanding the Human Tissue Act 2004 and much more.

Volume 7 of Progress in Pathology will be an essential addition to the shelves and laboratory benches of every practising pathologist.

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# Progress in Pathology

## Volume 7

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# Preface

In the years that have passed since the publication of the last volume of *Progress in Pathology* have seen many developments and changes in the practice of Pathology. The basis of our work in the diagnosis and understanding of disease processes remains much the same as ever but, as our society becomes more developed and more demanding of its healthcare workers, the challenges that we face grow beyond the basic task of diagnosis, to considerations of the place of the specialty of Pathology within that complex animal, the multidisciplinary team in the hospital, as well as more widely in society itself.

Some of these developments are reflected in the range of chapters to be found within these covers. The selection in this volume extends from basic science and issues in neuropathology and lymphoma, through the effects of drugs in causing, as well as treating, disease in the liver, to consideration of why small children come to die suddenly, and what may be found in the hearts of some adults who also come to autopsy after unexpected deaths. We also include contributions on the developing role of the pathologist as part of the multidisciplinary team and on the legislation that has been enacted in England as a reaction to some of the negative publicity that has impinged upon the specialty in recent years.

This review of the state of Pathology will, we hope, give you, our readers, an opportunity to develop your knowledge as well as to reflect upon some of the ways in which our work is changing, whether preparing for professional examinations or as part of continuing professional development. Once again we cannot disagree with the old adage that you cannot 'stand in the way of Progress'. We trust that you will find much of interest here.

The observant amongst you will have noticed that this, the 7th volume of *Progress*, sees us with, apparently, new publishers. In fact our new publishers, Cambridge University Press, have bought out our former publishers Greenwich Medical Media. We look forward to working with Cambridge for many editions to come.

N.K. and N.A.S
Newcastle upon Tyne and Gloucester
January 2007

# 1

# The microbiological investigation of sudden unexpected death in infancy

*James A. Morris and Linda M. Harrison*

## INTRODUCTION

Sudden unexpected death in infancy (SUDI) is simply defined as the death of an infant that is sudden and is unexpected. If a detailed post-mortem examination fails to reveal an adequate explanation for death then the term 'sudden infant death syndrome' (SIDS) is used. If the autopsy does not reveal an explanation for death, but there are suspicious features, the term 'unascertained' is often applied. The difference between SIDS, which legal authorities will regard as natural disease, and unascertained is, therefore, related to the level of suspicion. The latter term can cause distress to parents and lead to unnecessary inquests. It should be used sparingly. In strict logic, of course, the difference between 'I don't know' (SIDS) and 'I don't know' (unascertained) is unascertained and unascertainable.

The age distribution of SUDI and SIDS is the most consistent and characteristic feature of sudden infant death. The risk of SUDI and SIDS is low in the first few days of life, the risk then rises to a peak at two to three months, followed by a rapid fall so that the condition is uncommon after six months and rare after twelve months (Fig. 1.1). This risk profile is approximately reciprocal to infant serum IgG levels, and therefore sudden death occurs when infants have least protection against common bacteria and common bacterial toxins. For this reason it is important that careful microbiological investigation is carried out in all sudden infant deaths.

There are published protocols for the microbiological investigation of these cases, with which we broadly concur [1], [2]. Certain aspects of the investigation, however, are more important than others and the time when specimens are obtained is crucial. In this chapter we intend to concentrate on aspects of the investigation, giving background information as to why the tests are needed and placing emphasis on practical procedures as well as discussing the difficult task of interpreting the results obtained.



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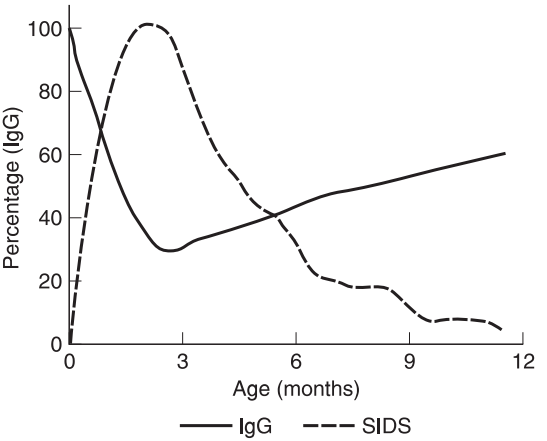


**Fig. 1.1** Serum IgG levels in infancy expressed as a percentage of the adult level. The age distribution of SIDS is for cases in England and Wales between 1993 and 1995 (1311 cases).

## CEREBROSPINAL FLUID

The diagnosis of meningitis in life depends on the examination of cerebrospinal fluid (CSF). In a suspected case of meningitis, CSF is obtained using an aseptic technique to avoid contamination by bacteria from the skin surface. The specimen is examined promptly; a white cell count and differential cell count are performed; protein, glucose and, in some laboratories, lactate levels are determined. The specimen is then examined for bacteria using Gram stain and bacterial culture. Part of the specimen is sent to a specialised laboratory for viral culture and some saved in case other specialised techniques are required, such as nucleic acid amplification or immunoassay to identify specific organisms.

We suggest that exactly the same approach should be used in cases of SUDI. The problem is, however, to obtain the specimen sufficiently soon after death for the investigation to be useful.

### WHITE CELL COUNT

Normal CSF contains no more than four mononuclear cells per cubic mm and no polymorphs [3]. Inflammation of the meninges (meningitis) leads to protein and white cell exudation so that the white cell count rises. The rise is predominantly lymphocytic in viral infection, while polymorphs predominate in bacterial infection. A major problem of interpretation of post-mortem CSF, however, is that the mononuclear cell count rises after death in the absence of inflammation. Thus Platt *et al*. [4] found CSF counts ranged between 37 and 3250 cells per cubic mm (mean = 647 per cubic mm) in 26 cases of SIDS, in which there was no evidence of meningitis. The post-mortem intervals were stated as 2 to 28 hours. In adult autopsies the CSF pleocytosis was less marked, with a mean of 28 white cells per cubic mm (mean post-mortem interval 15 hours). In SIDS cases the typeable cells were mononuclear and consisted of 60 to 70% lymphocytes and

**Table 1.1** Normal CSF protein ranges in infancy

| Term | |
|---|---|
| neonate | 0.2–1.7 g/l |
| 1–30 days | 0.2–1.5 g/l |
| 30–90 days | 0.2–1 g/l |
| 3–6 months | 0.15–0.3 g/l |
| 6 months–10 years | 0.15–0.3 g/l |

20 to 40% macrophages. The authors did not record the presence of polymorphs. When the post-mortem interval exceeded 12 hours the cells became vacuolated and could not be identified. The CSF cell count rises with the duration of the post-mortem interval, but the rate of rise is decreased if the body is stored at 4 °C as opposed to 20 °C [3].

The mononuclear cells in the CSF are apparently derived from the lining cells of the arachnoid membranes. In the absence of inflammation polymorphs should not cross the blood–brain barrier and therefore, if polymorphs are seen, they are, in theory, an absolute indicator of inflammation. This seems to be borne out in practice as neither of the above publications [3], [4] record the presence of polymorphs in the absence of meningitis. In order to gain maximum information, therefore, a differential cell count should be performed on post-mortem CSF samples, but they would have to be obtained within a few hours of death for this to be useful. The distinction between polymorphs and mononuclear cells in samples showing degenerative changes can be difficult, and modern staining techniques, using a range of specific antisera, on cytocentrifuge preparations should be deployed (a useful research project for someone).

## PROTEIN

Fishman [3] gives normal ranges for CSF protein in infancy (Table 1.1)

The blood–brain barrier is maintained in health so that the protein concentration in plasma is much higher than in CSF (i.e. 200-fold difference in adults). Meningeal inflammation, however, leads to increased permeability of meningeal vessels, and proteins leak into the CSF. The blood–brain barrier was first described in autopsy studies conducted in the nineteenth century [5]. Ehrlich and his students injected aniline dyes intravenously, the dyes became attached to albumin in blood. At autopsy it was found that the tissues were stained blue but not the CSF, which remained clear, nor the brain. Thus with rapid death and a short post-mortem interval there is no leakage of protein into the CSF in experimental animals.

There are relatively few studies of CSF protein changes in humans following death and no large systematic studies in infants [6]–[9]. The study by Mangin *et al*. [7] demonstrates a good correlation between the clinical history and the CSF protein concentration when the samples were obtained within 24 hours of death. If death was rapid, such as in cases of homicide, the CSF protein was within the normal range. If the process of dying was prolonged and associated

with inflammation and cytokine release, as in patients in intensive care, the CSF protein was raised. In the study conducted by Osuna *et al*. [9], in comparison, all the CSF protein values were raised and bore little relation to the premorbid condition. In this study, however, the samples were obtained after a mean interval of 48 hours. Thus it appears that the CSF protein estimation can be useful but only if obtained soon after death. This is another area in which more research is required.

## CSF GLUCOSE AND LACTATE LEVELS

Polymorphs are anaerobic cells, when activated they metabolise glucose and cause a fall in pH and a rise in lactate levels. Thus bacterial infection is associated with a fall in CSF glucose and a rise in CSF lactate. The same changes, however, can follow death in the absence of infection and therefore these analyses are of limited value in post-mortem samples.

## PRACTICAL CONSIDERATIONS

The usual practice of taking a CSF sample 24, 48 or even 72 hours after death results in loss of information. In most cases of sudden death the infants are taken urgently to A&E departments and a consultant paediatrician attends to supervise resuscitation and to decide when to desist. The consultant paediatrician should be empowered by the coroner to commence the investigation by obtaining samples for microbiology, including CSF. The sample should then be sent to the laboratory and treated urgently, as for a case of suspected meningitis in life. There is no point in getting the specimen early and then allowing it to deteriorate over several hours. The sample should be taken with full aseptic precautions to reduce the possibility of bacterial contamination. The result of the analysis will then be ready prior to the autopsy. The pathologist should also take a sample when the autopsy is undertaken, preferably by cisternal puncture (after careful cleaning of the skin with alcohol wipes) so as to obtain sufficient fluid in case further studies are required (see below). Paediatricians are skilled at taking clean samples but pathologists are better at getting large volumes of fluid.

## HISTOPATHOLOGICAL CORRELATION

There is a school of thought that meningitis can be diagnosed on the basis of histological examination alone, and that therefore counting white cells in the CSF and measuring the protein level is unnecessary. In my opinion this view is misguided for the following reasons:

1. Histological examination of the meninges is 100% specific for meningitis, i.e., by definition, those without meningitis will not show lymphocytic and polymorph infiltration of the meninges. Since no biological test is both 100% sensitive and 100% specific it follows that histological examination cannot identify every case of meningitis (sensitivity is the percentage of individuals with meningitis who show diagnostic histological features of meningitis).

2. In viewing a section of meninges on microscopy we would expect to see at least one polymorph per high power field in a case of meningitis. The mean diameter of a high power field is 0.5 mm and the section thickness is 0.005 mm. Thus one polymorph per high power field is equivalent to 800 polymorphs per cubic mm of tissue. Eighty polymorphs per cubic mm of CSF is more than enough for a diagnosis of meningitis, but the equivalent (one polymorph per 10 high power fields) is not enough. Thus, simple calculations indicate that in the early stages of meningitis examination of the CSF will allow a diagnosis to be made before diagnostic changes are seen in tissue sections. The CSF white cell count is a more sensitive indicator than histology.
3. Sonnabend *et al*. [10] conducted a very careful microbiological study of 70 cases of SUDI. They found evidence of overwhelming infection in eleven. They comment that 'the post-mortem cultures were of diagnostic value, providing the sole means of identifying the cause of death in 8 (11%) of the 70 infants, in whom the presence of an infection could be established only after repeated and extensive histological investigations'. Thus, initial histological investigations were negative but further sections revealed foci of inflammation. This principle applies generally, in the earliest stages of infection the changes may be focal and missed with standard samples.

Sadler [11] reviewed 95 infant deaths examined according to a detailed protocol in which the autopsy was conducted between 2.5 and 53 hours (mean 11 hours, median 5 hours) after death. The author records 'ten (16%) of the apparent cot deaths were explained on the sole basis of unexpected positive microbiological findings, mostly meningococcal or pneumococcal meningitis and/or septicaemia'. Thus, infection, particularly meningitis, can be missed if reliance is placed on histology alone.

## INTERPRETATION OF POSITIVE MICROBIOLOGICAL CULTURES

The interpretation of positive microbiological cultures from blood or CSF is fraught with problems[12]. In theory, positive cultures can be due to:

1. Bacteria invading the blood and tissues in life. This is usually associated with inflammation and in the absence of another explanation it is assumed that the bacteria cause or contribute to death.
2. Bacteria entering the blood in the agonal phase or during attempts at resuscitation.
3. Bacterial growth after death followed by tissue invasion. This is reduced but not eliminated by storage of the body at 4 °C.
4. Contamination of the samples, when they are obtained, by surface organisms. This can also be reduced, but not eliminated, by careful technique.

The following is a useful working rule:

A. A pure growth of a pathogen in the CSF in association with inflammation is regarded as the likely cause of death.
B. A pure growth of a pathogen without evidence of inflammation is regarded as a possible cause of death.

C. Other results, such as a mixed growth of organisms in the absence of inflammation, are more likely to be a consequence of mechanisms 2, 3 or 4.

If the specimen is obtained soon after death using a good technique, the chance of false positives due to mechanisms 3 and 4 is reduced.

## VIROLOGY

A specimen of CSF should be sent for virological studies. This also applies to blood, upper and lower respiratory secretions and bowel contents. The interpretation of the results is even more difficult than with bacteria. Infants will encounter a number of viruses in the first year of life, and therefore their presence does not necessarily imply significant disease. Isolation of a virus, together with evidence of inflammation at an appropriate site, should be regarded as a possible explanation for death. Isolation of a virus without other evidence of disease leaves the case unexplained.

## TOXICOLOGY

The idea that common bacterial toxins could have a role in the pathogenesis of SUDI and SIDS [13] has been strengthened by the discovery that over 50% of SIDS cases have detectable staphylococcal pyrogenic enterotoxins in brain and other tissues [14]. These toxins (toxic shock syndrome toxin (TSST)), and staphylococcal enterotoxins A, B and C) are only produced by staphylococci when the temperature is raised to between 37 and 40 °C. Thus, the presence of these toxins in tissues indicates production in life rather than after death. These results need to be confirmed, but there is a good case for measuring staphylococcal toxins in CSF in every case of SUDI and SIDS.

## RESEARCH POINTS

1. The concept that a post-mortem mononuclear cell pleocytosis in the CSF has no pathological significance is open to challenge. The CSF post-mortem cell counts in SIDS are much higher than in adults and there is a possibility that meningitis is being missed. Enumeration of cell sub-types using antisera against the wide range of cluster differentiation antigens (CD) now recognised should aid in distinguishing blood–borne mononuclear cells from the lining cells of the arachnoid membranes.
2. Proteomics is replacing genomics as the vanguard of biological science [15]. Proteins in fluids, such as CSF, can be separated by two-dimensional electrophoresis and then analysed by mass spectroscopy, and their amino acid sequence determined. This entire process can be automated, and in the future it will be possible to recognise foreign proteins, such as bacterial toxins, and products of inflammation. This is big science, rather than the small-scale science we are accustomed to in pathology, but it should be brought to bear on the problem of SUDI.

**Table 1.2** Causes of bacteraemia and meningitis in infancy

| Under 1 month old | 1–3 months old | Over 3 months old |
|---|---|---|
| Group B streptococcus | *Streptococcus pneumonia* | *Streptococcus pneumoniae* |
| *Enterobacteriaceae* | Group B streptococcus | *Haemophilus influenzae* |
| *Listeria monocytogenes* | *Neisseria meningitidis* | *Neisseria meningitidis* |
| *Streptococcus pneumonia* | *Salmonella* spp. | *Salmonella* spp. |
| *Haemophilus influenzae* | *Haemophilus influenzae* | |
| *Staphylococcus aureus* | *Listeria monocytogenes* | |
| *Neisseria meningitidis* | | |
| *Salmonella* spp. | | |

This table is adapted from Brook [16]

## BLOOD CULTURE

A blood culture should also be taken prior to the autopsy and as soon after death as possible. The specimen should be obtained by the consultant paediatrician who attends in A&E. The subsequent assessment of any bacterial growth must take into account the four possible routes of bacterial access noted in the previous section: invasion prior to death, invasion during the agonal phase or as part of resuscitation, post-mortem growth, or contamination. But if the specimen is obtained soon after death using a careful aseptic technique, the possibility of post-mortem growth and contamination is greatly reduced.

Table 1.2 lists the organisms that cause bacteraemia and meningitis in infants [16]. If one of these organisms is isolated in pure growth, then it is a likely explanation for death. If the culture produces a mixed growth of skin commensals then contamination should be suspected. A mixed growth of organisms from the gut or respiratory tract points towards an agonal ingress of bacteria. None of these rules, however, is absolute.

Correlation of the microbiological findings with histology can aid interpretation. If there is evidence of inflammation on serosal surfaces or in the liver or lung, then this increases the likelihood of genuine infection. The absence of inflammation, however, as in meningitis, does not exclude infection.

Table 1.3 shows the results of positive blood cultures obtained in Lancaster, UK from all age groups for one year ($n = 371$). Positive cultures judged to be a result of contamination are excluded. *Escherichia coli* and *Staphylococcus aureus* together account for 40% of serious infections in all age groups except infants aged 1 month to 12 months. This is in spite of the fact that colonisation of the upper airways by *S. aureus* is maximal in the first few months of life [17] and infant serum lgG levels reach their lowest levels at two to three months of age. Brook states [16] 'Most young children who develop bacteraemia are immunologically intact. The process is initiated by nasopharyngeal colonisation and followed by bacterial invasion of the blood and rare systemic dissemination. Both colonisation and bacteraemia are often associated with a preceding viral respiratory tract infection'. It appears that *S. aureus* bacteraemia and meningitis is not observed at the very time one would expect it to be most common.

**Table 1.3** Clinically significant blood culture isolates in one year from Royal Lancaster Infirmary, UK ($n = 371$)

| Organism | Percentage |
|---|---|
| *Escherichia coli* | 24.5 |
| *Staphylococcus aureus* | 15.9 |
| *Streptococcus pneumoniae* | 9.2 |
| *Klebsiella pneumoniae* | 5.7 |
| *Enterococcus faecalis* | 4.0 |
| *Proteus mirabilis* | 2.9 |
| Group B streptococci | 2.9 |
| *Pseudomonas aeruginosa* | 2.4 |
| Group A streptococci | 2.4 |
| *Serratia marcescens* | 1.8 |
| *Enterococcus faecium* | 1.8 |
| *Candida albicans* | 1.8 |
| Other organisms | 24.7 |

The absence of significant *S. aureus* infection in infancy is also at variance with the finding of staphylococcal toxins in the brain of over 50% of SIDS cases [14]. Is it that staphylococcal toxaemia kills before bacteraemia occurs? Is it that termination of bacteraemia by polymorphs leads to toxin release and sudden death? Or is it that the microbiological investigation of SUDI is too cursory, and disseminated staphylococcal infection is missed or ignored?

A specimen of blood should also be obtained at autopsy for possible virological, toxicological or genetic analyses. It is best to clean the skin with alcohol wipes, then cut down to a vein with a new scalpel blade and obtain blood by venepuncture. The specimen of blood should be separated and stored.

## SPLENIC CULTURE

A specimen of spleen can be obtained at autopsy for bacterial culture, and this is a useful adjunct to blood culture. The specimen should be obtained immediately the abdomen is opened. One approach is to push a swab into the spleen. A second is to cut off a small piece using a clean knife and send it for microbiology. The piece is subsequently placed in boiling water to sterilise the surface, and then splenic tissue from the centre is used for culture.

## RESEARCH POINT

*E. coli* and *S. aureus* are common causes of bacteraemia except in infants between one and twelve months of age. Either these organisms do not cause bacteraemia in this age group, or they do, but the infection progresses rapidly to death and is missed. The observation that staphylococcal enterotoxins and *E. coli* endotoxin can interact synergistically to cause death in experimental models [18] is pertinent to answering these questions. Nucleic acid amplification techniques

(polymerase chain reaction) need to be used on blood and CSF specimens from cases of SUDI to see if the bacteria have been present, even if they cannot be grown. If blood can be obtained soon after death, then endotoxin measurements may also be useful [19].

## UPPER RESPIRATORY TRACT

The nasopharyngeal bacterial flora of SIDS infants is different from that in healthy live infants matched for age, gender and season [20]. A study conducted in the 1980s, when most infants slept prone, revealed increased carriage of staphylococci, streptococci and Gram negative bacilli in infants at autopsy [20]. Although the possibility of Post-mortem change cannot be discounted as an explanation for the difference, a similar pattern is found in the early morning in infants suffering from a viral upper respiratory infection who have slept prone in the night [17]. Enterobacteriaceae, such as *E. coli* and *Klebsiella* spp., are rarely found in healthy infants, occur in less than 3% of those with clinical upper respiratory tract infections (URTI) who sleep supine, but are commonly found in the early morning in those with URTI who sleep prone, and were found in up to 45% of SIDS cases in the 1980s [17], [20].

*S. aureus* is found in approximately 50% of normal healthy infants in the first three months of life. The carriage rate thereafter gradually falls to 30% by 6 months of age. *Streptococcus pneumoniae*, by comparison, occurs in 5% in the first month of life and then gradually rises to 30% by 6 months. *Haemophilus influenzae* is rarely found in the first month but then rises to 10% at 6 months. Enterobacteriaceae remain under 3% throughout the first six months of life [17]. Streptococci are commonly found but group A haemolytic streptococci are rare. Other pathogens, such as meningococci and *Bordetella pertussis* are also rare.

In SUDI a pernasal swab should be taken as soon after death as possible, once again this is best obtained in A&E rather than waiting till the autopsy is undertaken. A full analysis of the nasopharyngeal flora requires examination using a wide range of culture media. In practice, most laboratories will concentrate on the identification of possible pathogens, but this should include *S. aureus* and at least partial identification of the enterobacteriaceae.

If *S. aureus* is isolated, the organism should be sent for genetic analysis to see if it produces any of the pyrogenic enterotoxins such as toxic shock syndrome toxin (TSST) and the staphylococcal enterotoxins A, B, C or D. These toxins are superantigens, their production is switched on when the temperature rises above 37 °C. They cause a polyclonal proliferation of T-lymphocytes, leading to an outpouring of cytokines, and this in turn can cause profound shock as in the toxic shock syndrome. These toxins have been found in the brain tissue of over 50% of SIDS cases, as noted above [14] and, therefore, are leading contenders for a causative role in SIDS.

In my opinion, if a toxigenic *S. aureus* is isolated, then the CSF and blood should be analysed for the respective toxin using an antibody probe. This test is not routinely available but can be performed. A positive result would point strongly to a mechanism of death based on toxic shock.

A pernasal swab, passed through the nose to touch the posterior nasopharyngeal wall, is the best method of sampling the flora of the upper respiratory tract. A throat swab is less useful overall but may give a higher isolation rate for pathogenic streptococci. Thus, either a pernasal swab alone or a pernasal swab plus a throat swab should be obtained, but not a throat swab alone.

## LOWER RESPIRATORY TRACT

Microbiological investigation of the lower respiratory tract depends on samples obtained at autopsy. If the autopsy cannot be undertaken immediately the body should be stored at 4 °C. The samples should be obtained as soon as the chest is opened and before the abdomen is opened. A clean scalpel blade should be used to obtain a sample of lung, and swabs should be passed down the trachea and into the bronchi as soon as the chest contents are removed. The specimens obtained can be used for both bacteriology and virology. If, during the subsequent dissection of the lungs, a focal area of consolidation is noted, a further sample should be obtained for bacteriology, but the chance of contamination will inevitably be increased.

The results of bacterial culture must be interpreted with care. In life the upper airways have a resident bacterial flora but the lower airways do not, and therefore the presence of bacteria in the lower respiratory tract is of significance. The problem, however, is that the lower airways may be contaminated from the upper airways during attempts at resuscitation. This is where careful analysis of the flora of the upper airways can help. If the mixture of organisms in the upper and lower airways is the same, then contamination is the more likely explanation. But if there is a pure growth of a pathogen in the lower respiratory tract and a mixed growth in the upper respiratory tract, a genuine infection is more likely. Furthermore, if the same organism is found elsewhere, e.g. in the blood or CSF, then infection is clearly the most likely explanation.

Correlation of bacteriology and histology is an important, but a vexed, problem. If there is histological evidence of pneumonia and a pathogen is isolated, then the diagnosis is clear. The difficulty arises when the bacteriological findings point to infection and histology is negative. The process of inflammation is defined by the histological appearance and histology is 100% specific; therefore, it cannot be 100% sensitive or we would be denying the essential uncertainty and variability that underlies biology and pathology. Thus, there will be false negatives and cases of genuine infection will be missed if we regard histology as the gold standard. This is particularly important in SUDI, as recent evidence suggests that infants can go from being apparently well to death in under 20 minutes [21]. This does not give time for the classical signs of inflammation to arise.

## THE GASTROINTESTINAL TRACT

The microbial flora of the gastrointestinal tract are much more complex than those of the respiratory tract. In fact, there are close to ten times more bacterial

cells in the gut than there are human cells in the body. The number of different species far exceeds those found in the respiratory system, and many, perhaps most, cannot be grown using conventional techniques. It is, therefore, not possible to analyse the gastrointestinal flora in the way that the respiratory tract flora can be assessed.

If bacteria and bacterial toxins have a role in the pathogenesis of SUDI then the numerical superiority of the gut flora makes it a prime candidate for the site of origin of the infection. The seasonality of SUDI, however, with an increased risk in the winter months, points more to the respiratory tract. Furthermore, epidemiological evidence indicates that breast feeding is not protective, which would be a surprising finding if the gut flora were important in pathogenesis.

Thus, there are theoretical as well as practical reasons for concentrating on the respiratory tract rather than the gastrointestinal tract in attempting to elucidate the cause of SUDI. There is nothing to gain from any attempt to analyse the gut flora in detail, but it is possible to look for specific pathogens such as salmonella, shigella and campylobacter organisms. In addition tests can be done for verotoxin-producing *E. coli* and toxigenic clostridia [10]. This is particularly useful if any of these organisms are isolated from the blood or CSF, or if any lesions of the bowel are found at autopsy.

## VIROLOGY

In the CESDI SUDI study [2], viruses were isolated from 4.5% of nasopharyngeal aspirates (5 of 112 cases), 4.2% of lung tissue (11 of 264 cases) and 18% of gastrointestinal samples (bowel contents) (43 of 237 cases). There were six cases in which virology confirmed the main cause of death, and one in which the virology confirmed a probable contributory cause. This is from a total of 450 autopsies. The cases included myocarditis (Coxsackie B2 in nasopharyngeal aspirate and gut), transverse myelitis (Coxsackie B1 in gut contents) and neonatal HSV infection (HSV1 in gut).

Polio viruses was isolated in 22 cases, rotavirus in 5, adenovirus in 4, echovirus in 4, coxsackie virus in 3 and enterovirus not specified in 2. These isolates were not associated with significant clinical illness or pathology.

The message is that virological studies are of only limited value, and the results must be interpreted in conjunction with the clinical picture and the pathological findings.

## CONCLUSION

The microbiological investigation of SUDI requires specimens of CSF, blood, upper respiratory tract secretions, lower respiratory tract tissue and bowel contents for both bacteriological and virological studies.

A specimen of CSF should be obtained within a few hours of death and processed urgently, as for the investigation of meningitis in life. Examination should

include a white cell count, a differential cell count, a protein estimation and bacterial culture.

A blood culture should be obtained within a few hours of death. Techniques based on nucleic acid amplification (PCR) and antigen immuno-recognition should be developed and used to seek evidence of infection by *S. aureus* and *E. coli*.

A pernasal swab should also be obtained within a few hours of death and examined for pathogens including *S. aureus* and enterobacteriaceae. If *S. aureus* is isolated, the toxigenic profile should be determined and the toxins measured in CSF and blood.

Tissue and swabs from the lower respiratory tract should be obtained, and the results of culture carefully compared with histology and with the upper respiratory tract flora. Histology aids diagnosis but cannot be regarded as the gold standard for infection.

Examination of bowel contents should be undertaken but provides only limited information.

The results of virology need to be interpreted in the light of clinical and pathological findings.

The investigation of SUDI in the twenty-first century must be taken beyond the concept that morphological assessment of tissues two to three days after death is sufficient, and is a gold standard against which other investigations are to be measured. Specimens must be obtained soon after death, examined urgently and the full range of modern techniques employed. Evidence of bacteraemia should be sought by culture and by antigen and genomic analysis. Evidence of bacterial toxaemia should be sought using antibody probes, and in the future by the emerging techniques of proteomics. Morphological analysis would also benefit from the wider use of immunohistological methods to type inflammatory cells and distinguish normal from the earliest stages of inflammation.

SUDI poses an intellectual challenge to pathology. There are big scientific questions to address: the acquisition of protective immunity to the microbial flora, the role of bacteraemia and bacterial toxaemia in switching on or off physiological systems, the specification of those systems by genes, and the effect of deleterious genetic mutations on physiological function. The science of genomics and proteomics will provide answers to these questions, but only if pathologists learn to take the correct samples at the correct time.

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# 2

# An overview of childhood lymphomas

*Jyoti Gupta and Keith P. McCarthy*

## INTRODUCTION

Lymphomas encompass a diverse group of neoplasms that arise from the clonal proliferation of lymphoid precursor cells. They are triggered by a series of genetic events that may be related to genetic predispositions, viral and other environmental factors, or may simply be accidental genetic rearrangements.

Lymphomas are the third most common cancer in children in the UK, behind acute leukaemias and brain tumours. They account for approximately 11–13% of all childhood malignancies with an annual incidence of 13.2 per million children [1]–[4]. The age-specific incidence rates and clinical features of paediatric lymphomas in white children in the USA correspond to those in Europe [5]. In equatorial Africa, 50% of all childhood cancers are due to lymphomas, because of the high incidence of Burkitt's lymphoma.

Paediatric lymphomas can be classified into Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL). The main subtypes are defined in the REAL classification [6], but the more recent WHO classification updates and expands this [7]. NHLs represent 60–70% of all childhood lymphomas in developed countries [2], [8], affecting children mainly between 7 and 10 years of age. The male-to-female ratio recorded in a Dutch study was 2.5 [5]. There is, however, an increased incidence in children with inherited or acquired immunodeficiencies [9], [10]. HL represents the remaining 30–40% of childhood lymphomas. Their incidence increases steadily throughout life and studies show a male-to-female ratio of 2.7 [5].

Unlike adult NHLs, which are often low-to-intermediate grade, the paediatric NHLs are frequently high-grade lymphomas and are clinically aggressive. However, low-grade tumours of both B- and T-cells do occur rarely in children [11]. Childhood NHLs are also more likely to present as extranodal disease in comparison to adults, where they tend to present as peripheral lymphadenopathy. A leukaemic phase is also more common in children, particularly in lymphoblastic lymphoma.



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The subtypes of NHL in children are predominantly of three main types, Burkitt's and atypical Burkitt's lymphomas (together accounting for about 35–50% of cases), lymphoblastic lymphoma (about 30–40%) and large cell lymphoma (15–25%). Individually, anaplastic large cell lymphomas are reported as the most common NHLs in children, in some studies accounting for even 25% of paediatric lymphomas [12]. Other subtypes of NHL are rare in children [3], [13] and in particular follicular lymphoma is very rare.

These groups are distinguished from one another by their clinical presentation, histological appearance, immunophenotype and cytogenetic profile.

Burkitt's lymphoma is a B-cell neoplasm. The WHO recognises three subtypes, including endemic BL, non-endemic (sporadic) BL and immunodeficiency associated BL [7]. The Revised European–American Lymphoma classification originally provisionally included a lymphoma subtype – 'Burkitt-like Lymphoma' – that was essentially defined as a variant of diffuse large B-cell lymphoma with features resembling Burkitt's lymphoma [6]. The new WHO classification of haematolymphoid diseases has dropped the term 'Burkitt-like lymphoma', replacing it with 'atypical Burkitt's lymphoma'; this is defined as a variant of Burkitt's lymphoma [7]. The difference between Burkitt's and atypical Burkitt's is based on subtle morphologic differences, but they do not appear to have different aetiology or clinical behaviour, and their diagnostic reproducibility is open to serious question. For these reasons, it must be questioned whether atypical Burkitt's lymphoma is a real entity.

Lymphoblastic lymphomas are derived from precursor B- or T-cells and are commonly associated with a leukaemic manifestation. Histologically, they are indistinguishable from ALL (acute lymphoblastic leukaemia), since they derive from precursor B- or T-cells that are analogous to the blasts seen in ALL. Although ALL predominantly involves B-cells, 80% of the lymphomas are of T-cell origin and only 20% are of B-cell origin [14]. The number of blasts in the bone marrow and presence or absence of mediastinal or nodal disease is used to distinguish lymphoblastic lymphoma from ALL. If more than 25% of blasts are present in the bone marrow, this implies ALL.

Large cell lymphomas can be of B-cell, T-cell, or intermediate (null) origin. They include diffuse large B-cell lymphomas, peripheral T-cell lymphomas (subtype; large cell) and anaplastic large cell lymphoma (T- and null-cell type). In general, B-cell lineage LCLs have centroblastic or immunoblastic morphological features, lack CD30 expression, affect older patients and involve less-advanced disease. In contrast, T-cell lineage LCLs in children usually express CD30 and are often of the anaplastic LCL subtype.

Other subtypes of NHL have been described in children, but are rare. For instance, although follicular lymphomas are one of the most common lymphomas in adults, they are rarely found in children and they tend to present extranodally [15].

Hodgkin's lymphoma represents 30–40% of childhood lymphomas. As with adult HL, they can be divided into classic HL (nodular sclerosis, mixed cellularity, lymphocyte depletion, lymphocyte rich) and nodular lymphocyte predominant. The histologic subtype does not influence survival [16].

Prompt diagnosis and staging of paediatric lymphomas is important due to the rapid growth of lymphomas. In addition to pathological typing

and staging, various clinical investigations are required before commencing treatment. These include blood count, electrolytes, liver function tests, chest X-ray and possibly CT scan of the chest, abdominal and pelvic ultrasound (US) or CT scan, bone scan, bone marrow biopsy and occasionally spinal fluid analysis.

As with other malignancies, the two groups of genes involved in the pathogenesis of malignant transformation are proto-oncogenes and tumour suppressor genes.

In paediatric NHL, the mechanism by which oncogenes are activated usually involves genetic translocations that correlate with cell lineage. Recent data have suggested that the role of tumour suppression genes and its inactivation may be associated with the loss of the multitumour suppressor (MTS1) gene, in the pathogenesis of certain lymphoblastic lymphomas [2].

In paediatric Hodgkin's disease, there is now evidence to suggest that its pathogenesis may be due to deregulated cytokine production [2], [17].

## BURKITT'S LYMPHOMA

Burkitt's lymphoma (BL) was previously referred to as small, non-cleaved cell, Burkitt's lymphoma according to the Working Formulation and Kiel classification.

### CLINICAL FEATURES

BL was first recognised as the endemic form presenting as tumours in the jaws of children [18]. It occurs endemically in Africa and parts of New Guinea, but is seen sporadically elsewhere in the world. The sporadic form is the predominant type in USA and Europe. Whereas the endemic form affects children ranging in age from 3 to 15 years, the sporadic form affects older children and young adults [12]. The male:female ratio is approximately 2–3:1, such that in the American Burkitt Lymphoma Registry, the incidence of BL was 1.4 per million in white males compared to only 0.4 per million for white females during 1973–1981 [19].

BL is associated predominantly with extranodal disease, but occasionally can present as leukaemic, and is then synonymous with ALL L3 [6], [20].

The endemic form is strongly associated with the Epstein–Barr virus (EBV) and can be found in up to 95% cases, but EBV is seen in only 20% of sporadic cases and 30–40% of HIV-positive cases. It still remains unclear how EBV contributes to the pathogenesis of Burkitt's lymphoma. However, a high antibody titer to EBV antigen is associated with a better prognosis [21]. They often show a dramatic response to chemotherapy, but spontaneous remission rarely occurs [22]. BL can also be found less frequently in ovaries, retroperitoneum, bones, salivary glands, thyroid and breast.

The sporadic cases usually present as intra-abdominal tumours. The commonest presentation is as a right iliac fossa mass and not infrequently a right hemi-colectomy is performed, usually because of intussusception or bleeding; needless to say, such surgery is not curative. Such cases are assumed to arise from Peyers patches in the ileo-caecal region or possibly from mesenteric nodes.

Rarely, there may be other intra-abdominal presentations, possibly because of involvement of kidney, liver or spleen. Sporadic BL may also present as CNS disease or in Waldeyer's ring, or even in the jaw [23], [24]. In sporadic cases, Burkitt's lymphoma may occur in AIDS patients and other cases of immunosuppression. The clinical course is aggressive, but as with the endemic form, cures can be achieved with chemotherapy. However, occasionally relapses may occur in the CNS.

## PATHOLOGICAL FEATURES

Usually, the normal architecture, whether it be of intestinal wall or lymph node, is completely effaced by monomorphic population of cohesive, medium-sized lymphocytes admixed with macrophages containing apoptotic debris, which give a 'starry sky' appearance. Although the growth pattern is usually diffuse, occasionally a more follicular arrangement of cells is seen (due to follicular colonisation), and this rare pattern should be borne in mind should a nodular lymphoma present in childhood.

The neoplastic cells have large round–oval nuclei, often angulated, containing dense, clumped chromatin, which obscures the nucleoli (usually 2–5) and abundant basophilic cytoplasm that may contain characteristic cytoplasmic vacuoles; however, it is important to remember that these may also seen in other lymphoma subtypes, particularly when cells are degenerate. Characteristically, there is a high proliferation fraction of very nearly 100%; this is an extremely important diagnostic feature, since no other lymphoma has such a high proliferation rate (except possibly atypical Burkitt's lymphoma, see below). The neoplastic cells often appear to infiltrate around nerves, muscles and other structures, thereby compressing them rather than destroying them [25].

The morphological features of sporadic and endemic types of Burkitt's lymphoma are indistinguishable.

## DIFFERENTIAL DIAGNOSIS

This includes other types of high-grade lymphomas such as B- and T-lymphoblastic lymphoma and the small-cell variant of diffuse large B-cell lymphoma. Poorly fixed BL may well lose its characteristic cytological and morphological features (such as the 'starry sky' pattern, and the monotonous appearance). Under these circumstances, the high proliferation index may help to distinguish BL from diffuse large B-cell lymphoma (DLBCL) (which generally has a lower percentage of cells in cycle), although atypical BL/Burkitt-like lymphoma will remain a problem. Another useful diagnostic feature is the demonstration of cytoplasmic vacuoles. Although these are best demonstrated in cytological preparations, they may be seen at the edge of sections, where cells are tending to separate in areas of relatively good preservation.

Should BL grow in an apparent follicular or nodular pattern (due to follicular colonisation), there may be superficial resemblance to follicle centre cell lymphomas, and this may be strengthened by the positivity for CD10. High-power examination should reveal, at least in areas, the characteristic cytology of the cells.

True lymphoblastic lymphomas are Terminal deoxynucleotide Transferase (TdT) positive whereas BL is always negative. However TdT is a technically difficult antibody and care should be taken in interpreting a negative result. If the lymph node is well fixed, then the morphological characteristics should enable distinction from BL.

### IMMUNOHISTOCHEMISTRY

The immunophenotype of the BL cell is essentially that of a peripheral B cell, although CD10 is also often present, suggesting derivation from germinal centre cells. Neoplastic cells express monoclonal surface IgM, pan B-cell markers (CD19, CD20, CD22 and CD79a) and CD10. They are CD5 and CD23 negative. Ki67/MIB-1 is a useful marker in the differentiation of BL from other high-grade lymphoma subtypes due to the characteristically extremely high proliferation index (see above). As might be assumed, the tumour cells are TdT negative, allowing their distinction from true lymphoblastic lymphoma. The tumour cells may be positive for Epstein–Barr virus (EBV) associated antigens such as LMP-1 and may, similarly, be positive on in-situ hybridisation for EBERs.

### GENETICS

Almost all cases, from whatever the subtype, show a chromosomal translocation involving the C-MYC gene on chromosome 8, which appears to be the critical event in the pathogenesis of Burkitt's lymphoma [2]. The most common abnormality [t (8:14)(q24;q32)] results in the translocation of C-MYC into the heavy chain (IgH) gene locus. The IgH gene locus is actively transcribing in B-cells and the translocation means that C-MYC comes under the influence of the IgH gene promotor, with consequent constitutive expression of C-MYC protein. C-MYC protein is a powerful cell-proliferation protein.

Variant chromosome translocations include the t(2;8)(q11;q24) and t(8;22)(q24:q11), in which the C-MYC gene is translocated, in an exactly analogous manner to the t(8;14), in the actively transcribing immunoglobulin kappa light chain gene and the immunoglobulin lambda light chain gene.

As will be obvious from the foregoing discussion, BL invariably shows rearrangements of the IgH and IgL gene loci. It may also show promiscuous rearrangements of T-cell receptor genes, especially of the T-cell receptor gamma chain genes. Such rearrangements are usually only partial and rarely complete.

## ATYPICAL BURKITT'S LYMPHOMA/BURKITT-LIKE LYMPHOMA

Although both the REAL classification and new WHO classification consider that there is a subtype of high-grade lymphoma with features intermediate