# FACTORS ASSOCIATED WITH MORTALITY FROM MENINGOCOCCAL DISEASE IN MERSEYSIDE CHILDREN.

Thesis submitted in accordance with the requirements of
the University of Liverpool
for the degree of Doctor of Medicine
by Frederick Andrew Ian Riordan

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## DECLARATION

This thesis is the result of my own work. The material contained in the thesis has not been presented, nor is currently being presented, either wholly or in part for any other degree or other qualification.

The clinical work was carried out in Royal Liverpool Children's Hospital (Alder Hey) and in the Paediatric departments of Arrowe Park, Countess of Chester and Whiston Hospitals. The research work took place in the Departments of Child Health and Medical Microbiology, University of Liverpool.

Measurement of cytokine levels was done by Mr N Hood, Dept of Medical Microbiology; cortisol levels were measured by Dr M Diver, Dept of Clinical Chemistry; DNA sequencing was done by Dept of Genetics and Microbiology, University of Liverpool. Vitamin A levels were measured by Dr K Fletcher, Dept of Tropical Paediatrics, Liverpool School of Tropical Medicine and fibronectin levels were measured by Mr K Bestwick, Dept of Biochemistry, Royal Liverpool Children's Hospital (Myrtle Street).

Signed

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**ABSTRACT**

Meningococcal disease (MCD) primarily affects children and has a high incidence on Merseyside. The mortality from MCD has remained at around 10% for the past 30 years and new strategies to decrease mortality are thus required.

Two studies of MCD in Merseyside children have been performed. A retrospective study of cases admitted to the Royal Liverpool Children's Hospitals between 1977 and 1993, aimed to study factors associated with mortality and the possible impact of a group C meningococcal conjugate vaccine. A prospective study in four Merseyside hospitals, from September 1992 until April 1994, aimed to determine the features of early MCD that parents and doctors notice and to relate plasma interleukin-10 (IL-10), tumour necrosis factor-α (TNF-α), cortisol, fibronectin, vitamin A and nutritional status to outcome. Laboratory diagnosis using polymerase chain reaction techniques (PCR) was also explored.

The retrospective study included 449 cases, 50 children died (11%). The proportion of cases with septicaemia alone increased from 7% in 1977-85 to 36% in 1990-3 ($p<0.0005$). Mortality was highest in children with septicaemia alone (19%). Group C meningococci caused 78 cases, 11 of whom died. A conjugate group C vaccine administered between 2 and 4 months of age could have prevented 68 cases, including all fatal cases.

During the prospective study 126 children with MCD were seen, 13 died (10%). Parents were the first to notice a petechial rash in 86 children (69%), and rash was the commonest reason for calling a doctor (52%). Delays in treatment occurred when doctors did not recognise the rash, especially if it was maculopapular, and if a diagnosis of "meningitis" rather than MCD was made. Cortisol and fibronectin levels were significantly lower in those who died, whilst IL-10 levels were significantly higher. Survivors had higher IL-10 levels for a given level of TNF-α. Nutritional status and vitamin A levels were not associated with disease severity or death. The PCR techniques used were not sensitive enough to detect meningococci in clinical samples.

Life threatening MCD does not present as meningitis, but as septicaemia. The proportion of cases presenting as septicaemia is increasing. Parents notice and seek medical advice about the rash of septicaemia, but not the features of meningitis. Doctors may not recognise the maculopapular rash of MCD. Pre-admission penicillin is rarely given if meningitis is diagnosed. Low fibronectin and cortisol levels and an imbalance between IL-10 and TNF-α, may lead to the development of shock and death. Trials of IL-10, fibronectin and replacement steroids in fulminant MCD may be of value. A conjugate vaccine could prevent most cases of group C infection, and decrease the mortality from MCD by 22%.

**ABBREVIATIONS**

| | |
|---|---|
| ACTH | Adrenocorticotrophic hormone |
| APPT | Partial thromboplastin time |
| cfu/ml | colony forming units per millilitre |
| CI | 95% confidence interval |
| CRP | C-reactive protein |
| CSF | Cerebrospinal fluid |
| DIC | Disseminated Intravascular Coagulation |
| DNA | Deoxyribose nucleic acid |
| dNTPs | deoxyNucleotide Tri-phosphates |
| ELISA | Enzyme linked immunosorbant assay |
| FFP | Fresh frozen plasma |
| GMSPS | Glasgow meningococcal septicaemia prognostic score |
| GP | General practitioner |
| HPLC | High performance liquid chromatography |
| HRP | Horseradish peroxidase |
| IgM | Immunoglobulin M |
| IL | Interleukin |
| IL-1ra | Interleukin-1 receptor antagonist |
| MAC | Mid-arm circumference |
| MCD | Meningococcal disease |
| MM | Meningococcal meningitis |
| MM+MS | Meningococcal meningitis + septicaemia |
| MRDR | Modified relative dose response |
| MS | Meningococcal septicaemia |
| nt | Non type-able |
| P1 | Class 1 outer membrane protein |
| PBS | Phosphate buffer solution |
| PCR | Polymerase chain reaction |
| PICU | Paediatric intensive care unit |

| | |
|---|---|
| PT | Prothrombin time |
| RDR | Relative dose response |
| RLCHs | Royal Liverpool Children's Hospitals |
| RNA | Ribose nucleic acid |
| sTNFr | Soluble tumour necrosis factor receptor |
| TAE | Tri Acetate EDTA buffer |
| *Taq* | *Thermus aquaticus* |
| TMB | Tetramethylbenzidine |
| TNF-α | Tumour Necrosis Factor-α |
| TSF | Triceps skin fold thickness |
| WBC | White blood cell count |

# CHAPTER ONE. INTRODUCTION

# CHAPTER ONE. INTRODUCTION

Disease caused by *Neisseria meningitidis* is a worldwide problem (Peltola 1983). Epidemics of meningococcal disease (MCD) regularly occur in the "meningitis belt" of sub-Saharan Africa and high or increasing levels of endemic MCD have recently been reported in Cuba, Brazil and Norway. There has also been an increase in MCD in England and Wales in recent years (Jones & Kaczmarski 1991). Within England and Wales certain areas have a high incidence of disease, with Merseyside having among the highest (Abbott et al 1985). MCD predominantly affects children and has a high mortality, which has remained unchanged for the past 30 years despite more potent antibiotics and advances in intensive care (Abbott et al 1985). MCD is thus a major cause of mortality in children on Merseyside, and new strategies are needed to decrease the mortality from this infection. This thesis includes a number of different studies of children with MCD, all of which seek the ultimate goal of decreasing the mortality from MCD.

To combat MCD it is first necessary to understand the meningococcus and the spectrum of disease it causes.

## 1.1 THE MENINGOCOCCUS.

*Neisseria meningitidis* is a gram-negative diplococcus and resembles other gram negative bacteria by having an outer and an inner cell membrane on either side of a peptidoglycan layer (Figure 1.1).

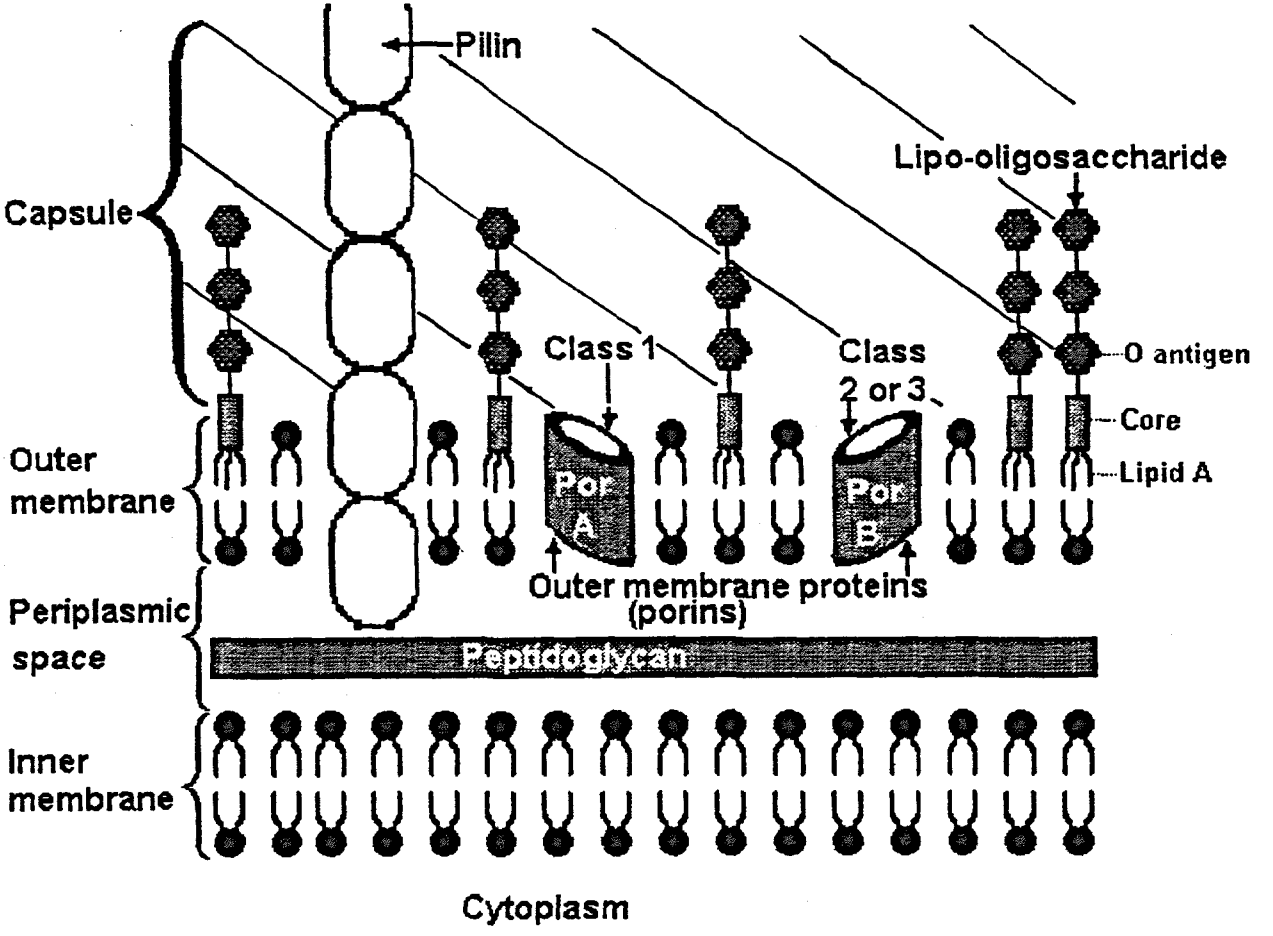
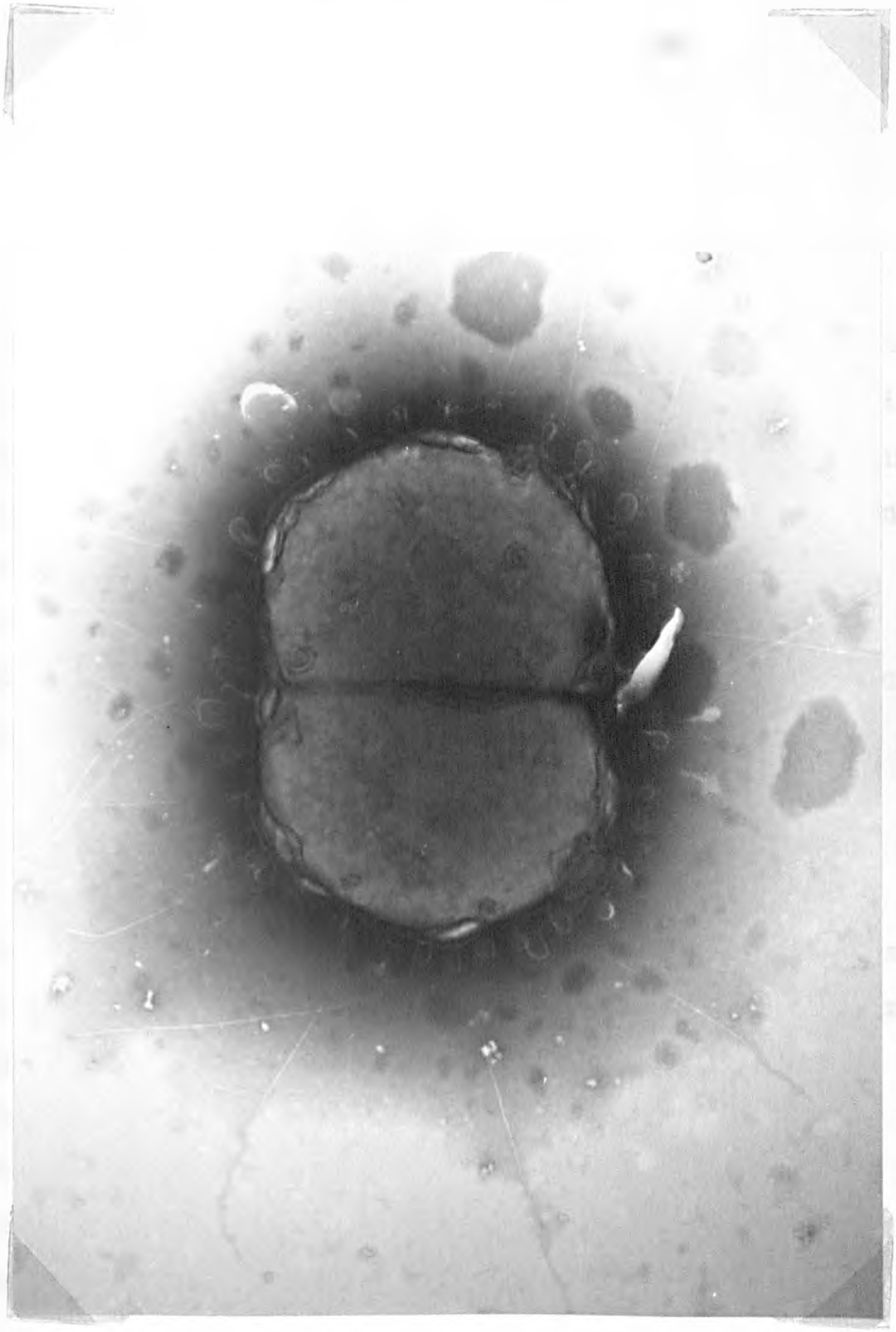



Figure 1.1 Diagram of the cell wall of the meningococcus (After Hart & Rogers 1993).

The outer cell membrane interacts closely with the human host. It is made up of a number of structures which are responsible for colonisation, invasion and pathogenicity, and which also allow typing of the organism (Reviewed by Hart & Rogers 1993; Poolman et al 1995). Approximately half of the outer leaflet of the outer cell membrane is made up of the lipo-oligosaccharide; endotoxin. The outer membrane continuously produces small outpouchings, or blebs, which therefore comprise large amounts of endotoxin (Figure 1.2). These are released as vesicles which then block antibody and release endotoxin. Outside of the outer membrane the meningococcus has a capsule made up of polysaccharide. The capsule helps the organism to evade detection within the body, and gives some protection against host defences. The major subdivisions of meningococci (ie the serogroups) are identified by their different polysaccharide capsules. Further typing and subtyping of meningococci is based on the outer membrane proteins. Typing is dependant on the Class 2 or Class 3 proteins, whilst subtyping depends on Class 1 proteins. These proteins act as porins and allow molecules into and out of the cell. Class 4 and Class 5 proteins, as well as other outer membrane proteins (eg transferrin binding protein) are also present, but are not used for typing or subtyping. The outer membrane of virulent meningococci also contain pili. These filamentous structures allow meningococci to adhere to the mucosal epithelium and to endothelium .

Figure 1.2 Electron micrograph of meningococcus showing "bleb" formation.



1.1.1 Serogroups.

On the basis of the capsular polysaccharide at least twelve serogroups have been described. However three serogroups are responsible for 90% of invasive MCD, these are serogroups A, B and C (Peltola 1983).

1.1.1.a Group A.

Group A meningococci are characteristically associated with epidemics. Group A predominates in the "meningitis belt" of sub-Saharan Africa (Lapeyssonie 1963), and has also caused recent epidemics in New Zealand (Lennon et al 1989) Nepal, Saudi Arabia and Chad (Moore et al 1989). Group A meningococci have caused few cases of MCD in the UK over recent years and affect young children much less frequently than the other serogroups (Abbott et al 1985).

1.1.1.b Group B.

The predominant serogroup seen in England and Wales since 1980 has been group B (Abbott et al 1985). This serogroup is associated with endemic disease and rarely causes explosive outbreaks. Certain serotypes of group B have recently caused prolonged, grumbling outbreaks in Norway (Bøvre et al 1977) and Gloucestershire (Cartwright et al 1986).

1.1.1.c Group C.

Group C meningococci can also cause outbreaks and clusters of disease (de Morais et al 1974), and cause between one quarter to one third of all cases of MCD in England and Wales (Jones & Kaczmarski 1991). The incidence of group C disease has been rising in Australia, Canada and the United States (Clements & Gilbert 1989; Whalen et al 1995; Jackson et al 1995), as well as in England and Wales (Jones & Kaczmarski 1991). Mortality from group C disease is higher than that due to groups A (Evans-Jones et al 1977) and B (Scholten et al 1994) meningococci.

1.1.2 Serotypes and serosubtypes.

Meningococci can be further subdivided into a number of different serotypes on the basis of their Class 2 and Class 3 outer membrane proteins (Frasch et al 1985). Group B disease is associated with only a small number of serotypes; mainly types 2, 4 and 15 (Jones & Kaczmarski 1991). Type 15 is the most important as it has some unusual characteristics. B15 strains have been responsible for the prolonged outbreak of MCD seen in Norway over the past 15 years (Bøvre et al 1977). They have also caused local outbreaks in Gloucester and Plymouth as well as in Liverpool (McGuinness et al 1991). The age distribution of disease due to type 15 suggests that this is a new serotype introduced into a community. All ages have equal susceptibility, the attack rate being highest in teenagers rather than in infants (Cartwright et al 1986).

The serosubtypes defined by the Class 1 outer membrane proteins (P1.) are also numerous, but again only a few are found in invasive disease. These include subtypes 4, 10, 15 and 16, of which P1.16 is the subtype found in the recent B15 outbreaks (McGuinness et al 1991). Patients with P1.16 disease have neck stiffness less often than those infected with other subtypes (Palmer et al 1992). Classification of meningococci is thus dependant on their capsular type and outer membrane proteins. Such classification is useful for epidemiological purposes and also because the strain can influence the virulence of the organism, the age group affected and the clinical presentation of disease.

## 1.2 EPIDEMIOLOGY OF MENINGOCOCCAL DISEASE

The incidence of MCD varies with age, location and season.

### 1.2.1 Age Distribution.

In industrialised countries, during non-epidemic periods, MCD is commonest in pre-school children (Peltola 1983). In England and Wales half the cases are under 4 years of age, with one quarter occurring during the first year of life (Jones & Kaczmarski 1991). The case fatality ratio is also highest in infants under 1 year of age (Abbott et al 1985). Immunity to meningococci is conferred by bactericidal antibodies, susceptibility to the disease being inversely proportional to the level of antibody (Goldshneider et al 1969).

At birth neonates have high levels of anti-meningococcal antibodies, acquired transplacentally. Maternal antibody levels fall during the first 3 months of life and the peak attack rate is at 6 months (Jones & Mallard 1993). Antibodies are stimulated by carriage of the meningococcus in the nasopharynx, but only 2% of children aged 1 to 4 years carry meningococci. Thus children under 4 are most susceptible to MCD.

However different strains affect different ages. Only 20% of group A infections occur in children under 4 (Abbott et al 1985). Group C disease is less common in infants than group B, and has been reported in significantly older children than group B (Baker & Griffis 1983). As already stated B15 affects older children as frequently as those under 4 years. Also the age of children with MCD shifts upwards at the start of an epidemic (Peltola et al 1982). The ages of children with MCD thus depend on the prevailing serogroups and serotypes, and on whether the disease is endemic or epidemic.

### 1.2.2 Geographical distribution.

MCD is known on all continents, but the classical endemic area is the "meningitis belt" of Africa (Peltola 1983). In this sub-saharan area the estimated annual incidence is 70 cases per 100,000 persons (Peltola 1983). There is no other comparable endemic area, but large epidemics of MCD have occurred in practically all areas of the world.

In the UK notification of meningococcal meningitis was begun in 1912, and provides national data (although under notification by up to 50% occurs (Harvey et al 1989)). During the first half of the 20th Century, 5-10 yearly cycles of MCD occurred. Large epidemics occurred during the two World Wars in both civilians and military personnel (Abbott et al 1985). After World War II the disease became sporadic with minor outbreaks. Since 1985 however, there has been a steady rise in the incidence of the disease (Jones & Kaczmarski 1991).

The first report of MCD on Merseyside was in 1847 by Whittle (1847), just 40 years after the first description of MCD (Vieusseaux 1805). Merseyside consistently has one of the highest incidences of MCD (Abbott et al 1985; Jones & Kaczmarski 1991; Jones & Kaczmarski 1992). The reasons for this are not clear, but may depend on a number of factors: the age distribution of the population, the social conditions and the predominant serogroups and serotypes (Abbott et al 1985).

### 1.2.3 Seasonal variation.

Seasonal variations in MCD is seen most markedly in the "meningitis belt" of Africa. Epidemics start at the end of the hot, dry season and end abruptly with the onset of the rains (Lapeyssonie 1963). Conversely the highest incidence of MCD in England and Wales is in the first 3 months of the year (Abbott et al 1985). This may reflect more overcrowding, close personal contact and intercurrent infections during the winter months.

Season does not appear to affect the number of meningococcal carriers, but it may affect the carrier to case ratio (Greenwood et al 1984). The case fatality ratio may also be affected by season, with less deaths occurring during the summer months (Halstensen et al 1987).

The likelihood of certain groups developing MCD can thus be influenced by their age, the season, the geographical location and the prevailing strain of meningococcus. Mortality may also be affected by these factors, although host characteristics are more strongly associated with death than strain characteristics (Scholten et al 1994). However, in most people the meningococcus colonises the nasopharynx and is eliminated after a few weeks or months with no ill effects. In a small number of people an invasive infection develops which can kill them in a number of hours. The meningococcus thus produces a wide spectrum of disease, and it is this which has the greatest influence on mortality.

## 1.3 SPECTRUM OF DISEASE.

### 1.3.1 Nasopharyngeal colonisation.

Man is the only natural host for the meningococcus and thus the human nasopharynx acts as a reservoir for MCD. The organism is passed on by droplet spread when coughing, sneezing, kissing etc. Airborne organisms are able to adhere to the nasopharyngeal epithelium by means of pili and establish colonisation (DeVoe 1982).

The host begins developing antibodies against meningococci within 7-10 days of colonisation. Invasive disease, if it occurs, therefore usually does so within ten days of acquiring meningococci (Edwards et al 1977).
Rates of carriage vary within populations. Carriage is low in young children and the elderly, and peaks at 15-20 years of age (Gold et al 1978). Carriage is also increased by smoking (Stuart et al 1989). However the rate of infection is not related to the carriage rate (Wenzel et al 1973), with a low carriage rate of invasive strains found even during outbreaks (Cartwright et al 1986). The development of MCD depends on both the host resistance and the virulence of the organism (Frasch & Mocca 1982).

### 1.3.2 Invasion of the nasopharyngeal epithelium.

MCD mostly affects previously healthy people. Those with deficiencies of complement C5-9 or properdin (Ross & Densen 1984) may be particularly susceptible to the disease, but these patients are in the minority.
A recent "flu-like illness" or respiratory infection may pre-dispose individuals to MCD (Moore et al 1990; Cartwright et al 1991; Hubert et al 1992). Invasive disease is also more likely in those exposed to passive smoking (Haneberg et al 1983; Stanwell-Smith et al 1994), non-secretors of ABO antigens (Blackwell et al 1989) and in those of lower socioeconomic status (de Wals 1984).

After mucosal adhesion encapsulated meningococci are transported through the epithelial cell (Stephens et al 1983). Once meningococci have penetrated through the mucosal barrier and gained access to the blood stream they may cause a variety of infections. The severity of the disease caused correlates with the number of bacteria per millilitre of blood (Reviewed by Brandtzaeg 1995). Rarely meningococci may remain within the circulation in low numbers and cause little systemic effect (occult and benign meningococcaemia). Mostly the low number of bacteria in the blood seed to parts of the body and cause local infection (meningitis, arthritis etc). High numbers of meningococci in the blood can cause systemic inflammation (septicaemia). The highest mortality is in those with septicaemia (Andersen 1978; Fallon et al 1984).

### 1.3.3 Occult and benign meningococcaemia.

Occult meningococcaemia, with no clinical signs other than fever, is uncommon (Alario et al 1989; Baltimore & Hamerschlag 1977). Occult meningococcaemia may just be the very early stages of MCD, since without treatment such children can develop severe disease and die (Dashesky et al 1983). Benign meningococcaemia is also relatively rare and is characterised by episodes of fever, rash and arthralgia which may recur until antibiotics are given (Olcén et al 1978). The child however remains in a good general condition throughout.

### 1.3.4 Localised infection.

The commonest local infection is meningococcal meningitis (MM). Meningococci gain entry to the CSF from the blood stream, possibly via the choroid plexus (Feigin et al 1992). The clinical presentation of MM is similar to other forms of bacterial meningitis, with fever, irritability, vomiting, headache and neck stiffness. Occasionally meningoencephalitis with a rapidly deteriorating conscious level, is present. Without antibiotic treatment this infection is invariable fatal, however mortality with antibiotic treatment is only 2-10% (Peltola 1983). Survivors may suffer sequelae such as sensorineural deafness, mental retardation or seizures (Dawson et al 1990; Fortnum 1992; Baraff et al 1993).
Meningococci may also cause pneumonia, arthritis, opthalmitis or pericarditis (Peltola 1983). These other focal infections are rare and mortality is low.

### 1.3.5 Septicaemia.

The sudden onset of meningococcal septicaemia (MS) and the rapid progression to death was well described by Herrick (1919); "No other infection so quickly slays." Septicaemia is the presentation with the highest mortality (Andersen 1978; Fallon et al 1984) and attempts to decrease mortality should focus on it. A rash occurs in over 70% of cases (Wong et al 1989). Initially the rash may be maculopapular (Marzouk et al 1991a), but the hallmark of MS is a petechial or purpuric rash, with the larger lesions being seen in those with the most severe disease (Toews & Bass 1974).

### 1.3.6 Meningitis + Septicaemia (MM+MS).

Despite the classification into either MM or MS (Niklasson et al 1971), there is a marked overlap between the two clinical presentations. Many children with "meningitis" have the petechial rash of MS or positive blood cultures. This group has clinical features of both meningitis and septicaemia and constitute the largest clinical group. Mortality in this group falls between that of meningitis alone and septicaemia alone (Thomson et al 1990).

Mortality from MCD is thus dependant on disease presentation and studies of mortality should take into account disease presentation and severity. The highest mortality in MS is in those in shock.

### 1.3.7 Septic Shock.

Sepsis is a systemic response to a possible infection (Sáez-Lorens & McCracken 1993). Altered organ perfusion may follow. At a cellular level inadequate perfusion leads to cell dysfunction (Zimmerman & Dietrich 1987). Clinically this is manifested by hypoxia, acidosis, oliguria or mental changes; the sepsis syndrome (Sáez-Lorens & McCracken 1993). If this persists, poor skin perfusion or hypotension may result producing septic shock.

Release of meningococcal endotoxin within the blood stream leads to endothelial damage, capillary leakage, intravascular thrombosis, abnormal vascular tone and myocardial dysfunction (Mercier et al 1988; Heyderman et al 1991)).

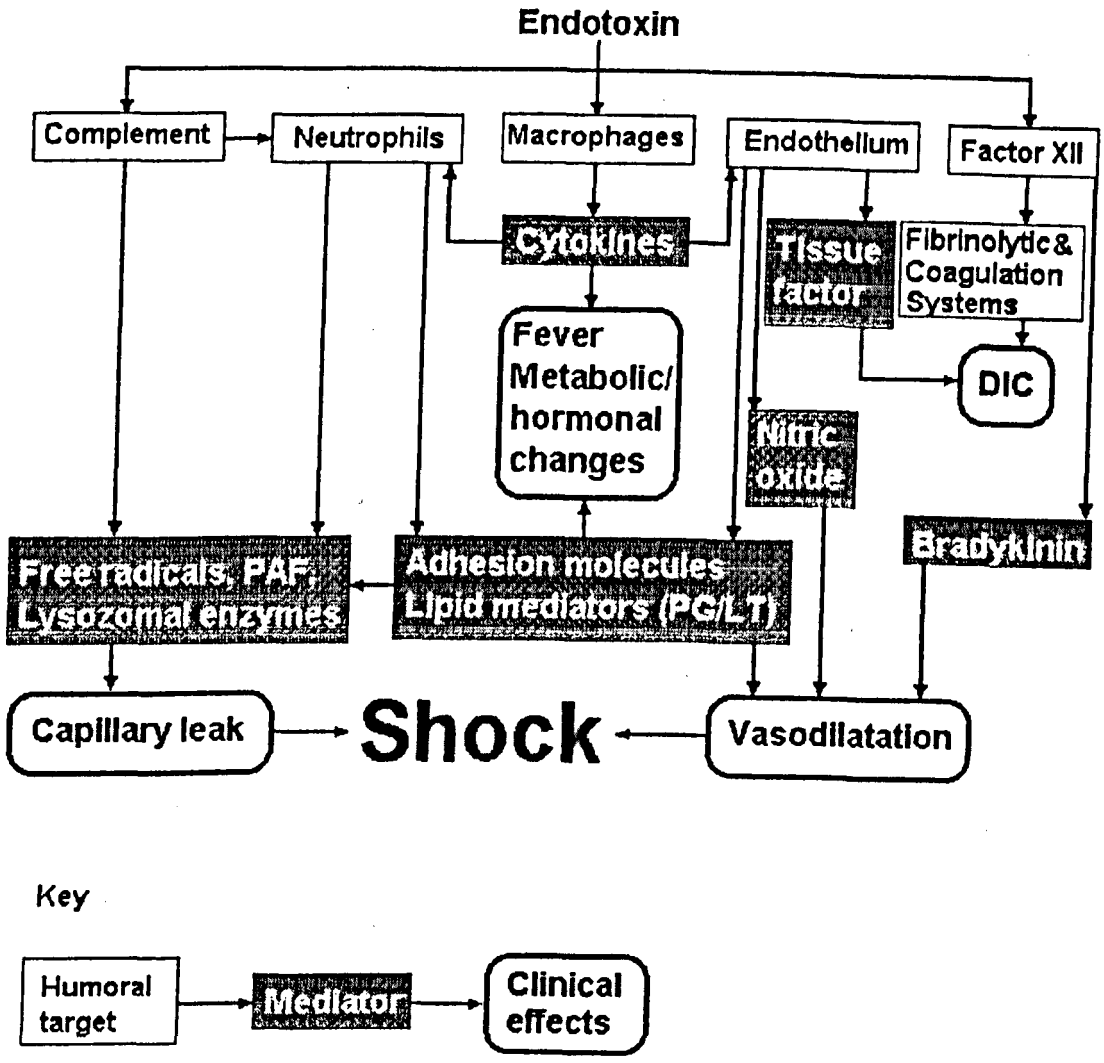



Figure 1.3 Pathogenesis of meningococcal septic shock. DIC, disseminated intravascular coagulation; PAF, platelet activating factor; PG/LT, prostaglandins and leukotrienes. (After Glauser et al 1991).

These processes lead to decreased systemic and cutaneous perfusion, focal thrombosis and tissue oedema. These can then cause septic shock and multi-organ failure (Heyderman et al 1993). These patients therefore present with the features of life threatening MCD; poor perfusion, hypotension and coagulopathy (Figure 1.3).
Understanding the pathological mechanisms that lead to septic shock, may suggest ways in which the mortality from MCD could be decreased.

## 1.4 PATHOPHYSIOLOGY OF MENINGOCOCCAL SEPTIC SHOCK.

The meningococcus releases endotoxin into the bloodstream via the "blebs" which it produces both continuously and when the bacterial cell wall is disrupted by antibiotic therapy (Andersen & Solberg 1980). Endotoxin triggers a cascade of events which ultimately produces the clinical features of meningococcal septic shock. High levels of meningococcal endotoxin are found in severe MCD and levels correlate strongly with mortality (Brandtzaeg et al 1989; Marzouk 1995). However it has recently become clear that host derived factors (cytokines) are responsible for the progression of septic shock (Tracey & Cerami 1993).

### 1.4.1 Cytokines.

Cytokines are low molecular weight glycoproteins. The systemically active cytokines, involved in septic shock, are produced by macrophages. The pro-inflammatory cytokines (Interleukin-1β, Tumour Necrosis Factor-α) promote inflammation. Their production is blocked by the anti-inflammatory cytokines (Interleukin-4, Interleukin-10, Interleukin-13, Transforming growth factor ß) (Dinarello 1991; de Waal Malefyt et al 1993).
Tumour necrosis factor-α (TNF-α), Interleukin-1β (IL-1β) and Interleukin-6 (IL-6) all have a role in MCD (Waage et al 1989b; Girardin et al 1988; Marzouk 1995).

### 1.4.2 Tumour Necrosis Factor-α

TNF-α is secreted in response to endotoxin, bacterial exotoxins or viruses (Beutler & Grau 1993), and reaches peak levels between 90-120 minutes after stimulation (Cannon et al 1990) Production of TNF-α can be down regulated by other cytokines (IL-4, IL-10) and by corticosteroids (Tracey & Cerami 1993).
Low levels of systemic TNF-α protect against death from infection (Havel 1989; Mestan et al 1986), but increasing levels are harmful to the host. High levels of systemic TNF-α are associated with high mortality rates in MCD (Waage et al 1987; Girardin et al 1988; Marzouk 1995).

### 1.4.2.a Tumour Necrosis Factor in septic shock

Most of the sequelae of septic shock are thought to be due to TNF-α. Evidence to support this is as follows; a. Macrophages stimulated by endotoxin produce TNF-α (Beutler et al 1985), b. TNF-α is detected in the serum of human volunteers given endotoxin (Michie et al 1988), c. the features of septic shock (coagulopathy, leucopenia, vascular leak and vasoconstriction/dilatation), can all be produced in animals by TNF-α (Tracey et al 1986), and d. passive immunisation against TNF-α (by giving anti-TNF antibodies) protects animals from lethal doses of endotoxin or bacteraemia (Tracey et al 1987a). TNF-α is the only cytokine that will trigger the spectrum of metabolic, haemodynamic and cytokine changes that are seen in septic shock. TNF-α has a prothrombotic, proinflammatory effect on endothelial cells and can also induce adrenal haemorrhage when injected into animals (Tracey et al 1986). Other cytokines (such as IL-1, IL-6, IL-8) amplify the effects of TNF-α, although production of these cytokines is also triggered by TNF-α.

### 1.4.2.b Tumour Necrosis Factor-α in Meningococcal disease.

Tumour Necrosis Factor-α has been detected in serum from both adults and children with MCD. Higher levels were found in those with severe disease (Waage et al 1987; Girardin et al 1988; Marzouk 1995). Cases of MM were less likely to have high serum TNF-α levels, although levels were raised in CSF (Waage et al 1989a). Serum levels of TNF-α correlated with a number of risk factors for death and with mortality.

Tumour Necrosis Factor-α levels were also negatively correlated with fibrinogen levels, supporting a relationship between TNF-α and DIC (Girardin et al 1988). High levels of TNF-α were thus of prognostic value, but were less predictive of outcome than clinical features (Girardin et al 1988; Marzouk 1995). Raised levels of systemic TNF-α may be responsible for many of the features of fulminant MCD, but other cytokines play a role.

### 1.4.3 Interleukin-1β

Interleukin-1β is mainly produced by macrophages and monocytes, but also by endothelial cells, stimulated by microbial products or TNF-α. IL-1β stimulates fever and corticosteroid production and both of these down regulate IL-1β production by negative feedback (Besedovsky et al 1986). An essential role for IL-1β in normal homeostasis has not yet been found, but IL-1β does stimulate the release of pituitary hormones, particularly ACTH. Increasing amounts of IL-1β produce hypotension, leucopenia and thrombocytopenia in animals (Okusaura et al 1988). These effects were all augmented by the addition of TNF-α (Okusaura et al 1988; Waage & Espevik 1988). TNF-α is the main stimulus for IL-1β production in sepsis (Fong et al 1989). However IL-1β can be produced when TNF-α is absent, if large enough doses of endotoxin are given.

1.4.3.a Interleukin-1β in septic shock

Like TNF-α, IL-1β can cause the features of septic shock; coagulopathy, vascular leak and hypotension. IL-1β suppresses cell surface anticoagulants and increases factors that lead to thrombosis. It also increases the adhesion of leucocytes to the endothelium (Bevilacqua et al 1989) and produces vasodilatation by inducing prostacyclin release and inhibiting vascular smooth muscle contraction.

1.4.3.b Interleukin-1β in Meningococcal disease.

In MCD IL-1β has only been detected in patients with high levels of TNF-α, and correlates with TNF-α levels (Girardin 1988; Waage 1989b). IL-1β levels were also significantly higher in patients who died. These studies fit with the theory that IL-1β production is stimulated by TNF-α and the two act synergistically to produce shock and death.

1.4.4 Interleukin-6

High levels of IL-6, in combination with TNF-α and IL-1β, are also associated with mortality in MCD.
IL-6 production is stimulated during infection both by endotoxin (Nordan & Potter 1986) and by other cytokines (Shalaby et al 1989; van Damme et al 1987). IL-6 appears later than IL-1β and TNF-α (Shalaby et al 1989).

Interleukin-6 produces a spectrum of effects that may be helpful in acute infection; stimulating antibody synthesis (Hirano et al 1985), ACTH release and acute phase response protein synthesis (Marinkovic et al 1989) by stimulating hepatocytes (Castell et al 1989). Animal studies have shown a lack of toxicity from IL-6 alone and its harmful effects in septic shock are probably due to amplification of the effects of TNF-α and IL-1β (Jablons et al 1989).

1.4.4.a Interleukin-6 in Meningococcal disease

High levels of IL-6 were found in the serum of patients with MCD (Waage et al 1989b; Marzouk 1995). Levels were a thousand times higher in those with septic shock compared to those with meningitis. Significantly higher levels were also found in those who died. Peak levels of IL-6 were found 1-4 hours after admission, but levels were detectable up to 36 hours later.

The pro-inflammatory cytokines thus play an important role in the pathogenesis of meningococcal septic shock. What role do the anti-inflammatory cytokines, such as IL-10 play?

1.4.5 Interleukin-10

Interleukin-10 is an important regulator of immune function and controls cytokine production. IL-10 is produced by T helper 2 cells, macrophages, activated B cells and keratinocytes (Moore et al 1993).

Interleukin-10 was produced some hours after TNF-α, IL-1β and IL-6 *in vitro*, becoming detectable 6 hours after endotoxin stimulation and reaching a peak level at 24-48 hours (de Waal Malefyt et al 1991b). It has now been shown that macrophages stimulated with endotoxin rapidly release IL-10 in a dose dependent manner, IL-10 being produced within 90 minutes of endotoxin stimulation (Marchant et al 1994b). This release is predominantly mediated by TNF-α (Wanidworanun & Strober 1993). IL-10's production can be inhibited by IL-4 or interferon gamma (de Waal Malefyt et al 1991a; Fiorentino et al 1991), as well as by negative feedback on itself.

IL-10 inhibits a number of macrophage functions responsible for the harmful effects of septic shock; the production of TNF-α, IL-1ß and IL-6 (de Waal Malefyt et al 1991b; Fiorentino et al 1991); the induction of procoagulant activity and tissue factor release (Pradier et al 1993; Ramani et al 1993) and the release of toxic oxygen radicals (Bogdan et al 1991). IL-10 also enhances the production of natural TNF and IL-1 antagonists (de Waal Malefyt et al 1993, Joyce et al 1994). Most interest focuses on IL-10's role as a cytokine synthesis inhibiting factor (Fiorentino et al 1989). IL-10 protects mice against endotoxin by decreasing TNF-α production, even when given up to 2 hours after endotoxin challenge (Howard et al 1993). The mechanism by which IL-10 inhibits macrophages is unknown.

IL-10's ability to suppress the production of, and increase the natural inhibitors to, the proinflammatory cytokines makes it of great interest in septic shock.

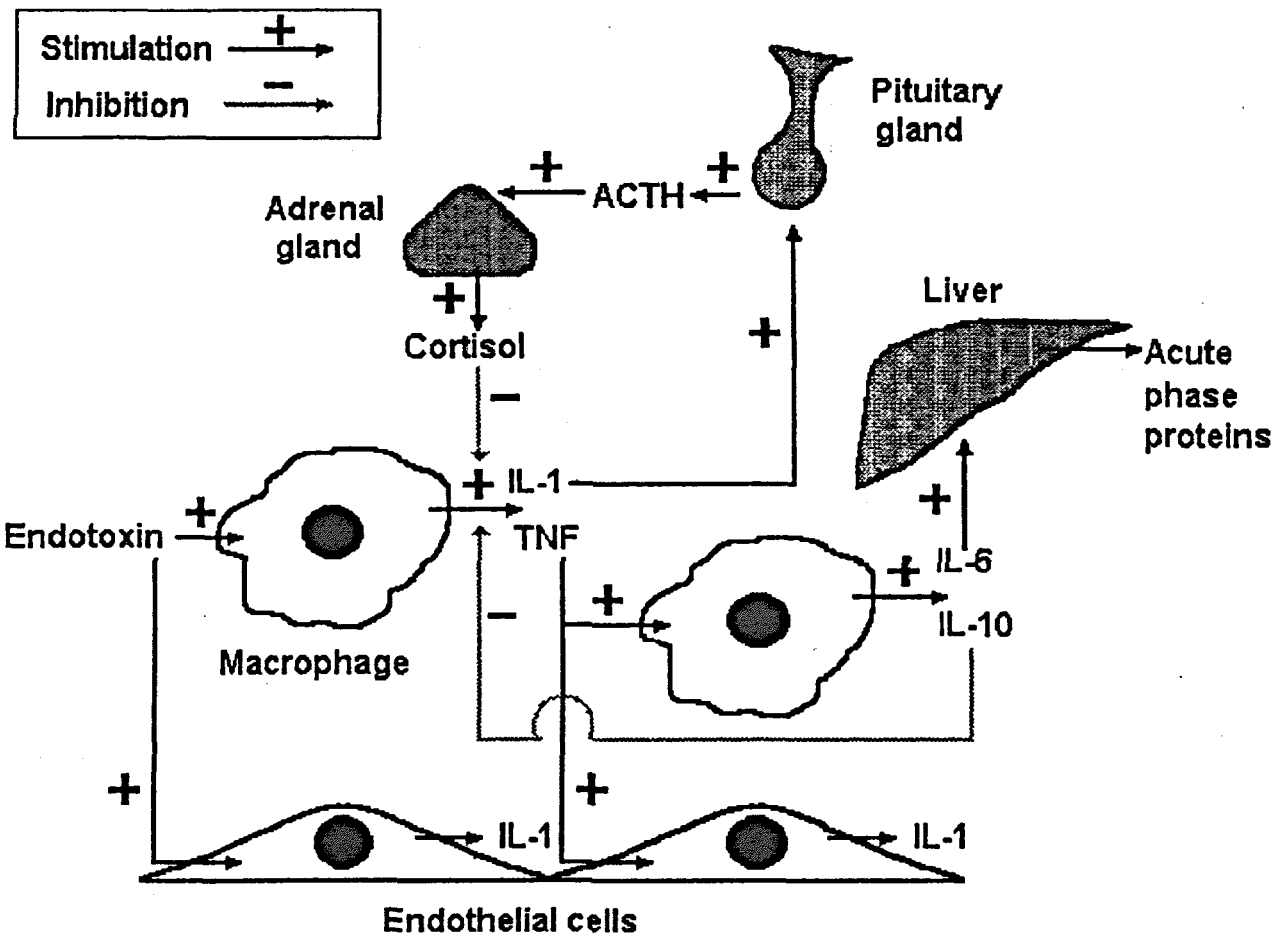



Figure 1.4 Interaction of cytokines and cortisol in Meningococcal disease.

High levels of IL-10 have been found in adults with septic shock (Marchant et al 1994a) which correlate with levels of the pro-inflammatory cytokines (Gómez-Jiménez et al 1995). High levels of IL-10 have also been found in children with meningococcal septic shock (Derx et al 1995). IL-10 levels were found to correlate with GMSPS in the 25 children studied, but were not significantly associated with mortality. These findings need confirming in a larger cohort with MCD, with a broader spectrum of disease. No studies correlating IL-10 levels with other cytokines have been performed in MCD.

### 1.4.6 Interaction of cytokines in Meningococcal disease.

The systemic release of TNF-α, IL-1β and IL-6 are all associated with meningococcal septic shock and death (Waage et al 1987; Waage et al 1989b; Girardin et al 1988). Although during in vitro experiments the effects of individual cytokines can be studied, a complex interplay of cytokines exists in MCD (Waage et al 1989b)(Figure 1.4).

TNF-α appears to be the first cytokine released and reaches its peak level around the time of hospital admission (Waage et al 1989b). TNF-α stimulates the production of IL-1β and the two have a synergistic effect. IL-1β is only found in those with high levels of TNF-α, the group most likely to die (Girardin et al 1988; Waage et al 1989b). IL-6 appears later reaching its peak 1-4 hours after admission. IL-6 has a longer half life than TNF-α (Waage et al 1989b). IL-6 may therefore reflect cytokine activation more accurately, since endotoxinaemia is often transient and TNF-α is cleared rapidly.

The systemic circulation and the subarachnoid space are functionally separate compartments with respect to the production and effects of these cytokines. High levels in CSF are not associated with death and do not cross into the bloodstream (Waage et al 1989a).

High systemic levels of all three cytokines are associated with a high mortality, and it is speculated that a they share a common stimulus in MCD. The most likely stimulus is endotoxin stimulating TNF-α and IL-1β, which then stimulate IL-6.

TNF-α, IL-1β and IL-6 are all produced following an injection of endotoxin (Cannon et al 1990), as are their natural inhibitors; IL-1 receptor antagonists, IL-1ra (Dinarello & Wolff 1993), and soluble TNF-α receptors, sTNFr (Spinas 1992). Raised levels of these inhibitors have been found in MCD (van Deuren et al 1994) and the balance between cytokines and their antagonists may influence the outcome of MCD (Girardin et el 1992). IL-10 levels are also raised in meningococcal septic shock (Derx et al 1995). The highest levels measured were at admission and fell rapidly within 24 hours, suggesting IL-10 peaked either at or before admission. Its role in the pattern of cytokinaemia in MCD remains to be elucidated.

This pattern of endotoxin and cytokine release is similar to animal models where an injection of endotoxin or bacteria produces bursts of TNF-α and then IL-6. However this pattern is very different from that found in the septic shock following trauma or burns, commonly seen in adults. Here the release of endotoxin from a focus of infection may be more sustained and subacute (Glauser et al 1991).

In adult septic shock levels of endotoxin are a thousand-fold lower, bioactive TNF-α is undetectable and the levels of cytokines fluctuate over a number of days (Waage & Ansgar 1992). Drawing conclusions about meningococcal septic shock from studies on post trauma septic shock in adults is thus unwise.

Since the pattern of cytokines in MCD is complex, attempts at improving the outcome by blocking just one cytokine may not be successful. What may be required is a way of blocking the action of a number of cytokines, possibly by a cocktail of antibodies and antagonists (Mercier 1993), or by cytokine inhibitors, such as IL-10.

## 1.5 METHODS FOR DECREASING MORTALITY.

With the increased understanding of the pathophysiology of septic shock, the leading cause of death in MCD, new forms of treatment may become available. However for these therapies to be effective they will need to be given early in the course of the disease. Decreasing the mortality from MCD will thus not only require new treatments, but also early presentation and appropriate treatment of cases, as well as early identification of those with life-threatening disease who might benefit from novel therapies.

## 1.5.1 Early detection of Meningococcal disease.

### 1.5.1.a Features of meningococcal disease noted by parents.

Early detection requires parents to be aware of the features of life threatening MCD. Parents of children who die from MCD may delay seeking medical advice because they do not recognise the severity of the illness (Slack 1982; De Wals et al 1984).

Many studies and standard texts report the symptoms and signs in children with MCD on admission to hospital (Wong et al 1989; Palmer et al 1992; O'Reilly 1992; Feigin & Snider 1992). Information based on these sources can be misleading (Thomson & Hayhurst 1993), since signs noted on admission may not have been present or not noticed by parents when the child was at home. Few studies record the signs noticed by parents before admission. In one study fever was the commonest feature noted by patient's relatives at the start of MCD (74%), but only vomiting was significantly more common in patients with MCD compared to controls (Tønjum et al 1983). Non-specific signs are often the first features of MCD (Steihm & Damrosch 1966), it is more helpful to know which features parents notice later in the disease and why they seek medical advice.

### 1.5.1.b Parent's reasons for seeking medical advice.

Information for parents about MCD advises them to look for signs of meningitis or septicaemia (Department of Health & National Meningitis Trust 1994).

Table 1.1 Reasons for seeking medical advice in meningococcal disease and childhood bacterial meningitis. Advice was sought for up to 3 features. Data shown as %.

| Study | Olcén et al 1979 | Tønjum et al 1983 | Valmari et al 1987 |
|---|---|---|---|
| No of patients | 69 | 115 | 110 |
| Age range (years) | 0.4-65 | 0.25-24 | 0.1-14 |
| Disease | MCD | MCD | BM |
| Fever | 96 | 44 | 60 |
| Vomiting | 61 | 8 | 31 |
| Impaired LOC | 57 | 16 | 22 |
| Headache | 51 | 16 | 6 |
| Rash | 49 | 23 | 0 |
| Neck stiffness | 23 | 2 | 3 |
| Irritability | 23 | 3 | 6 |
| Seizures | 1 | 1 | 6 |

Key

MCD=Meningococcal disease

BM=Bacterial meningitis (28/110 had meningococcal meningitis)

LOC=Level of consciousness

Signs of meningitis.

The parents of children admitted with bacterial meningitis commonly consult a doctor for non-specific symptoms such as fever or vomiting (Table 1.1). Parents either do not appreciate the significance of specific signs such as neck stiffness, or these signs are not present or not noticed before admission. Neck stiffness was noted by the patient or their relatives in only 27% of cases of MM (Olcén et al 1979). However only 3% of parents sought advice because of neck stiffness, although it was present in 67% of children with meningitis (Valmari et al 1987). Neck stiffness is a late sign of meningitis and is often absent in young children (Valmari et al 1987). Expecting parents to recognise neck stiffness and then seek medical advice because of it, thus seems unrealistic.

Signs of septicaemia.

A haemorrhagic rash is a common diagnostic feature of MCD (Raman 1988), and is often present in life-threatening disease (Toews & Bass 1974). Medical advice was sought because of a petechial rash in 23-49% of cases of MCD in Scandinavia (Table 1.1). However advice was sought more frequently for non-specific features such as fever and vomiting.

Parents sometimes delay seeking medical advice because they do not want to disturb a doctor (Slack 1982). If parents were aware of the significance of a petechial rash they might seek medical advice earlier in life-threatening MCD.

## 1.5.2 Early treatment of Meningococcal disease.

Early treatment requires doctors to recognise MCD in it's early stages. The difficulty for general practitioners (GPs) is to differentiate the two or three cases of MCD they may see in a lifetime (Strang & Pugh 1992), from the majority of febrile children with less serious illnesses.

### 1.5.2.a Difficulties in diagnosis.

General practitioners correctly diagnose MCD in 70-80% of the cases that they admit (Sørensen et al 1992a; Mathiassen et al 1989; Strang & Pugh 1992). However around 50% of cases are seen by a doctor, but not sent for admission during the early stages of their illness (Sørensen et al 1992a; Nadel et al 1994), implying that the diagnosis is often made at a late stage. The early symptoms of MCD are non-specific (Tønjum et al 1983), but parents often seek medical advice because of them (Olcén et al 1979; Tønjum et al 1983). However a GP is significantly more likely to make a diagnosis of meningitis or MCD if specific signs such as neck stiffness or petechiae are present (Sørensen et al 1992a). These signs are not always recognised however and a delay in diagnosis may contribute to a fatal outcome (Oakley & Stanton 1979).

Further diagnostic difficulties occur when MCD presents with a maculopapular rash (Baxter & Priestley 1988). This rash is seen in up to 38% of cases of MCD (Marzouk et al 1991a), and significantly decreases the chance of a GP making a diagnosis of MCD (Sørensen et al 1992a).

Young children pose an even greater diagnostic difficulty for GPs. A correct diagnosis is least likely in children under 2 years of age (Rømer 1977; Goldacre 1977; Mathiassen et al 1989).

To help GPs diagnose MCD it is necessary to know which features are present in those correctly diagnosed, and which features lead to misdiagnosis. Education about these specific features could then be targeted to GPs.

1.5.2.b Delays in hospital treatment.

The diagnosis of MCD, and thus appropriate treatment, is delayed following admission to hospital in 8-12% of cases (Borchsenius et al 1991; Olcén et al 1979) and in 15-20% of those who die (Oakley & Stanton 1979; Slack 1982). Immediate diagnosis and treatment on admission may thus help decrease mortality. Again it is important to identify those factors which lead to delayed diagnosis on admission. These factors could then be highlighted for Casualty officers and other hospital junior medical staff.

1.5.3 Early administration of antibiotics.

Mortality from MCD could be decreased if parenteral penicillin was given to suspected cases before admission to hospital (Slack 1982; Oakley & Stanton 1979).

1.5.3.a Pre-admission antibiotics in bacterial meningitis.

There is little evidence available to assess the effect of a short delay in antibiotic treatment on the mortality from bacterial meningitis (Talan et al 1988). Mortality from meningitis was significantly lower in patients taking antibiotics before admission (Goldacre 1977; Romer 1977). These patients probably received oral antibiotics, prescribed when the diagnosis of meningitis was not obvious. Pre-admission antibiotics are significantly more likely to be given to children with a longer history before admission. These children have a significantly better outcome than those with a shorter history (Kilpi et al 1991). It is unclear whether the better outcome is due to partial treatment with antibiotics or to the fact that the prognosis is better in children with an insidious onset, who are given antibiotics more frequently (Kilpi et al 1991). An analysis of studies of meningitis found that a short delay in antibiotic treatment appears to increase the risk of sequelae only in children with clinically overt meningitis. Delays in children presenting with a non-specific illness or fulminant meningitis do not appear to alter the risk of sequelae or death (Radetsky 1992).

1.5.3.b Pre-admission antibiotics in Meningococcal disease.

Studies of pre-admission treatment of MCD are also confounded by disease severity (Gedde-Dahl et al 1990b).

Effectiveness of early penicillin in Meningococcal disease contacts.

Parenteral antibiotics prevented occult meningococcaemia progressing to meningitis or septicaemia in 16 febrile contacts of cases of MCD (Wall et al 1986). From previous studies Gedde-Dahl et al(1990a) calculated that 43% of those with occult meningococcaemia might have developed MCD if untreated (Dashefski et al 1983; Shapiro 1986). Treating febrile MCD contacts may therefore decrease the risk of MCD and death. However as most cases of MCD in the UK are unrelated (Cooke et al 1989), such treatment is impractical in most cases.

Effectiveness of early penicillin in Meningococcal disease.

Despite this lack of proven effect the Chief Medical Officer advised all doctors in the UK to consider giving parenteral penicillin in cases of suspected MCD before transfer to hospital (Department of Health & Social Security 1988). This advice has been repeated a number of times (Welsby & Gollege 1990; Cartwright et al 1992a). Two recent studies showed a trend towards increased survival in those given penicillin before admission, although neither achieved statistical significance (Strang & Pugh 1992, Cartwright et al 1992b). A meta-analysis of these studies, combined with one reported in the subsequent correspondence (Gossain et al 1992), found six deaths in the 129 cases given penicillin (4.7%), compared with 41 deaths in the 358 not given penicillin (11.5%).

The odds ratio for increased survival with penicillin was 2.61 (95% confidence interval 1.04 to 7.18)(Cartwright et al 1992c). However a study from Denmark, reported at the same time, found a significantly higher mortality amongst those given early penicillin (6/25), compared with those not given penicillin (4/73). The odds ratio for increased death with penicillin was 5.4 (95% confidence interval 1.5 to 19.2)(Sørensen et al 1992b). The authors suggest that the high mortality amongst those given pre-admission treatment was because those with fulminant disease were most likely to receive penicillin. No data is presented to support this, but this highlights the confounding variable of disease severity once again.

Both Strang & Pugh (1992) and Cartwright et al (1992b) suggest that penicillin was more likely to be given to those with more severe disease (i.e. those with DIC or a purpuric rash), although neither study assessed disease severity on admission by a valid system. In contrast a recent UK study found that pre-admission antibiotics were given more often to those with meningitis without a rash (Research Committee of the BSSI 1995).

### 1.5.3.c Improving delivery of pre-admission antibiotics.

Despite the Chief Medical Officer's advice, pre-admission penicillin is given infrequently in MCD (Rouse 1992), although its use can be increased by regular encouragement (Cartwright et al 1992b; Strang & Pugh 1992).

All doctors received the CMOs letter about MCD (DHSS 1988), but some may still be unaware of it (Rouse 1992). Injectable penicillin was carried by 80-91% of GPs questioned (Ong & Dunbar 1988; Colbridge et al 1995).

Reasons for not carrying penicillin were that it was difficult to obtain, may deteriorate before it was used, or the expense (Rao & Selby 1992). Despite carrying injectable penicillin GPs may not use it because of the proximity of a hospital or concerns about reactions to penicillin or affecting culture results (Crowe 1994). A history of penicillin allergy is usually unfounded (Surtees et al 1991). Where there is doubt or proven hypersensitivity chloramphenicol could be used. Giving pre-admission antibiotics does significantly reduce the chances of positive blood or CSF cultures, but nasopharyngeal swabs are unaffected, and other methods may help to confirm the diagnosis (Strang and Pugh 1992; Cartwright et al 1992b). When faced with the choice between keeping the patient or the meningococcus alive, there should be no delay in treatment (Farmer 1993). Uncertainty about the diagnosis also discourages pre-admission penicillin (Rao & Selby 1992; Rouse 1992). Pre-admission penicillin is given least often to young children, the group with the highest mortality (Cartwright et al 1992b). The diagnosis of MCD is most difficult in this age group (Mathiassen et el 1989), and there may be reluctance or perceived difficulty in giving injections to young children (Rao & Selby 1992; Rouse 1992).

Is there any justification for not giving pre-admission penicillin?

1.5.3.d Increased endotoxin release.

A major concern about pre-admission penicillin is that it may cause the sudden lysis of many meningococci, resulting in a massive release of endotoxin leading to septic shock (Buxton Hopkin 1978). Increased levels of endotoxin after antibiotic administration have been found in animal experiments (Andersen & Solberg 1980), and one case report (Berkowitz et al 1983). However in a series of patients with MCD Brantzaeg and co-workers (1989) found that endotoxin levels decreased after the first dose of antibiotics in every case. Thus the theoretical concern about increasing endotoxin levels have not been born out in clinical practice.

Early penicillin treatment may help to decrease the mortality from MCD, to improve it's usage it is necessary to know the reasons why it is not given, and then to target education to these areas.

1.5.4 Severity assessment.

Once the child is admitted to hospital mortality may be decreased by appropriate early management. This requires an assessment of disease severity so that those with life-threatening disease can be given optimal management immediately.

1.5.4.a Severity scores.

A number of severity scores have been devised to help identify those with life threatening MCD. To be useful scores should be simple, made up from rapidly available data and easy to compute. Clinical scores (Bjark et al 1987; Stockland et al 1985) are likely to be the most rapidly applied. Most scores consist of a mixture of clinical and laboratory features. Those scores requiring laboratory data to be included in a lengthy equation (LeClerc et al 1985; Emparanaza et al 1988) may not be easy to calculate rapidly.

Scores have been devised in a number of different subgroups with MCD, some for children (Sinclair et al 1987), some for those in shock (LeClerc et al 1985) and some for those on intensive care (Khan & Blum 1978).

The first score, devised in 1966 (Steimh & Damrosch 1966), has been added to by others (Ansari et al 1979), but may no longer be reliable (Tesoro & Selbst 1991). The predictive value of a score may change as treatment within a hospital improves, and scores therefore need to be validated for the population in which they are to be used. However few scores have been well validated.

Two comparisons of a number of scores found those by Niklasson et al (1971) and Kahn and Blum (1978) to have the best sensitivity and specificity (Gedde-Dahl et al 1990c; LeClerc et al 1991). Both these scores require data on peripheral white cell count and platelet count, and the Niklasson score also needs data on CSF white cell count.

These laboratory results may take time and it is inappropriate to perform lumbar punctures on all children with MCD (Heyderman et al 1993). Neither score is thus suited to rapid assessment of disease severity. However neither of the comparative studies examined one of the best validated scores, the Glasgow Meningococcal Septicaemia Prognostic Score (GMSPS).

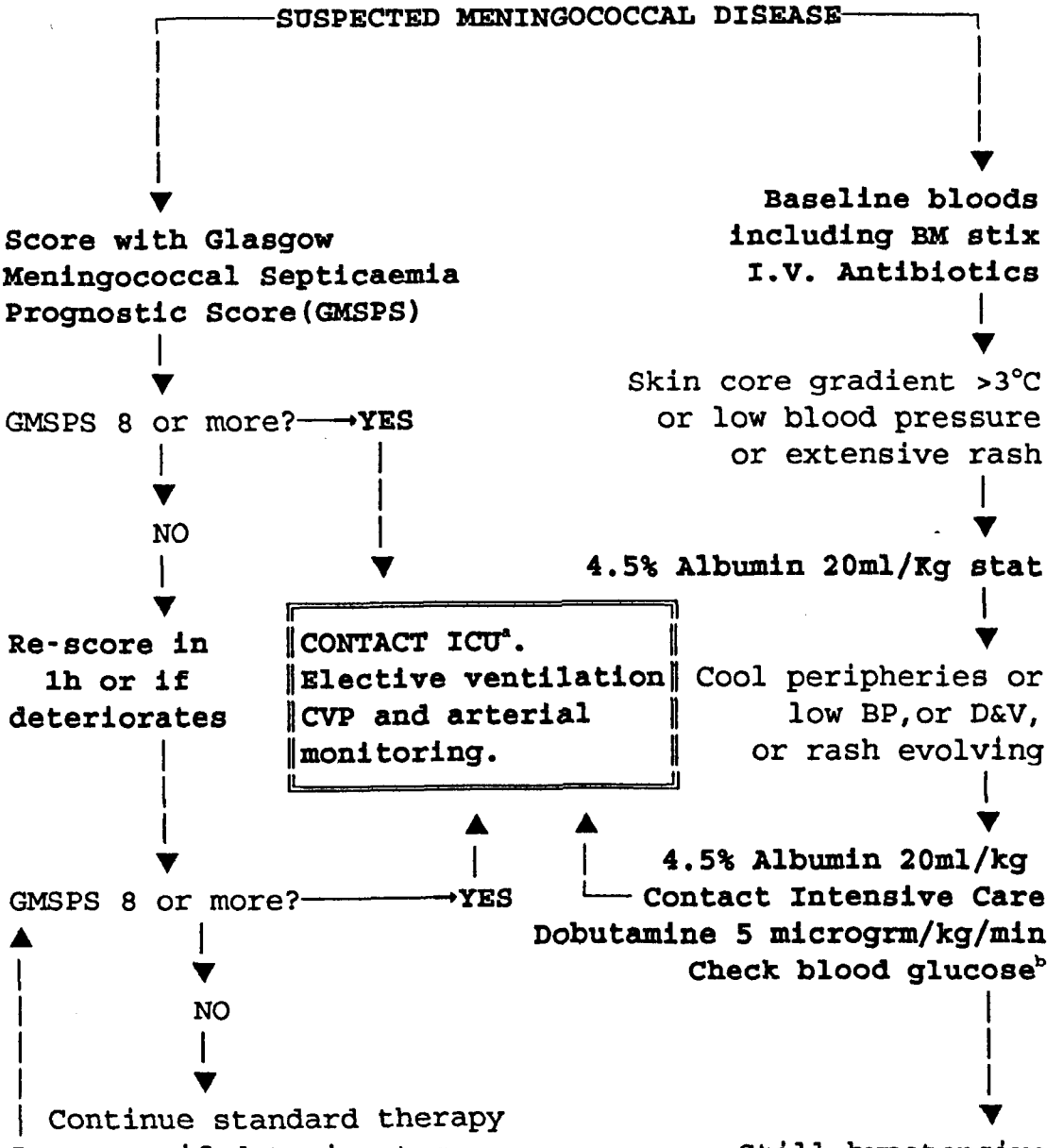
1.5.4.b The Glasgow Meningococcal Septicaemia Prognostic Score.

The GMSPS consists of six clinical variables and the base deficit (rapidly available on most ICUs)(Table 1.2). A score of 8 or more identifies those with a high mortality. As the score is mainly clinical it can be rapidly performed and easily repeated if there is clinical deterioration. It has been validated on Merseyside children both retrospectively (Thomson et al 1991a) and prospectively (Marzouk 1995). A prospective study found it to be as good if not better than other scoring systems in this population (Marzouk 1995). Used prospectively in Merseyside children a GMSPS of 8 or more predicts a 30% risk of death (Marzouk 1995). It is this group who require appropriate management on Intensive Care (Figure 1.5), and who may be offered novel treatments.

Table 1.2. Glasgow meningococcal septicaemia prognostic score (differing minimally from original desciption by Sinclair et al Lancet 1987;ii:38).

| | ARRIVAL | 1 HOUR |
|---|---|---|
| 1. SYSTOLIC BLOOD PRESSURE. | | |
| If < 75 mmHg age < 4 years | | |
| or < 85 mmHg age > 4 years | | |
| **Score 3 points.** | | |
| 2. SKIN/RECTAL TEMP DIFFERENCE. | | |
| If > 3 degrees Centigrade. | | |
| **Score 3 points.** | | |
| 3. MODIFIED COMA SCALE. | | |
| If initial score < 8, or | | |
| deterioration of 3 or more | | |
| points at any time. | | |
| **Score 3 points.** | | |
| 4. DETERIORATION IN LAST HOUR. | | |
| Ask parents or nurses; if yes | | |
| **Score 2 points.** | | |
| 5. ABSENCE OF NECK STIFFNESS. | | |
| **Score 2 points.** | | |
| 6. EXTENT OF PURPURA. | | |
| Widespread ecchymoses, or | | |
| extending lesions on review | | |
| **Score 1 point.** | | |
| 7. BASE DEFICIT. | | |
| If > -8 | | |
| **Score 1 point.** | | |
| **TOTAL** | | |

Figure 1.5 Immediate management of meningococcal disease
After Marzouk et al. Care Crit Ill 1991;7:186-7
(N.B.The two arms should run simultaneously)




Notes:

[a]Elective ventilation even in the presence of normal gases and adequate respiratory function is advisable.

[b]Hypoglyceamia is common.
Correct with 25% Dextrose-2ml/kg.

[c]Increase dobutamine to a dose of 20μg/kg/min(if central venous access obtained), while maintaining renal perfusion using dopamine up to a maximum of 5μg/kg/min. If still hypotensive despite adequate CVP, dopamine can be increased up to a maximum of 20μg/kg/min. If central venous access not available maintain blood pressure by infusing colloid.

## 1.5.5 Conventional treatment.

### 1.5.5.a Intensive Care

Familiarity with treating children with severe MCD leads to a decrease in mortality (Sinclair et al 1989). This may be due to early treatment with appropriate antibiotics (Kreger et al 1980), prompt cardiorespiratory resuscitation, with 40 ml/kg of fluid in the first hour (Carcillo et al 1991), elective ventilation (Ledingham & McArdle 1978; Rasmusen et al 1988), and improved training of ICU staff (Reynolds et al 1988); all of which have been shown to decrease mortality in septic shock. Other commonly advocated therapies such as corticosteroids and FFP may be beneficial in MCD, but have not been shown to be so in clinical trials.

### 1.5.5.b Corticosteroids.

One of the first reports of the successful use of corticosteroids in MCD came from the University of Liverpool. Grace et al (1940) described a case of meningococcal septicaemia which survived after treatment with extracts of adrenal cortex combined with antibiotics. The amount of corticosteroid in adrenal cortical extract was variable and this treatment lead to few other recoveries. Once cortisone, a pharmacological steroid preparation, became available there were a number of reports claiming almost "miraculous" recoveries from MCD associated with it's use (Nelson & Goldstein 1951; Newman 1951; Bauman et al 1953; Breen et al 1952; Buzzard et al 1953).

There was thus initially great enthusiasm for the use of corticosteroids in MCD. However larger series did not demonstrate any beneficial effects in children with MCD (Koch & Carson 1958; Margaretten & McAdams 1958) and no satisfactory trials were carried out. Disappointingly the mortality for severe MCD remained unchanged over the next decade and it was concluded that;

> "the present evidence does not justify the use of steroids in treatment or 'prophylaxis' of fulminant meningococcal infection" (May 1960).

The use of corticosteroids in fulminant MCD therefore declined. There are however theoretical reasons for their use.

The Waterhouse-Friderichsen syndrome and adrenal insufficiency.

The post mortem appearances of bilateral adrenal haemorrhage in a patient presenting with shock and a purpuric rash was described by Voelecker (1894-5;Quoted in Waterhouse 1911) a century ago. Reviews of the literature were published by Waterhouse (1911) and Friderichsen (1918) and the syndrome now bears their names.

Neither Waterhouse (1911) nor Friderichsen (1918) identified a causative organism in their cases, although others isolated *N meningitidis* (Andrewes 1906; Maclagan & Cooke 1916).

About 75% of children dying from MCD have macroscopic adrenal haemorrhage at autopsy (Leclerc et al 1988; Neveling & Kaschula 1993). Since not all those who die have adrenal haemorrhage (Ferguson & Chapman 1948) it was suggested that the term "Waterhouse-Friderichsen syndrome" be reserved for those fatal cases with both the clinical syndrome and post mortem adrenal haemorrhage. The term "fulminating septicaemia" should be used for those with MCD with shock and extensive purpura (Kinsman et al 1946). The contribution of adrenal haemorrhage to the development of shock and death in MCD remains controversial.

During acute illness there is a shift in adrenal metabolism away from androgen and mineralocorticoid synthesis and towards corticosteroid synthesis (Parker et al 1985; Ducker & McLaughlin 1986). Survival in infectious disease requires an intact adrenal cortex (Bertini et al 1988). Adrenal insufficiency may be clinically indistinguishable from septic shock (Dorin & Kearns 1988) and it was assumed that adrenal insufficiency was the cause of the rapid clinical deterioration in fulminant MCD (Nelson & Goldstein 1951). The presence or absence of adrenal haemorrhage in MCD, and its possible contribution to the clinical presentation was much debated (Kinsman et al 1946). However few studies sought to confirm adrenal insufficiency by measuring plasma cortisol levels.

The rate of production of cortisol is increased in bacterial infection (Bassøe et al 1965; Midgeon et al 1967; Cornil et al 1968). Plasma cortisol levels are high in shock due to infection (Melby & Spink 1958), and especially raised in dying patients (Sandberg et al 1956). The degree of increase in cortisol levels in patients with septic shock however is variable (Schein et al 1990). Some children dying from MCD had lower cortisol levels than might be expected (Gardner 1956). This possible adrenal insufficiency in fulminant MCD has been much debated.

Cortisol levels in Meningococcal disease.

Few cases of MCD with low cortisol levels, suggesting true adrenal insufficiency, have been reported (Bosworth 1979; McWhinney et al 1989; Enriquez et al 1990). There are also few studies of cortisol levels in groups of patients with MCD (Table 1.3). In one of the few studies published, elevated plasma cortisol levels were found in children with meningitis due to *H influenzae* or *S pneumoniae*, especially those who died (Midgeon et al 1967). Re-interpreting the data from the group with MCD shows significantly lower cortisol levels in those who died. Zachmann et al (1974) also found significantly lower cortisol levels in children dying from fulminant MCD although Lewis (1979) did not (Table 1.3). Midgeon et al (1967) found that all survivors of severe MCD had initial cortisol levels above 800nmol/l. All children with MM had elevated plasma cortisol levels, most markedly in the child who died.

Table 1.3 Admission cortisol levels in previous studies of children with meningococcal disease. Results shown as median(range).

| | | | Cortisol (nmol/l) | | |
|---|---|---|---|---|---|
| Study | Setting | Method | Survived | Died | P value* |
| Midgeon et al (1967) | USA | Isotope dilution | (n=18) 987(353-4828) | (n=8) 484(0-2911) | 0.022 |
| Zachmann et al (1974) | Switzerland | Fluorimetry | (n=18) 1757(913-3449) | (n=7) 730(235-1162) | 0.0007 |
| Lewis (1979) | Nigeria | Competitive protein binding | (n=17) 695(440-925) | (n=12) 648(425-1500) | 0.71 |

* P value by Mann-Whitney U test.

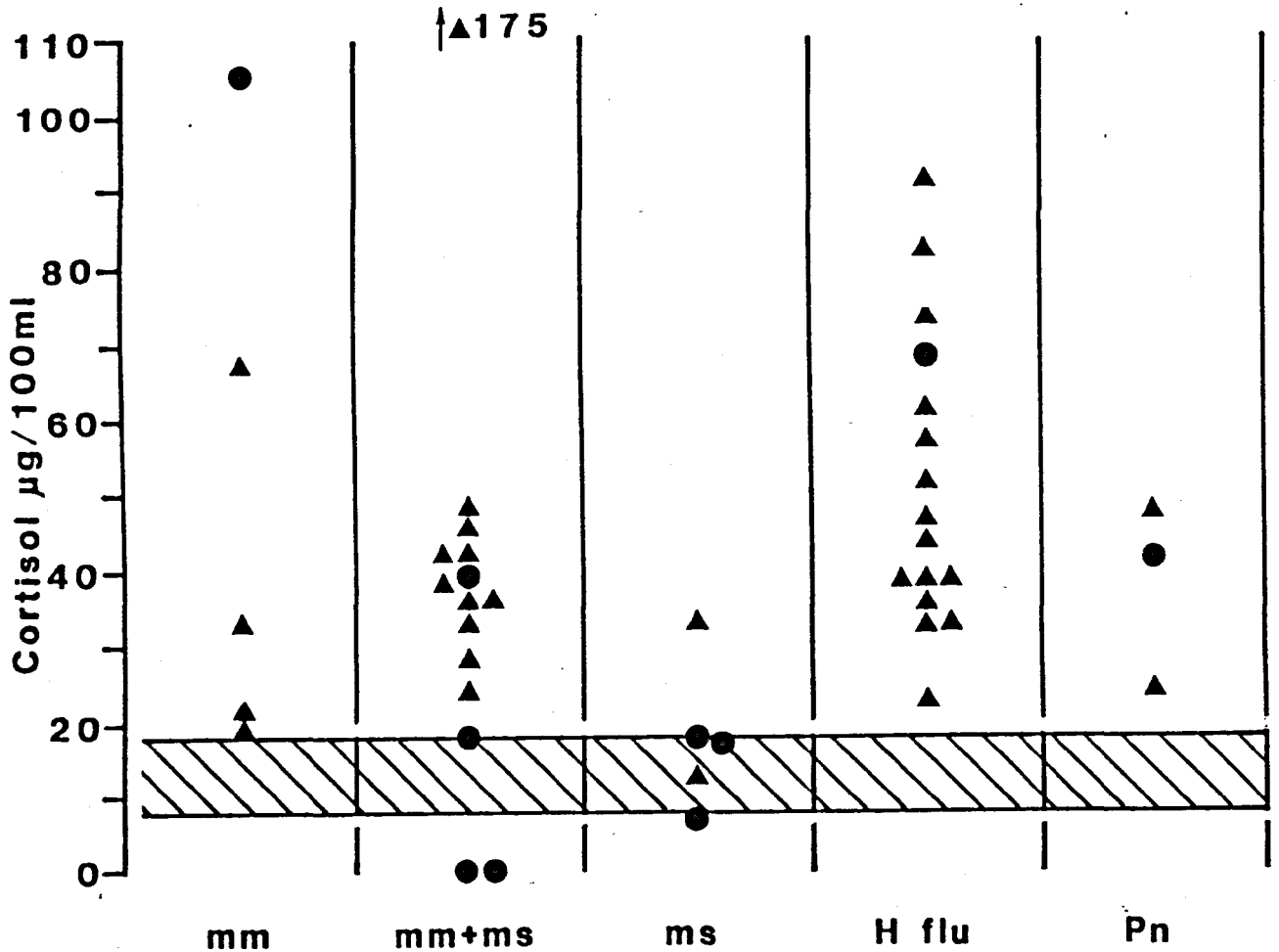



Figure 1.6 Admission cortisol levels in children with meningococcal disease and bacterial meningitis. After Midgeon et el (1967). Triangles represent survivors, circles represent deaths. H flu, *Haemophilus influenzae* meningitis; Pn, *Streptococcus pneumoniae* meningitis. Normal range is shown by the shaded area. To convert µg/100 ml to nmol/l multiply by 27.59.

However in the group with MS or MM+MS seven children had steroid levels within or below the normal range (Figure 1.6). One of these children had been treated for 26 hours prior to steroid levels being measured, and the lower level may represent a convalescent phase. The remaining 6 died and at post mortem were all found to have adrenal haemorrhage.

These small studies suggest that most children with MCD have raised cortisol levels. However a few children have true adrenal insufficiency and others may have inappropriately low cortisol levels for the severity of their illness; relative adrenal insufficiency. These findings need confirming. The mortality in those with relative adrenal insufficiency is high and autopsy confirms them as true cases of the Waterhouse-Friderichsen syndrome. Steroid treatment in this group may be beneficial (Bosworth 1979), but successful treatment would require early identification of those with low cortisol levels. Cortisol levels may not be urgently available, so other methods for identifying adrenal insufficiency in fulminant MCD are needed.

Cytokines and Cortisol.

The circulatory collapse in Waterhouse-Friderichsen syndrome may be due to high levels of endotoxin and cytokines and not to adrenal insufficiency. The two may be linked since cortisol and the cytokines regulate each other's production (Figure 1.4).

An IL-1β mediated rise in glucocorticoids is part of the normal host response to infection (Besedovsky et al 1986) which down regulates both IL-1β and TNF-α production (Sáez-Llorens et al 1990). Lack of the adrenal gland increases the toxicity of these cytokines (Bertini et al 1988). In sepsis, cytokines may prevent this negative feedback from cortisol, since TNF-α can cause adrenal haemorrhage (Tracey et al 1986) and cytokines may also inhibit cortisol production in sepsis (Catalano et al 1984). This balance between cytokines and cortisol may therefore influence survival in MCD.

1.5.5.c Blood Products.

The use of colloid blood products (especially fresh frozen plasma) in MCD is advocated by some (Sinclair et al 1989; Brandtzaeg et al 1989), but not others (Busund et al 1993). FFP and cryoprecipitate may be benefical not only as plasma expanders, but also because they contain substances which may modulate host defence, such as fibronectin.

Fibronectin

Fibronectin is a high molecular weight glycoprotein found in plasma, the extracellular matrix and on the surface of cells (Mosher 1984).
Most circulating fibronectin is synthesised by hepatocytes (Owens & Cimino 1982). Children over the age of 1 year have levels at the lower end of the adult range (200μg/ml), but levels are significantly lower in infants (McCafferty et al 1983).

During the first year of life plasma fibronectin concentration is thus dependant on age.

Other factors may affect circulating levels of fibronectin. Low levels of plasma fibronectin are found in children (Blanco et al 1990) and neonates (Gerdes et al 1983; Barnard & Arthur 1983; Dyke & Forsyth 1993) with septicaemia and septic shock.

Patients with serious liver dysfunction (Gonzalez-Calvin et al 1982) disseminated intravascular coagulation (Mosher & Williams 1978; Stathakis et al 1981; Bone 1992) or malnutrition (Sandberg et al 1990) also have reduced levels.

Infusions of cryoprecpitate (which is rich in fibronectin) leads to increased levels (Grossman et al 1983).

Function.

Fibronectin is a glycoprotein that binds and opsonises entities that can be phagocytosed by macrophages. Fibronectin can bind heparin, fibrin, complement, IgG and a wide variety of microorganisms including bacteria, viruses, fungi and parasites (Proctor 1987).

The main immune function of fibronectin is to act in conjunction with phagocytes. Fibronectin can enhance phagocytic defence without damaging the surrounding tissues by free radical production (Wright et al 1983; Yang et al 1993). Fibronectin also increases endothelial clearance of tissue debris (Snyder et al 1981; Saba 1986).

Fibronectin has an important role in the maintenance of microvascular integrity at the tissue level (Mosher 1984), and may help to regulate vascular permeability (Richards et al 1986). Fibronectin is also involved in coagulation, adhesion, migration and tissue repair (Reviewed by Proctor 1987). Such properties would have obvious benefits in the intense inflammation seen in meningococcal septicaemia.

Animal studies show that sepsis leads to an acute decrease in the level of plasma fibronectin, with a subsequent rise to normal levels (Grossman 1987). Treatment with fibronectin may decrease the capillary leak associated with sepsis (Charash et al 1991; Wheatley et al 1993) and improve survival. Of particular interest is a study where treatment with fibronectin was combined with specific immunoglubulins. Neither fibronectin nor immunoglobulin alone had an effect on mortality, but the combination increased survival significantly in neonatal mice with group B streptococcal peritonitis (Hill et al 1984). This study suggests that the effect of immunotherapy with monoclonal antibodies may be improved by the addition of fibronectin.

Studies in Human sepsis.

Effect of sepsis

Plasma fibronectin is low in patients with sepsis and low levels are related to the severity of the disease (Brodin et al 1986; Coulaud et al 1982).

Levels returned to normal without supplementation within 2 weeks of antibiotic treatment (Ahlgren et al 1985). Fibronectin levels may be lower in septic patients who die, but are poor predictors of prognosis in individual patients (Coulaud et al 1982; O'Connell et al 1984). Fibronectin shares this pattern with a number of other plasma proteins (Coulaud et al 1982; Rubli et al 1983, Mansberger et al 1989). The decrease in sepsis may thus be part of a broader pattern of protein depletion due to decreased hepatic synthesis (Pussell et al 1985; Velky et al 1984).

Replacement therapy with fibronectin has been used to try to decrease the mortality and morbidity associated with sepsis.

Fibronectin therapy.

Cryoprecipitate is enriched eight to ten fold with fibronectin, and has been used to increase plasma fibronectin levels in critically ill adults. A series of uncontrolled trials showed improvements in cardiovascular, pulmonary and renal function in critically ill adults given cryoprecipitate (Saba et al 1978; Scovill et al 1978; Scovill et al 1979).

However in controlled trials, cryoprecipitate or purified fibronectin administration showed benefit in some adult studies (Lundsgaard-Hansen et al 1985; Stevens et al 1986), but not others (Grossman et al 1983; Hesselvik et al 1989; Todd et al 1984; Mansberger et al 1989).

Fibronectin did improve survival in severly malnourished children and increase plasma protein concentration (Sandberg et al 1990), but there are no trials of the effect of fibronectin on the treatment of sepsis in children (Polin 1990).

Fibronectin in Meningococcal disease

Only one study of fibronectin in patients with MCD has been carried out. Plasma fibronectin was significantly lower in 44 children with MCD compared to controls (Blanco et al 1990). Fibronectin was also significantly lower in children who had disseminated intravascular coagulation, but not in the 4 children who died. No comparison of plasma fibronectin levels with disease severity or between those with meningitis or septicaemia was performed. If plasma fibronectin levels are found to be decreased in children with life-threatening MCD, then a trial of fibronectin therapy (perhaps combined with a monoclonal antibody) may be justified.

## 1.6 CONFIRMING THE DIAGNOSIS OF MENINGOCOCCAL DISEASE.

### 1.6.1 Standard methods

The diagnosis of MCD is normally first made on clinical grounds by recognising the rash or the presence of meningism. This diagnosis may be rapidly confirmed if gram-negative diplococci are seen during microscopy of the CSF. If this fails to identify the organism, subsequent confirmation of the diagnosis comes from isolating *N meningitidis* from blood or CSF.

These laboratory tests, however, confirm the presence of the meningococcus in only 62%-93% of clinically suspected cases (Gedde-Dahl et al 1983). Blood cultures are positive in about half of patients and CSF microscopy and culture are positive in 90% (Bohr et al 1983; Cartwright & Jones 1989).

Rapid confirmation of the diagnosis is important. Other organisms (both bacterial and viral), as well as non-infectious illnesses, can produce a similar clinical presentation (Jacobs et al 1983; Nguyen et al 1984; Baker et al 1989). Confirming the presence of the meningococcus allows confident management of the patient and of any contacts who need chemoprophylaxis. Proven cases can also be included in epidemiological studies of MCD which can help predict the need for, and efficacy of meningococcal vaccines (Riordan et al 1994).

Pre-admission antibiotic treatment decreases the likelihood of isolating meningococci from blood to less than 10%, and from CSF to 50% (Cartwright et al 1992b). Problems confirming the diagnosis also occur in those with septicaemia alone (only 50% are culture positive) and in those with meningitis in whom a lumbar puncture is deferred (Research Committee of the BSSI 1995).

Meningococci may be cultured from the skin lesions of MCD (Tompkins 1943; van Deuren et al 1993). Punch biopsy of skin lesions identified meningococci in 64% of cases who had received prior antibiotics (van Deuren et al 1993).

Meningococci can be isolated from the nasopharynx of less than 10% of cases (Gold et al 1978; Riordan, unpublished data), although some claim 30% of cases have positive swabs (Cartwright et al 1992b). As the number of pre-treated cases increases methods other than culture are needed.

### 1.6.2 Antigen Detection

Specific antisera can detect meningococcal antigens even after the administration of antibiotics. Techniques involve counter immununoelectrophoresis (CIE), latex agglutination (LA) or enzyme-linked immunosorbant assays (ELISA). All methods are reliable for groups A, C, Y and W135. However a major drawback is the lack of a high-quality commercial antisera against group B meningococci, the commonest group seen in the UK (Jones & Kaczmarski 1991).

Antigen detection of meningococci in CSF has a sensitivity up to 100% (Cuevas et al 1989), with LA being equally as sensitive as CIE (Whittle et al 1974), or more sensitive (Leionen & Kayhty 1978).

Serum antigen is more likely to be detected when there is a septicaemic component to the illness. Serum antigen was detected in 69% of cases with septicaemia with or without meningitis, but in only 38% of those with meningitis alone (Holland et al 1990). Again CIE and LA were comparable. Higher antigen concentrations are found in those with more severe disease (Lewis 1979).

However in routine practice Gram stain and microscopy of the CSF is more sensitive than antigen detection (Burans et al 1989; Coovardia et al 1989). Antigen studies can also give false positive results as they may cross react with antigens from *E. coli* (McCracken 1976; Coovardia et al 1989), especially in urine (Boyer et al 1993). Antigen detection is highly correlated with bacterial concentration (Feldman 1977). Feigin was unable to detect antigen in CSF or serum in 50% of cases of MM (Quoted in McCracken 1976). The concentration of meningococci in CSF ranges from 150 to 6 x $10^7$ colony forming units per ml(cfu/ml) (Feldman 1977), and in blood from 10 to more than 100 cfu/ml (La Scolea et al 1981). CSF microscopy is positive in 97% of those with concentrations above $10^5$ cfu/ml, but only 25% of those with less than $10^3$ cfu/ml (La Scolea & Dryja 1984).

Antigen detection is a useful adjunct to bacterial culture but still will not identify cases of MCD with low concentrations of bacteria.

### 1.6.3 Antibody Detection.

MCD can be confirmed by a rising antibody titre to the meningococcus (Flægstad et al 1990; Jones & Kaczmarski 1993). However this method requires the patient to survive long enough to mount a measurable antibody response. It is thus of limited value in patients who die from MCD, since they are likely to do so within 16 hours of admission (Niklasson et al 1971).

In MCD there is a need for a rapid, highly sensitive diagnostic test that can confirm the diagnosis, especially at low bacterial concentrations and when prior antibiotics have been given. The polymerase chain reaction may prove to be such a test.

## 1.6.4 The Polymerase Chain Reaction.

The polymerase chain reaction (PCR) was first described in 1985 (Saiki et al 1985), and is now probably the most widely used single technique in all branches of biological science. The technique allows millions of copies of a section of genome to be amplified from as little as one target DNA molecule (Saiki et al 1988). The presence of minute amounts of DNA from bacteria, viruses or tumour cells can thus be detected in samples which could otherwise have been thought free of them (Ou et al 1988; Schochetman et al 1988; Tompkins 1992).

### 1.6.4.a Method of PCR.

PCR is based on the repetitive cycling of three reactions; denaturation of DNA, primer annealing and primer extension (Figure 1.7). The temperature of the mixture determines which reaction takes place. All three reactions occur in the same tube using thermostable reagents; the method is thus self contained (Eisenstein 1990).

Denaturation.

A sample of DNA is denatured by heating to just below 100°C, so that the hydrogen bonds linking the 2 strands break (Figure 1.7).

Primer annealing

PCR is critically dependant on the primers used. These oligonucleotides are especially chosen so that they match two short sequences flanking the area of interest on the target DNA. The primers together with deoxynucleoside triphosphate bases are added in vast excess. The mixture is cooled after denaturation allowing the primers to bind, or anneal, to the single DNA strands (Figure 1.7).

Primer extension

A thermostable *Taq* DNA polymerase (isolated from the bacterium, *Thermus aquaticus*), able to make DNA at temperatures above 37°C is also added. This extends the primers by adding the deoxynucleoside triphosphate bases so that two double stranded DNA molecules result (Figure 1.7).

The cycle is then repeated doubling the amount of DNA each time, so that after 20 cycles over $10^6$ copies of the target area are present. Not all the DNA in the original sample is amplified. The DNA polymerase only attaches new residues to one end of the primers, this means that the DNA fragment whose ends are defined by the primers is mainly produced by PCR (Figure 1.7). As more copies are made, it becomes easier to copy the fragment of interest rather than the original DNA. The "short products" this produces far out number any longer products from PCR or the original DNA (Eisenstein 1990). These short products can then be visualised by gel electrophoresis.

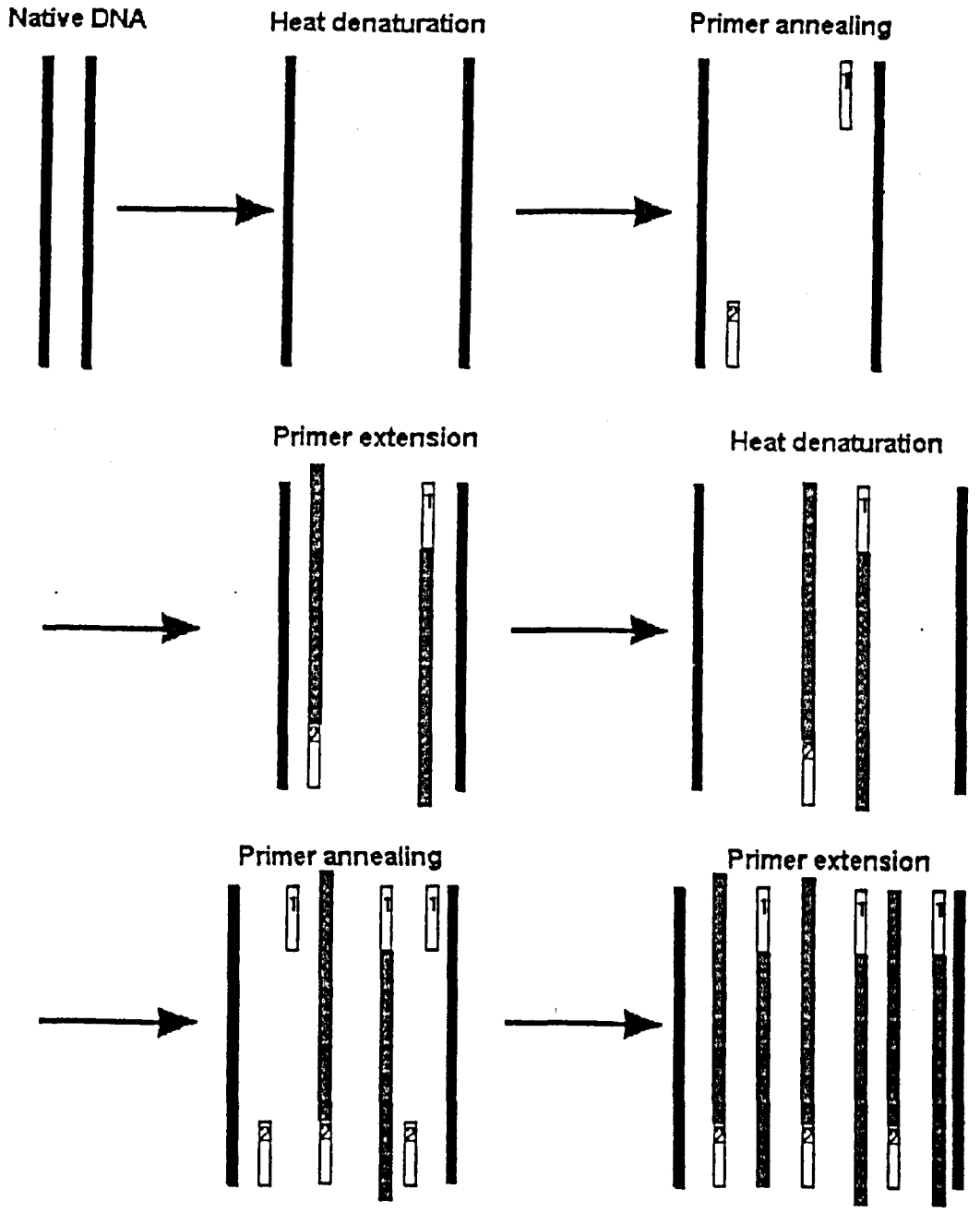



Figure 1.7 Diagram of the polymerase chain reaction. Primers 1 & 2 (white bars) anneal to the denatured native DNA (black bars) and are extended (hatched bars) by DNA polymerase. As the cycle is repeated copies of the area of DNA defined by the primers predominate; short PCR products.

### 1.6.4.b Potential Drawbacks

PCR is not without problems. The technique's greatest asset, the ability to generate many copies, is also it's major drawback. Any contamination of the sample by previously amplified DNA will produce false positive results (Lo et al 1988). Precautions must therefore be taken to avoid contamination (Kwok & Higuchi 1989). Another potential problem is primers binding to other sites on the DNA leading to spurious priming. This can lead to depletion of primer, but may be avoided by making the reaction conditions more stringent, with high annealing temperatures and low magnesium and nucleotide concentrations to destabilise partially mismatched primers (Arnheim & Erlich 1992). After a number of cycles the primer or deoxynucleoside triphosphates may become depleted or the activity of the DNA polymerase become reduced. Should this occur a small sample can be taken and the process repeated. If the target DNA has closely related sequences then a second PCR using primers just inside the original primers ("nested primers") can be performed so that only the area of interest is copied (Reviewed by Arnheim & Erlich 1992).

The ability of PCR to detect minute amounts of specific DNA in clinical samples makes it a potentially useful method for detecting the presence of meningococci in patients with suspected MCD. Three reports of it's use in detecting meningococci in CSF have been published.

1.6.4.c PCR in Meningococcal disease

Kristiansen et al (1991) reported successfully detecting meningococcal DNA, using primers flanking the dihydropteroate synthase gene, in culture negative CSF. This case report was followed by a study using PCR in CSF samples from patients with MM, other meningitides and non-infectious conditions (Ni et al 1992a). Primers were designed to amplify a fragment of the insertion sequence, IS*1106* (Knight et al 1992), present in multiple copies in all meningococcal strains (Ni et al 1992b). Using these primers positive results were obtained from group A, B, C, Y and W135 meningococcal DNA, but not from other pathogens or *Neisseria* species. The minimum amount of meningococcal DNA detectable was equivalent to that found in 10 meningococci.

The specificity and sensitivity of PCR for diagnosing MM in this series was 91%, compared to CSF culture which had a sensitivity of 82%, since 7 patients had received pre-admission antibiotics. The sensitivity of CSF microscopy and culture combined was 100%.

PCR for meningococci should ideally be able to detect very low concentrations (less than $1 \times 10^4$), since these samples are likely to be negative on microscopy and antigen studies. However when a sample contains less than 10 copies of the target DNA, PCR gives inconsistent results (Varas et al 1991). This is often due to mis-priming of other DNA sites, since there is so little target DNA for the primers to bind to. Mispriming can be reduced by using nested primers, and by adding the DNA polymerase once the DNA has been denatured by heating ("hot-start PCR").

Saunders et al (1993) used a nested PCR to detect less than 0.25 cfu of meningococci in CSF samples. They designed nested primers to detect the *por A* gene (which codes for the Class 1 outer membrane protein). They also designed specific primers to detect subtype P1.7,3. and were able to confirm the presence of this subtype in CSF, negative by culture, microscopy and antigen testing. These samples came from children vaccinated against P1.7,3 (Zollinger et al 1991). It was thus vitally important to determine the exact cause of their meningitis, so that vaccine efficacy could be determined.

By designing different primers other subtypes of meningococci could be detected, providing useful information for epidemiological studies as well as vaccine trials.

Meningococci have been identified in 15 CSF samples by PCR (Kristiansen et al 1991; Ni et al 1992a; Saunders et al 1993). Further evaluation of this technique is required on a large cohort of patients with a clinical suspicion of MCD. Lumbar puncture may now be undertaken less frequently in MCD (Harper et al 1985), so that CSF is not often available to perform PCR on. The previous studies recognise this and the need to develop PCR for use with other specimens in MCD, the most obvious candidate being blood.

## 1.7 NOVEL THERAPIES.

The mortality from meningococcal septic shock has remained high despite conventional treatments (Abbott et al 1985). This has stimulated research into novel methods of treatment, most aimed at decreasing the levels of endotoxin or the circulating inflammatory mediators by means of specific antibodies.

### 1.7.1 Anti-endotoxins

The most toxic part of endotoxin is the lipid component, lipid A (Glauser et al 1991). The structure of lipid A is highly conserved across all gram negative bacteria, whilst the polysaccharide O side chains are highly variable (See Figure 1.1). Antibodies against the core glycolipid of endotoxin might therefore bind endotoxin from all gram-negatives, including meningococci. The J5 mutant of *Escherichia coli* O111:B4 has endotoxin which lacks O side chains and thus presents the core glycolipid on its surface. Volunteers immunised with *E coli* J5 vaccine produce antibodies to endotoxin core glycolipid. This antiserum reduced mortality from gram-negative bacteraemia and shock in adults (Ziegler et al 1982). However when used in children with severe MCD anti-J5 plasma did not affect mortality (J5 Study Group 1992).

Two trials of anti-endotoxin therapy in children with severe MCD have been performed in Merseyside.

Both trials gave Pentaglobin, an IgM-enriched pooled, polyvalent immunoglobulin preparation with a high antibody titre to *E coli* J5 endotoxin. This was combined with Polymixin E, a cationic detergent which binds and inactivates endotoxin. The first open trial found a significant decrease in mortality in those given anti-endotoxin therapy compared to historical controls (Thomson et al 1991b). This pilot study was followed by a prospective randomised double blind placebo controlled trial. This did not show any difference in mortality in those given Polmyxin/Pentaglobin (Marzouk 1995). However it underlines the fact that novel treatments for MCD should be assessed by prospective randomised double blind placebo controlled trials.

Using bio-technology a human monoclonal IgM antibody against Lipid A, HA-1A, has now been produced (Teng et al 1985). This antibody decreased mortality in adults with gram-negative bacteraemia and shock, but not in those with other infections (Ziegler et al 1991). A case report of the successful use of HA-1A in MCD has appeared (Syed et al 1992), and a European multicenter randomised double blind placebo controlled trial of HA-1A in MCD is underway to confirm whether it is beneficial (Nadel et al 1992). Children from Merseyside with severe MCD are being entered into this trial, alongside the current study.

Other methods of modifying the host inflammatory response might be by nutritional supplements (Grimble 1990). Vitamin A supplementation may have a role in decreasing mortality from MCD.

## 1.7.2 Vitamin A Supplementation.

Shortly after it's discovery Vitamin A was described as an "anti-infective vitamin" (Green & Mellanby 1928). However until recently vitamin A research has focused on preventing xeropthalmia and blindness. In the past 10 years the potential of vitamin A for decreasing mortality from infectious disease has been re-discovered.

### 1.7.2.a Dietary sources and metabolism

Vitamin A comes from two natural sources; retinyl esters found in animal tissues (liver, fish liver oil) and plant carotenoid pigments (found in carrots, dark green leafy vegetables, orange and yellow fruits and vegetables). After ingestion ß-carotene is converted into retinol, whilst retinyl esters are hydrolysed to retinol. Retinol is absorbed into the mucosal cell and then transported via chylomicrons to the liver, where over 90% of the body's vitamin A reserves are stored. Vitamin A is transported from the liver to other organs bound to its carrier protein, retinol binding protein. Retinol enters target cells via specific receptors and activates genes in the nucleus through other specific receptors, similar to those for steroid and thyroid hormones (Reviewed by Semba 1994). Retinol is needed for vision in dim light. Deficiency results in morphological changes in epithelial surfaces, night blindness and decreased immunity.

### 1.7.2.b Vitamin A deficiency.

Severe vitamin A deficiency predisposes to infection.

Vitamin A is needed to maintain the integrity of epithelial surfaces (Tomkins & Hussey 1989) and deficiency leads to increased bacterial binding to respiratory mucosa (Chandra 1988). Retinol is an important co-factor in T cell activation (Garbe et al 1992) and deficiency adversely affects cell-mediated immunity (Beisel 1982). Children with vitamin A deficiency have low lymphocyte counts (Bhaskuran & Reddy 1975) and abnormal proportions of T cell subsets (Semba et al 1993). Humoral immunity is however intact in vitamin A deficient animals (Tomkins & Hussey 1989), although the ability to mount an IgG response to T cell-dependent antigens is improved by vitamin A (Semba 1994).

Immunity is thus impaired in children who are deficient in vitamin A. Such children are common in the developing world, but may also be found in the developed world.

Vitamin A deficiency in the developed world.

Increasing concern is being expressed about the nutritional adequacy of the diets of children and adolescents in developed countries. Recent dietary studies have found low intakes of vitamin A in children in the lower socio-economic groups and in inner cities (McNeill et al 1991; Doyle et al 1994). Measuring serum levels of retinol in these children showed that 5-17% were at risk of clinical vitamin A deficiency (Malvy et al 1989; Doyle et al 1994). Despite this possible biochemical deficiency, no children had evidence of clinical deficiency (ie keratomalacia). Clinical vitamin A deficiency has been described in the UK, but only in those with gastrointestinal and liver disease (Watson et al 1995).

Assessment of vitamin A status remains controversial, and may not be wholly reflected by serum retinol levels which may be affected by other factors.

1.7.2.c Assessment of Vitamin A status.

Since most vitamin A is stored in the liver and secreted as necessary, plasma vitamin A levels do not correlate well with body stores. However they may reflect vitamin A status when liver stores are fully saturated or depleted (Underwood 1990), with levels below 100 μg/L (0.35 μmol/L) indicating deficiency (World Health Organisation 1976). Plasma retinol levels can also vary for other reasons. Decreased retinol levels have been found in children with febrile infections; measles, chickenpox, bronchitis, diarrhoea and malaria (Thurnham 1989; Arroyave & Calcano 1979). Levels returned to normal without supplementation within 8 weeks (Bhaskaram 1985). This implies that these low levels are not due to low body stores. However the cause for the low levels during infection is unknown. Possible causes include; impaired absorption of vitamin A (Sivakumar & Reddy 1972), inadequate mobilisation of liver stores (Hussey & Klein 1990), redistribution of vitamins (Vitale 1977) or retinol leaking through the vascular endothelium with its binding protein (Thurnham 1989). The decrease may be due to the acute phase response to infection (Thurnham & Singkamani 1991), since retinol levels mirror CRP levels (Louw et al 1992) and correlate negatively with α-1-acid glycoprotein (Filteau et al 1993). Vitamin A levels have also been found to correlate with IL-6 levels (Tabone et al 1992), again suggesting a link with the acute phase response.

An increased urinary excretion of vitamin A during infection may also explain the decreased levels (Stephensen et al 1994).

Infection may produce an accelerated depletion of liver retinol stores (Campos et al 1987). Thus a child with borderline deficiency may be precipitated into overt deficiency by infection. In children with initially low vitamin A levels the very low levels produced by infection may impair recovery (Thurnham 1989). This is supported by a study of children with measles in Zaire in which mortality was associated with low retinol levels (Markowitz et al 1989).

Low retinol levels during infection are not only found in the developing world. Children in the United States have been shown to have low retinol levels during measles and respiratory syncitial virus infections (Frieden et al 1992; Butler et al 1993; Arrieta et al 1992; Neuzil et al 1994). Vitamin A levels also correlated with disease severity in these children (Butler et al 1993; Frieden et al 1992; Neuzil et al 1994).

Low retinol levels are thus associated with severity of infectious disease. Do vitamin A supplements decrease disease severity?

1.7.2.d Vitamin supplements and Infection.

Community Intervention Trials.

A number of controlled trials of vitamin A supplements in children living in areas where vitamin A deficiency is endemic have been done. The evidence from these trials was inconsistent, some showing a highly significant decrease in mortality (Rahmathullah et al 1990), but not others (Herrera et al 1992; Vijayaraghavan et al 1990). Two meta-analyses of these trials showed an overall 30% decrease in mortality in those given vitamin A (Fawzi et al 1993; Glasziou & Mackerras 1993).

In one study (Rahmathullah et al 1990) mortality associated with convulsions was markedly reduced by vitamin A supplements. These convulsions may have been associated with meningitis, and vitamin A may thus decrease mortality from meningitis. Keusch (1990) commenting on this study states: "It would be of great interest and importance to know whether vitamin A status has an effect on mortality from meningitis."

In these at risk populations Vitamin A supplementation does not appear to decrease the number of infectious episodes a child has (Stansfield et al 1993), only the severity of the episode and the risk of dying from it (Arthur et al 1992; Sommer 1993; Ghana VAST Study Team 1993).

Hospital Treatment Trials.

If low vitamin A levels are associated with mortality from infectious disease, then vitamin A supplementation in the acute phase of the illness may be beneficial. This has proved to be the case with measles. Four trials of vitamin A supplementation in measles have been reported (Coutsoudis et al 1991; Ellison 1932; Barclay et al 1987; Hussey & Klein 1990). Meta-analyses of these trials found a 60-66% decrease in mortality in those given vitamin A (Glasziou & Mackerras 1993; Fawzi et al 1993) as well as a decrease in morbidity (Coutsoudis et al 1991). More importantly some of these trials were carried out in the UK (Ellison 1932) or parts of Africa where clinically apparent vitamin A deficiency was rare (Hussey & Klein 1990). Vitamin A thus protected against death from measles, even in populations with no clinical signs of vitamin A deficiency.

No studies of retinol levels in meningitis or MCD have been carried out. Some children in Merseyside may be at risk of Vitamin A deficiency, as found in other deprived parts of the UK (Doyle et al 1994). If MCD causes a further fall in retinol levels, vitamin A supplementation may be beneficial.

## 1.7.3 Nutritional status.

### 1.7.3.a Nutrition's effects on infection.

Nutritional status affects a child's susceptibility to infection. Malnutrition leads to impaired host defences and an increased risk of infection. Paradoxically undernutrition can also hinder the infectious process of certain organisms (Scrimshaw et al 1968). Mortality from infectious disease is also influenced by nutritional status (Berkowitz 1992).

Malnourished children have a high incidence of, and mortality from, Gram negative bacteraemia. However in a study comparing malnourished and normally nourished children with bacteraemia, meningococcaemia was only found in well nourished children (Berkowitz 1984). A further study found that children dying from MCD had significantly better nutritional status (as measured by weight-for-age), compared to children dying from other causes (Neveling & Kaschula 1993). These studies suggest that MCD may have its disease process hindered by poor nutrition. This may be due to the decreased cytokine production in malnutrition (Bhaskaram & Sivakumar 1986; Grimble 1990). Other studies however, have found no association between nutritional status and morbidity or mortality from MCD (Ryder et al 1987) and no association between malnutrition and meningitis (Rosen & Davis 1980).

1.7.3.b The effect of infection on nutrition.

Infection can also adversely affect a child's nutritional status (Scrimshaw et al 1968), although only minor changes were found in children with *H influenzae* meningitis (Sherry et al 1989). The catabolic changes of muscle protein loss, lipolysis and enhanced gluconeogenesis seen in sepsis, are thought to be mediated by the pro-inflammatory cytokines, TNF-α and IL-1β (Grimble 1990). These cytokines stimulate catecholamine and glucocorticoid release which lead to muscle proteolysis. The amino acids produced from proteolysis are used in gluconeogenesis and to make acute phase proteins. Changes in nutritional status may thus be associated with cortisol and CRP levels.
If mortality in MCD is associated with nutritional status, then nutritional modulation of cytokine production may improve the outcome (Grimble 1990).

## 1.8 PREVENTION OF MENINGOCOCCAL DISEASE.

The best way of decreasing mortality from MCD is by preventing the disease. The two main methods of prevention are chemoprophylaxis of MCD contacts and vaccination. Unfortunately neither is likely to prevent many cases at present.

### 1.8.1 Chemoprophylaxis of Meningococcal disease contacts.

The risk of developing MCD is one hundred to one thousand fold higher for household contacts of MCD compared to the general population (DeWals et al 1981; Cooke et al 1989).

Co-primary cases develop within 24 hours of the first case and will not be prevented by prophylaxis. Secondary cases mostly occur within a week of the primary case (Cooke et al 1989). Prophylaxis can be either by pre-emptive treatment with penicillin during the first week, or by giving antibiotics that eliminate carriage of the organism in the oropharynx (Cooke et al 1989). This latter approach prevents further transmission and is assumed to prevent secondary infection. Prophylaxis may fail if the organism is not sensitive to the antibiotic used or if the source of the infection is outside the treated group. Even after optimal chemoprophylaxis there remains an increased risk of MCD (Cooke et al 1989). Most cases of MCD are not secondary cases (Cooke et al 1989), prevention of MCD in the population will require vaccination.

### 1.8.2 Meningococcal vaccines.

Immunity to the meningococcus is conferred by bactericidal antibodies against the polysaccharide capsule (Goldschneider et al 1969). Unfortunately the group B polysaccharide is similar to antigens found on foetal brain cells, and does not stimulate antibody production in man (Finne et al 1987). Vaccines made from purified groups A and C polysaccharide have proved highly effective in controlling epidemics of MCD (Peltola et al 1977, Lennon et al 1992). However group C polysaccharide vaccines are ineffective in children under 2 years of age (Taunay et al 1974). Polysaccharide vaccines also do not produce long lasting immunity or a booster response in older children (Gold et al 1979).

A tetravalent vaccine against groups A, C, Y and W135 is available, but as most MCD in the UK is group B disease in infants, such a vaccine would have little impact on mortality.

1.8.2.a Conjugate vaccines.

The poor immunogenicity of polysaccharide vaccines in young children is due to their immature B cell function. If the polysaccharides are conjugated to protein carriers known to induce immunity in young children (eg diptheria or tetanus toxoid), then long lasting, boostable, T cell dependant immunity can be induced (Dintzis 1992). These conjugate vaccines have proved highly effective in decreasing invasive disease due to *H influenzae* (Peltola et al 1992). A conjugate meningococcal A and C vaccine has been developed and is now in clinical trial (Costantino et al 1992; Anderson et al 1994). It's effectiveness in decreasing mortality from MCD will depend on the age distribution and predominant serogroups within a population.

An effective vaccine against all meningococci will thus not be available for some time. Other methods of decreasing mortality are therefore necessary.

## 1.9 CURRENT RESEARCH

### 1.9.1 Current Position.

The high incidence of MCD on Merseyside, compared to other areas of the British Isles (Abbott et al 1985), has stimulated a programme of research into a number of aspects of the disease. Conclusions drawn so far include: that MCD presents in 3 distinct clinical groups - septicaemia, septicaemia plus meningitis, and meningitis alone (Thomson et al 1990). These three groups differ in respect of a number of clinical and laboratory variables (Thomson et al 1990; Marzouk et al 1993) as well as having different prognoses (Thomson et al 1990; Marzouk et al 1991b). The patients at highest risk of dying are in the 2 groups with a septicaemic component to their illness. Those at risk of dying can be identified by means of a clinical prognostic score, GMSPS, (Marzouk et al 1991b). This score also correlates with the mediators of severe MCD, endotoxin, TNF-$\alpha$ and IL-6 (Marzouk 1995).

### 1.9.2 Indications for current research

The high mortality from MCD has remained unchanged for 30 years (Abbott et al 1985). A decrease in mortality may be achieved by a number of different approaches:

Delays in seeking medical advice, in diagnosis and in treatment may all contribute to the high mortality from MCD (Oakley & Stanton 1979; Slack 1982). Parents and doctors of first contact thus need accurate information on the features of early life threatening MCD. Rapid laboratory tests to confirm the diagnosis could also be valuable. Fibronectin levels or PCR techniques may provide such a test.

Once a diagnosis has been made appropriate treatment needs to start immediately. The value of commonly used therapies, such as corticosteroids and fibronectin (contained in FFP and cryoprecipitate) remains controversial (May 1960). The role of novel therapies such as anti-endotoxin treatment (such as HA-1A), cytokine inhibitors (such as IL-10) or nutritional interventions (such as vitamin A) remain to be defined.

Mortality could also be decreased by an effective vaccine. The potential impact of a conjugate group C vaccine on the incidence and mortality from MCD needs to be evaluated.

### 1.9.3 Aims of the current study

#### 1.9.3.a Aims of the retrospective study.

1) To study a possible increase in meningococcal septicaemia, and determine if changes in the incidence of septicaemia, disease severity or seasonality have been associated with changes in mortality from MCD.

2) To study the possible impact of a conjugate group C meningococcal vaccine on the mortality from MCD seen at RLCHs.

1.9.3.b Aims of the prospective study.

A number of interlinked studies of MCD in children aim to:

1) determine the features of early MCD that parents and doctors notice, to provide accurate and appropriate information about early life-threatening MCD;

2) relate interleukin-10 to disease severity and outcome and to other cytokines;

3) determine the presence of true or relative adrenal insufficiency and a method for rapidly identifying those with it;

4) relate plasma fibronectin to disease severity and outcome and evaluate it's role as a marker for MCD;

5) relate vitamin A and nutritional status to disease severity and outcome;

6) explore the possibility of early, accurate, laboratory diagnosis by polymerase chain reaction techniques.

This study runs in tandem with a prospective, randomised, double-blind study of anti-endotoxin monoclonal antibody treatment (Centoxin, Centocor BV).

# CHAPTER TWO. MATERIALS AND METHODS

# CHAPTER TWO. MATERIALS AND METHODS

## 2.1 RETROSPECTIVE STUDY.

Data were collected on children with MCD admitted to the Royal Liverpool Children's Hospitals (RLCHs) between January 1977 and December 1993. The Royal Liverpool Children's Hospitals (Alder Hey and Myrtle Street) admit children from the Liverpool and South Sefton health districts. Since January 1990 all inpatient facilities have been at Alder Hey. The Regional Paediatric Intensive care unit (PICU) at Alder Hey receives referrals from other local district hospitals.

Since 1987 there has been an active program of research into MCD in the RLCHs. Data on children admitted between 1977 and 1987 had already been collected (Thomson et al 1990). Further cases of MCD seen between January 1988 and December 1993, were identified from microbiology and post-mortem records, the Intensive Care register, and from the records of two prospective studies of MCD (Marzouk 1995; See prospective study). Case notes or the Research Fellow's data for these children were examined.

Children were included in the study if they had positive cultures for *N meningitidis* in blood, CSF or synovial fluid; or a clinical presentation compatible with MCD together with either; detection of meningococcal antigen in serum or CSF, Gram negative diplococci seen in the CSF or a positive throat swab for *N meningitidis*. Isolates of *N meningitidis* were sent to the Meningococcal Reference Laboratory, Manchester for serotyping and subtyping.

Table 2.1. Definitions of clinical presentations of meningococcal disease (After Thomson et al 1990).

Meningococcal meningitis (MM): A child with positive cerebrospinal fluid (CSF) antigen or cultures; or a CSF white cell count (WBC) >10 x $10^6$/l with a positive throat swab, but with no rash and negative blood cultures or antigen.

Meningococcal septicaemia (MS): A child with a petechial or purpuric rash with a positive throat swab; or positive blood cultures or antigen, but with no CSF changes.

Meningococcal meningitis plus meningococcal septicaemia (MM+MS):
A child with a petechial or purpuric rash with positive CSF culture or antigen; or
A petechial or purpuric rash with >10 WBC in the CSF and a positive throat swab; or
Positive blood culture or antigen with positive CSF culture or antigen or >10 WBC in the CSF; or
A petechial or purpuric rash with positive throat swab or positive blood culture or antigen, with neck stiffness if lumbar puncture was not performed.

2.1.1 Clinical presentation.

Children were divided into three groups; meningitis alone, septicaemia alone and meningitis plus septicaemia (See table 2.1). A petechial or purpuric rash was used as a marker for septicaemia.

2.1.2 Disease severity.

The severity of illness was assessed retrospectively by a single observer, using the score devised by Kahn and Blum (1978). This score was designed for children with MCD admitted to an intensive care unit, and selects those at a high risk of dying. The score consists of 5 factors (Table 2.2). The presence of 3 or more of these factors is associated with a 79% risk of mortality. This score has been shown to have better sensitivity and specificity than most other scoring systems for MCD (Leclerc et al 1991).

Table 2.2 Factors associated with poor prognosis in Meningococcal disease (Khan & Blum 1978).

| | |
|---|---|
| Coma | (unrousable with no spontaneous eye movement) |
| Shock | (blood pressure <60 mmHg) |
| WBC≤10,000/mm$^3$ | |
| DIC | (Platelets≤150,000/mm$^3$ or prolonged PT or APPT, or fibrin degradation products >40mg/ml) |
| Acidosis | (Bicarbonate ≤15mmol/l or pH≤7.30) |

WBC=White blood cell count

PT=Prothrombin time

APPT=Partial thromboplastin time

## 2.2 PROSPECTIVE STUDY.

The prospective study took place in 4 hospitals on Merseyside. These were Alder Hey Children's hospital, and the paediatric departments of 3 surrounding district general hospitals; Arrowe Park, Whiston and the Countess of Chester hospitals. These hospitals admit children from five health districts. Alder Hey also receives tertiary referrals to the Regional Paediatric Intensive Care Unit (PICU) from other districts.
Children were recruited at Alder Hey from September 1992. Recruitment began in January 1993 at Arrowe Park and Whiston, and in May 1993 at the Countess of Chester Hospital. The study ended in April 1994. The study was approved by the Local Research Ethics Committee of each hospital.

### 2.2.1 Notification of Cases and Controls.

Children were included in the study if they were initially treated for MCD at any of the participating hospitals. The research fellow was contacted as soon as MCD was suspected. These children were seen by me on admission and their clinical course followed. Children not thought to have MCD on admission, but who later had *N meningitidis* isolated from blood or CSF (n=15), were also seen and followed for the rest of their illness.

## 2.2.2 Clinical assessment and prognostic score.

The parents of the referred children were interviewed using a verbally administered questionnaire. Parents were asked about specific symptoms and signs they had noticed and their reasons for seeking medical advice. The admitting GPs diagnosis was noted, as was the outcome of any contact with a doctor before admission. Any pre-admission antibiotics given were recorded.
An independent examination was carried out and the type of rash, vital signs and GMSPS were recorded. If there was a deterioration in clinical condition the GMSPS was recalculated. For late referrals an estimate of the GMSPS on arrival and on deterioration was made.

## 2.2.3 Nutritional Status.

Children were weighed on admission by members of the nursing staff using ward scales. The circumference of the left arm was measured midway between the acromion and the olecranon (mid-arm circumference). The Triceps skinfold thickness was also measured at this point using skinfold callipers. Both these latter measurements were made by myself. Weight-for-age z scores were calculated using Epi Info version 5 computer software. Measurements were repeated on children with MCD 5-7 days after admission.

2.2.4 Case Definition

Meningococcal disease was defined as an illness in a child who had *N meningitidis* isolated from either blood or CSF, or an illness with fever and petechiae, diagnosed as MCD by the local paediatrician in a child with no CSF or blood isolate of *N meningitidis* (Meningococcal Disease Surveillance Group 1976).
Confirmation of the diagnosis was sought using standard Gram stain, culture and antigen techniques.

Confirmed cases of MCD (n=74).

Children with positive blood or CSF cultures or antigen for meningococci.

Probable cases of MCD (n=4).

Children with clinically suspected MCD in whom *N meningitidis* was only isolated from throat swabs.

Possible cases of MCD (n=48).

Children diagnosed with MCD by the local paediatrician, but with negative blood, throat or CSF cultures or antigen for meningococci. These children consisted of 23 children with haemorrhagic rashes and no clinical or laboratory sign of meningitis (included with MS) and 25 with a rash and evidence of meningitis (included with MM+MS). Twenty five of these 48 children had received antibiotics (16 parenterally) before cultures were taken.

2.2.4.a Clinical presentations.

Disease presentations were classified into MS (n=34), MM+MS (n=34) or MM (n=10) as before (See Table 2.1).

Severe MCD (n=46).
Any child with MCD who scored 8 or more at any time on the GMSPS.

Meningococcal Septic Shock (n=33).
Any child with MCD and a systolic blood pressure less than the 5th centile for age, plus evidence of altered organ function; deteriorating mental status, oliguria (<0.5 ml/kg/hr), metabolic acidosis (pH <7.3, base deficit >5 or lactate >2mMol/l) or hypoxia ($PaO_2$ <75mmHg in air) (Jafari & McCracken 1992).

Other serious infections (n=10).
Ten children initially treated for MCD, had pathogens other than *N meningitidis* isolated from blood or CSF (*S pneumoniae* 3) or had culture negative CSF suggestive of bacterial infection (high neutrophil count and protein, low glucose), but no rash (n=7). These were considered as a separate group from the MCD group and controls.

Controls (n=77).
These were defined as children initially treated for MCD, but who were later diagnosed as having less serious conditions. Discharge diagnoses in this group were; viral illness 31, respiratory infection 23, urticaria/vasculitis 8, gastroenteritis 5, urinary tract infection 2 and 5 others.

## 2.2.5 Collection of samples.

Parental consent was obtained to collect the study samples.

### 2.2.5.a Blood Samples.

Blood samples were collected at the same time as routine samples using a sterile technique.
Blood samples were collected on admission or at the next time blood was taken after admission. If blood was taken for routine samples on day 5-7, a further sample was taken then. In children on PICU with indwelling arterial lines, further samples were taken 12, 24, 36 and 48 hours after admission.
Survivors in the Centoxin trial (see below) had a further sample taken 8 weeks after admission.

In addition to routine tests blood samples were collected on admission for cortisol, retinol, fibronectin and for PCR. Children on PICU had admission samples for cytokines taken, cortisol samples taken at 12, 24, 36 and 48 hours after admission and a fibronectin sample at 24 hours. Samples on day 5-7 were for fibronectin. Samples at 8 weeks were for cortisol (See table 2.3).

Samples were separated within 2 hours of collection and stored at -70°C until analysed in batches at the end of the study. Retinol samples were stored protected from light.

Table 2.3. Routine and study samples collected from children in prospective study.

| | | Day sample taken | | | | |
|---|---|---|---|---|---|---|
| Routine Samples; | | Arrival | 1 | 2 | 7 | 56[a] |
| Astrup/Arterial gas | ) | * | | | | |
| FBC, Platelets | ) | * | * | | | |
| U+E, Gluc, Creatinine | ) | * | * | | | |
| C-reactive protein | ) | * | | | | |
| Blood culture | ) | * | | | | |
| Clotting studies | ) | * | | | | |
| Serum Antigen | ) | * | | | | |
| CSF[b] | ) | * | | | | |
| Study Samples; | | | | | | |
| Fibronectin | (0.5ml) | * | * | | * | |
| Vitamin A[c] | (1ml) | * | | | | |
| Blood for PCR | (1ml) | * | | | | |
| Cortisol | (0.5ml) | * | (x2[d]) | (x2[d]) | | * |
| Cytokines[d] | (1ml) | * | | | | |
| TOTAL EXTRA BLOOD(ml) | | 4 | (1.5) | (1) | 0.5 | 0.5 |

NOTES:

[a] Week 8 specimens only taken from Centoxin trial survivors.

[b] If LP performed CSF sample collected for PCR.

[c] Sample protected from light after collection.

[d] Steroid profile 12 hourly and cytokines in children on PICU.

2.2.5.b Cerebrospinal Fluid Samples.

If a lumbar puncture was performed a sample of CSF was collected for PCR.

2.2.6 Methods for additional laboratory tests.

2.2.6.a Cortisol

Blood was collected as a 0.5ml sample in Lithium Heparin. Samples were assayed by a single technician blind to the clinical details, in batches at the end of the study. Analysis of samples was done by $I^{131}$ radio-immunoassay. The inter assay coefficient of variation was 7.2-8%. The assays were done in the Endocrine Laboratory of the Department of Clinical Chemistry, Royal Liverpool University Hospital.

2.2.6.b Fibronectin

Because of the sensitivity of fibronectin to protease, 0.5ml of blood was collected and added to 50 µl (ie. 500 Kallikrein inactivator Units) of the protease inhibitor, Aprotinin ("Trasylol" Bayer, UK). Aprotinin 70 mg dissolved in 50 ml 0.9% sodium chloride = 500,000 Kallikrein inactivator Units. Samples were assayed by a single technician blind to the clinical details, in batches at the end of the study.

Plasma fibronectin levels were measured using a commercially available turbidimetric immunoassay (Fibronectin Opsonic Protein, Boeringher Mannheim Biochemica). These tests were carried out by the Department of Biochemistry at the Royal Liverpool Children's Hospital, Myrtle Street.

2.2.6.c Retinol

Blood was collected as a 1 ml sample in a covered Lithium Heparin tube. Retinol can be degraded if exposed to light. Samples were therefore stored protected from light, at -70°C in sterile, clear plastic tubes (Sarstedt Micro Tubes). Later samples were stored in brown glass bottles. A small pilot study of retinol levels in MCD was done. Every third sample from children with either MS or MM+MS or from controls was analysed. Samples were assayed by a single technician blind to the clinical details, in a batch at the end of the study.
Retinol levels were measured by high performance liquid chromatography at the Department of Tropical Paediatrics, Liverpool School of Tropical Medicine.

2.2.6.d Polymerase Chain Reaction

Methods for the PCR techniques used will be described in Chapter 9.

2.2.6.e Cytokines.

Methods for cytokine analysis will be described in Chapter 5.

## 2.3 CENTOXIN TRIAL.

The hospitals involved in the study were also participating in a multicentre trial of the use of the anti-lipid A IgM human monoclonal antibody, HA-1A. Only those in meningococcal septic shock were eligible for this trial (Table 2.4). Twenty two children in the prospective study were enrolled in the trial. These children were randomised to receive either HA-1A or an albumin placebo. Interim analyses have not shown a highly significant effect of HA-1A. It will not be known which children received HA-1A until after the trial ends in May 1995.

## 2.4 STATISTICAL METHODS.

Standard statistical methods were used to calculate median and ranges for non-parametric data. For categorical data $Chi^2$ was used, with Yates correction if the total was less than 100 or any one cell was less than 10 (Swinscow 1983). If any expected value was less than 5, the Fishers Exact test was used (Altman 1991).

For non-parametric continuous data the Mann-Whitney U test and Spearman's rank correlation coefficient were used. A P value below 0.05 was considered significant.

Statistical analysis was performed on a personal computer using Arcus Pro-II version 2.15 (Buchan 1994) and SPSS/PC+ version 5 statistical software.

Table 2.4. Inclusion Criteria for HA-1A Study.

1. Presumptive diagnosis of fulminant meningococcaemia.
2. Purpura and/or petechiae.
3. Age >3 months and < 18 years.
4. (i) Persistent hypotension, (systolic B.P. <5th centile for age[a], or <100mmHg if over 12 years) in the presence of normal hydration and the absence of inotropes.

**OR**

(ii) Evidence of systemic toxicity or poor end-organ perfusion within 24 hours prior to enrolment (i.e. at least 2 of the following);

a. Unexplained arterial metabolic acidosis.
(pH < 7.3, or Base deficit > 5
or lactate > 2 mMol/l)

b. Hypoxia.
($PaO_2$ < 75mmHg (10kPa) in air, or
< 100mmHg (13kPa) in 40% oxygen)

c. Acute renal failure.
(Urine output <0.5ml/kg/hour for 1 hour, despite acute volume loading or evidence of adequate intravascular volume and without renal disease.)

d. Sudden deterioration of the patients baseline mental status.

5. Written informed consent has been obtained from Parent/guardian.

[a]Systolic Blood Pressure - 5th centiles;

| | |
|---|---|
| Less than 1 year | 75mmHg |
| 1-5 years | 80mmHg |
| 6-12 years | 85mmHg |

# CHAPTER THREE. RETROSPECTIVE STUDY

# CHAPTER THREE. RETROSPECTIVE STUDY

## 3.1 INTRODUCTION

### 3.1.1 Clinical presentations of Meningococcal disease.

Infection with *N meningitidis*, as its name suggests, commonly presents as meningitis (Heyderman et al 1993). The term "meningococcal meningitis" is thus widely, but loosely, used to describe all meningococcal infections (i.e. both meningitis and septicaemia)(Tarlow & Geddes 1992). Recent reports suggest that MCD is becoming more common, and is presenting less often as meningitis (Palmer et al 1992; Jones & Kaczmarski 1994). Nationally collected data, however, has only identified cases of meningococcal septicaemia since October 1988 (Baxter & Payne 1993). An accurate classification of disease presentation before this time is therefore difficult to obtain. It is important to be aware of an increase in meningococcal septicaemia since this presentation carries the highest mortality (Andersen 1978; Fallon et al 1984; Halstensen et al 1987; Thomson et al 1990).

Mortality from MCD may also be influenced by disease severity (Andersen 1978; Havens et al 1989) and by season, with fewer deaths occurring during the summer months (Halstensen et al 1987).

The 3 main clinical presentations of MCD in Merseyside children; meningitis alone, meningitis plus septicaemia and septicaemia alone, have previously been described (Thomson et al 1990). A rise in the proportion of cases with septicaemia alone was noted in the final year of that study, 1987.

The current study updates the previous study. The aim was to determine whether the proportion of cases of meningococcal septicaemia admitted has continued to increase and if, as suspected, there has been an increase in disease severity. Attention will focus on whether changes in the incidence of septicaemia, disease severity or seasonality have been associated with changes in mortality from MCD.

### 3.1.2 Serogroup C infections.

Meningococcal disease in the UK has mainly been caused by serogroup B meningococci (Abbott et al 1985; Cartwright et al 1986) for which an effective vaccine is not available (Jones 1993). However serogroup C meningococci have recently increased in prevalence (Jones & Kaczmarski 1991) and are currently responsible for 25% of MCD in England and Wales (Jones & Kaczmarski 1993). Group C infection could be prevented by the use of a polysaccharide vaccine. Unfortunately this vaccine is not effective in children under 2 years of age (Taunay et al 1974).

In the United States children with group C disease are significantly older than those with group B disease, with 73% aged over 2 years (Baker & Griffiss 1983). Children over 2 years of age with group C infection could have their disease prevented by the currently available vaccine. If children with group C disease are below this age then protein conjugation of the vaccine (Beuvery et al 1983), similar to the new *Haemophilus influenzae* type b vaccines, could offer protection to children as young as 4 months.

Such a vaccine is now in clinical trial (Costantino et al 1992; Anderson et al 1994).

The mortality from group C disease is higher than that from group A disease (Evans-Jones et al 1977), and group B disease (Scholten et al 1994). Data are needed to estimate the benefit of vaccinating children against group C meningococci. In order to evaluate the possible benefits of vaccination, this study aims to answer four questions. Firstly, what proportion of MCD, seen in the Royal Liverpool Children's hospitals (RLCHs) over a 17 year period, was due to group C? Secondly, what was the mortality in children with group C disease? Thirdly, how many of these children were over 2 years of age and could have been protected by the current vaccine? And finally, how many were 4 months or older and could be protected by a conjugate vaccine, given as part of the routine immunisations?

## 3.2 METHODS.

Full details of the methods have been given in Chapter 2. Data were collected from case notes, post mortem reports and Research Fellows' data, on children with MCD admitted between January 1977 and December 1993. Inclusion criteria excluded children without positive cultures or antigen for *N meningitidis* in blood, cerebrospinal fluid or throat swab. Isolates of *N meningitidis* were sent to the Meningococcal Reference Laboratory, Manchester for serogrouping and serotyping.

Children were divided into three groups; MM, MS and MM+MS (See Chapter 2). The severity of illness was assessed retrospectively using the score devised by Kahn and Blum (1978), in which the presence of 3 or more risk factors is associated with a 79% risk of mortality.

## 3.3 RESULTS

Four hundred and forty nine children were admitted with 451 episodes of MCD; 52 were transferred from other hospitals for intensive care. Information was available for 449 episodes in 447 children.
Fifty (11%) of these children died, 10 of whom were brought in dead, the diagnosis being made at post mortem. It was impossible to calculate the prognostic score for the 10 children brought in dead and for 2 other children who died, because of insufficient information.

### 3.3.1 Clinical Presentations.

The survey was divided into three time periods which contained approximately equal numbers of cases. There was a marked increase in the number of cases of MCD admitted from 1986 onwards (Figure 3.1); data were therefore compared for the years 1977-85, 1986-89 and 1990-1993. Table 3.1 shows the changes in clinical presentations, disease severity and mortality for each time period. There was a significant increase in the proportion of cases with septicaemia alone, rising from 7% in 1977-85 to 36% in 1990-93 ($p<0.0005$).

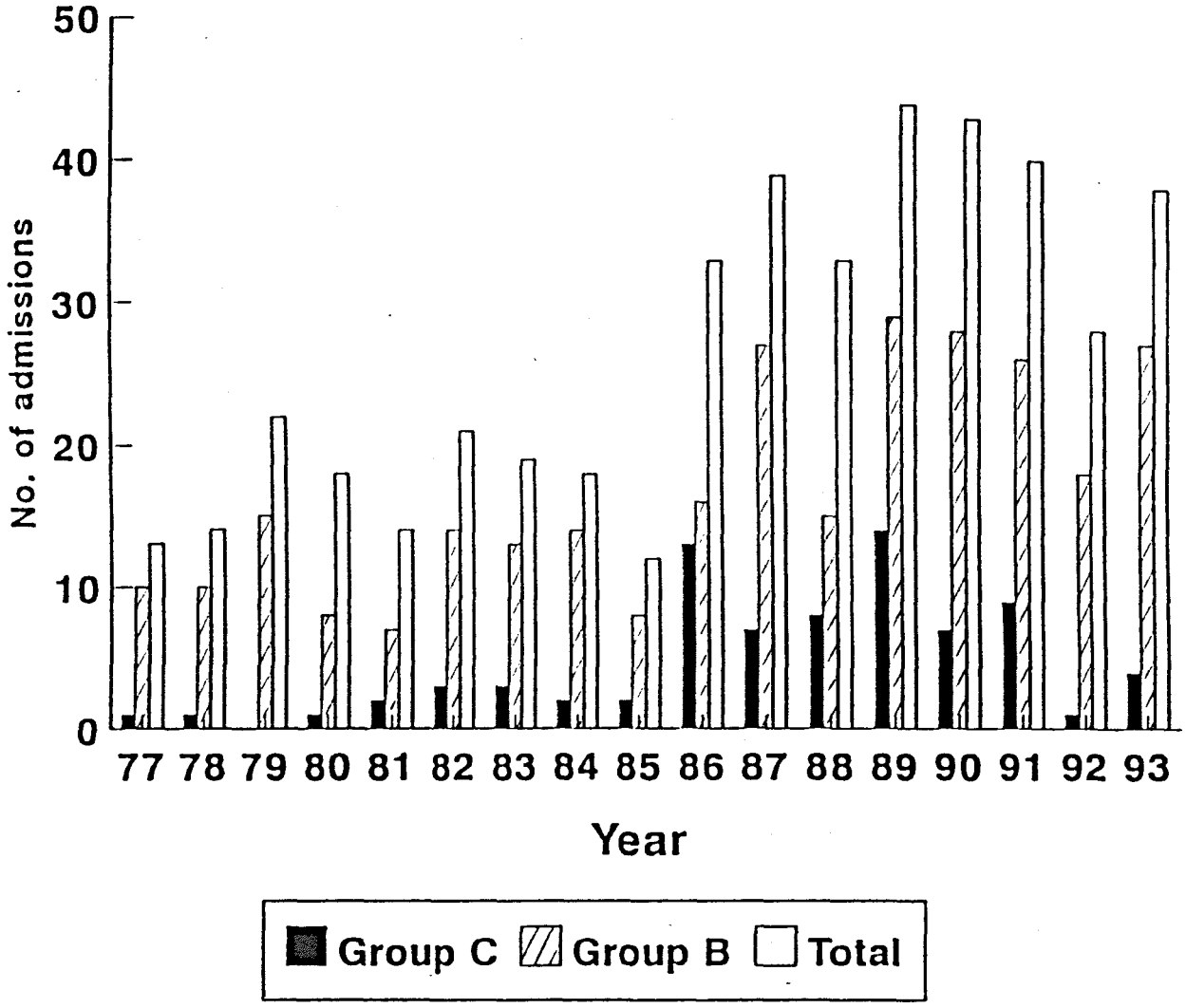



Figure 3.1. Annual number of admissions of children with meningococcal disease to the Royal Liverpool Children's Hospitals by serogroup, 1977-1993.

Table 3.1. Comparison of clinical presentation in all cases of meningococcal disease for 1977-85 vs 1986-89 vs 1990-93. No. of episodes(%), except age median(range).

| | 1977-85 (n=151) | 1986-89 (n=149) | 1990-93 (n=149) |
|---|---|---|---|
| Age (months) | 13(0.3-163) | 16(1-168) | 16(0.8-178)[c] |
| Deaths | 17(11.3) | 13(8.7) | 20(13.4) |
| MS | 11 (7)[a] | 41 (28) | 54 (36)[d] |
| MM+MS | 102 (68)[a] | 81 (54) | 74 (50)[c] |
| MM | 38 (25) | 26 (17) | 21 (14)[c] |
| Septic arthritis | 0 | 1 (0.6) | 0 |
| DGH* | 4 (3)[a] (n=146)# | 13 (9)[b] (n=146)# | 35 (24)[d] (n=145)# |
| Kahn ≥3 | 16 (11) | 18 (12) | 28 (19)[c] |

*DGH=Referral from other district general hospital.

#Kahn score not calculated on 12 children due to lack of data.

[a] Difference between 1977-85 and 1986-9 $p<0.05$

[b] Difference between 1986-89 and 1990-3 $p<0.0005$

[c] Difference between 1977-85 and 1990-3 $p<0.05$

[d] Difference between 1977-85 and 1990-3 $p<0.0005$

The annual incidence of meningitis alone stayed approximately stable throughout the 17 years (at 4 to 6 cases/year), but the proportion of cases with meningitis plus septicaemia and meningitis alone both showed significant decreases.

There was also a significant increase in disease severity (as measured by the Kahn score) and age at presentation in the last 4 years of the study. The proportion of tertiary referrals also increased significantly from 3% to 24%.

### 3.3.2 Changes in mortality

These increases in the proportion of children with the more severe and life threatening forms of MCD might be expected to lead to a rise in mortality. Mortality, however, was not significantly different in the three time periods.

Mortality from septicaemia alone fell from 36% (4/11) in 1977-85 to 17% (16/95) during 1986-93. Mortality from meningitis plus septicaemia also fell from 13% (13/102) to 10% (16/155), but neither fall achieved statistical significance. These changes in mortality may be confounded by the increasing disease severity. To remove this potential confounder mortality amongst those with severe disease, as defined by a Khan score above 3, was examined. During 1977-85, 10 of the 16 children with a Khan score of 3 or more died (62.5% [CI 35-85]). This is not significantly different from the expected 79% mortality (Kahn & Blum 1978). Mortality in those with a Khan score of 3 or more during 1986-93 was not significantly different from that in 1977-85 (20/46; 43% [CI 29-59)]), but was significantly less than the expected 79%.

### 3.3.2.a Influence of tertiary referrals

Another potential confounder is the increasing number of tertiary referrals. This could account for the rise in cases of septicaemia, disease severity and age at presentation. The data were therefore re-analysed to exclude tertiary referrals (See Table 3.2). The increase in cases of septicaemia remained highly significant ($p<0.0005$) despite excluding these children. However the increase in disease severity and age at presentation disappeared, suggesting that this increase was due to a rising number of older children referred for intensive care from other hospitals. Table 3.3 shows that mortality was significantly greater in the group with septicaemia alone (18.8%) compared with those children with meningitis plus septicaemia (11.3%) or meningitis alone (1.2%). Disease severity, as measured by the Kahn score, was also greatest in the group with septicaemia alone. The proportion of cases due to serogroups B or C meningococci, or those referred from other hospitals was not significantly different between the 3 types of presentation.

### 3.3.2.b Influence of season

Children with MCD were admitted less often between June and September (Figure 3.2). Mortality during these summer months was 11% and was not different from that for the rest of the year (12/107 vs 38/342). The proportion of children brought in dead was significantly higher during June to September (5/12 deaths), compared with the rest of the year (5/38 deaths, $p<0.05$ by Fisher's Exact Test)

Table 3.2. Comparison of clinical presentation in direct admissions with meningococcal disease for 1977-85 vs 1986-89 vs 1990-93.
No. of episodes(%), except age median(range).

| | 1977-85 (n=147) | 1986-89 (n=136) | 1990-93 (n=114) |
|---|---|---|---|
| Age (months) | 13(0.3-163) | 14(1-168) | 14(0.8-119) |
| Deaths | 15(10.2) | 12(8.8) | 12(10.5) |
| MS | 11 (8)[b] | 37 (27) | 43 (38)[d] |
| MM+MS | 99 (67)[a] | 73 (54) | 54 (47)[c] |
| MM | 37 (25) | 25 (18) | 17 (15)[c] |
| Septic arthritis | 0 (n=142)# | 1 (0.7) (n=133)# | 0 (n=111)# |
| Kahn ≥3 | 14 (9) | 12 (9) | 15 (13) |

#Kahn score not calculated on 11 children due to lack of data.

[a] Difference between 1977-85 and 1986-9 $p<0.05$

[b] Difference between 1977-85 and 1986-9 $p<0.0005$

[c] Difference between 1977-85 and 1990-3 $p<0.05$

[d] Difference between 1977-85 and 1990-3 $p<0.0005$

Table 3.3. Comparison of age, referral pattern, disease severity, causative serogroup and mortality for 3 presentations of meningococcal disease. No. of episodes(%), except age median(range).

| | MM (n=85) | MM+MS (n=257) | MS (n=106) |
|---|---|---|---|
| Age (months) | 11(0.8-178) | 14(0.3-168) | 19.5(3-163)[d] |
| DGH* (n=52) | 6(7) | 31(12) | 15(14) |
| Kahn≥3 (n=62) | 0[a] | 35(14)[c] | 27(25)[•] |
| Group B (n=284) | 53(62) | 163(63) | 68(64) |
| Group C (n=78) | 11(13) | 48(19) | 19(18) |
| Deaths (n=50) | 1(1.2)[a] | 29(11.3)[b] | 20(18.8)[•] |

*DGH=Referral from other district general hospital.

[a] Difference between MM and MM+MS $p<0.01$

[b] Difference between MM+MS and MS $p\leq0.05$

[c] Difference between MM+MS and MS $p<0.01$

[d] Difference between MM and MS $p\leq0.01$

[•] Difference between MM and MS $p<0.0005$

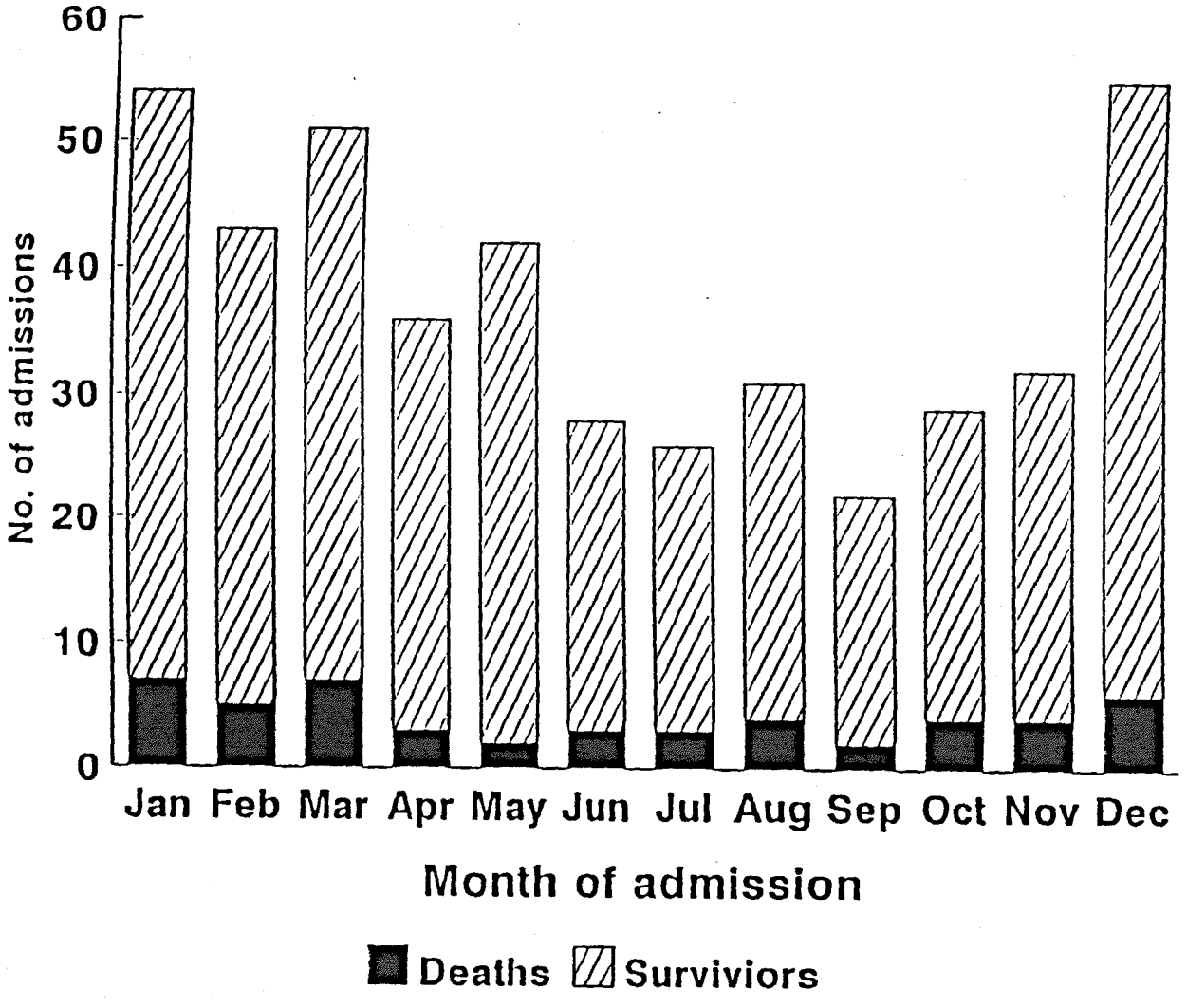



Figure 3.2. Number of episodes and deaths from meningococcal disease per month, Royal Liverpool Children's Hospitals, 1977-1993.

### 3.3.3 Group C Infections.

Serogrouping was available for 384 (86%) cases, 285 (74%) of which were group B. Group C disease occurred in 78 (20%) cases.

The total number of cases of MCD rose from 1986 onwards; serogroup data were therefore compared for the years 1977-85 and 1986-93. (See Figure 3.1)

The proportion of cases due to group C meningococci increased significantly from 1986 (10% vs 21% p=0.003), and there was a significant decrease in the number of cases due to serogroups A and W-Z (10% vs 2% p=0.0004) (See Table 3.4). There was no change in the overall proportion of cases due to group B meningococci over this time, although the total number of cases increased (99 vs 186). Mortality due to group C increased from 1 of 15 cases (6%) during 1977-85 to 10 of 63 cases (16%) in 1986-93. During the latter period group C meningococci were responsible for 10 (30%) of the 33 deaths due to MCD. All cases of fatal group C disease occurred in children 4 months of age or older.

Table 3.5 compares the age of children with group B and group C infection. The median ages (range) were 14 months (0.3-168) for group B and 14.5 months (1-162) for group C. These ages were not significantly different and the proportion of children aged less than 2 years or over 4 months was also similar.

Clinical presentations were also similar. Septicaemia without meningitis occurred in 68 cases with group B infection (24%) and 19 cases of group C infection (24%).

Table 3.4. Mortality by serogroup for 449 admissions with meningococcal disease. No. of cases (%).

| Serogroup | Year of admission | | | | | |
|---|---|---|---|---|---|---|
| | 1977-85 | Deaths | 1986-93 | Deaths | Total | Deaths |
| Group B | 99 (66) | 14 | 186 (62) | 19 | 285 | 33 |
| Group C | 15 (10) | 1 | 63 (21) | 10 | 78 | 11 |
| Other* | 15 (10) | 0 | 6 (2) | 1 | 21 | 1 |
| Unknown | 22 (14) | 2 | 43 (14) | 3 | 65 | 5 |
| Total | 151 | 17 | 298 | 33 | 449 | 50 |

*Other serogroup found; A (n=4), W135 (n=2), X (n=1), Y (n=4), Z (n=2), A-D (n=2), A/Y (n=1), X-Z (n=1) and non groupable (n=4, 1 died).

Table 3.5. Comparison of age and outcome in 363 cases of meningococcal disease due to group B or group C meningococci.

| | Group B | | Group C | |
|---|---|---|---|---|
| | No.cases (%) | Deaths | No.cases (%) | Deaths |
| Age: | | | | |
| In those >2 years | 98(34) | 12 | 24(31) | 6 |
| In those≥4 months | 256(90) | 28 | 68(87) | 11 |
| Totals | 285 | 33 | 78 | 11 |

Mortality was slightly higher in children with group C disease (14%) compared to children with group B (11.6%), but this difference did not achieve statistical significance.

## 3.4 DISCUSSION

This retrospective study shows that despite a significant increase in the proportion of cases of MCD presenting as septicaemia alone (the presentation with the highest mortality), mortality has remained constant. The use of a conjugate group C vaccine however could produce a significant decrease in mortality.

### 3.4.1 Changing clinical presentations.

The proportion of cases presenting with a septicaemic component, and therefore with a petechial or purpuric rash, rose from 75% in 1977-85 to 86% in 1990-93. Mortality was significantly greater in those with septicaemia alone compared to those with a meningitic component to their disease. There was also an increase in the severity and age at presentation of MCD seen in RLCHs, due to an increase in the number of tertiary referrals.

### 3.4.1.a Changes in mortality

Despite these increases in factors that are associated with an increased mortality, the mortality did not change significantly over the 17 years studied. This may actually represent an improvement in mortality, since an increase in disease severity has led to increased mortality in other centres (Andersen 1978; Havens et al 1989). There was a trend to decreased mortality in the MS group (Table 3.1), and in those with Kahn scores above 2. The active research program and more aggressive treatment in recent years may have caused this decreasing trend in mortality. There can be no room for complacency however, as one in ten children admitted with MCD died.

### 3.4.1.b Accuracy of data on clinical presentations

Accurate UK data on the presentations of MCD prior to 1988 are lacking, as meningococcal septicaemia was only notifiable after this time (Baxter & Payne 1993). Data based on notification figures may also be unreliable since under notification is commón (Harvey et al 1989).
The current study identified cases from clinical, laboratory and autopsy sources, as well as from prospective studies. The study included both confirmed and probable cases, and is therefore likely to be a true reflection of the changing presentations of MCD. This study has excluded "possible cases" of MCD (ie. those with fever and a haemorrhagic rash but no laboratory confirmation), because of the difficulties of reliable diagnosis and case ascertainment in a retrospective study.

During the prospective studies "possible cases" made up 23% of the total (Marzouk 1995; See Chapter Two), with half having septicaemia alone and the other half having meningitis plus septicaemia. The increase in MS may thus be even greater if possible cases were included.

There are a number of explanations for the increase in cases of septicaemia. Increasing concern over the dangers of lumbar puncture may explain the fall in cases of meningitis, with a reluctance to perform a lumbar puncture in a child with an obvious meningococcal rash (Harper et al 1985). To remove this possible bias children with a rash and meningitic signs, but who did not have a lumbar puncture, were included in the meningitis plus septicaemia group.

Septicaemia was not associated with tertiary referral or dependant on meningococcal serogroup. The rise in cases of septicaemia cannot therefore be explained by changes in referral pattern or serogroup prevalence. Infection with the subtype B15 P1.16, the Gloucestershire outbreak strain (Cartwright et al 1986), may be less likely to give neck stiffness (Palmer et al 1992). This strain has caused an increasing amount of infection recently (Jones & Kaczmarski 1992), and may explain the increase in septicaemia.

A true rise in septicaemia is supported by the fact that the proportion of cases of septicaemia has risen in other recent reports from the UK, being between 18% and 27% (Jones & Kaczmarski 1994; Palmer et al 1992).

An increase in meningococcal septicaemia is important since it carries the highest mortality (Andersen 1978; Fallon et al 1984). Nearly one in five children with septicaemia alone died in this study, compared to only one out of the 85 children with meningitis alone. (This diagnosis was made at post mortem as this child was brought in dead.)

3.4.1.c Focusing on Septicaemia.

The press (Thomson & Hayhurst 1993), the public and the medical profession (Tarlow & Geddes 1992) often refer to all meningococcal infections as "meningitis". Previously this was mostly correct as 93% of cases had a meningitic component to their disease prior to 1986 on Merseyside. However over a third of cases now do not have meningitis at all, but the more lethal septicaemia.

Focusing attention on "meningitis" can mean that the features of septicaemia are ignored (Thomson & Hayhurst 1993). Information for the public about MCD stressing the signs of meningitis is becoming increasingly inaccurate as the number of cases of septicaemia without meningitis rises. Information about MCD should therefore stress the features of the life threatening septicaemia, in particular the vasculitic rash (Thomson & Hayhurst 1993). Septicaemia has a different pathophysiology to meningitis (Heyderman et al 1993). New treatments based on an improved understanding of these disease processes are now being used (Tarlow & Geddes 1992). It is therefore necessary for doctors treating MCD to recognise the difference between septicaemia and meningitis because the treatments may differ.

3.4.1.c Influence of Season.

Mortality in this study did not vary with season in contrast to the study by Halstensen et al (1987), which found no deaths during the summer months. However the proportion of deaths that occurred outside hospital in the current study, was greater during the summer months (5/12, 42%) compared to the rest of the year (5/38, 13%). The reasons for this are not clear, but may be due to a decreased awareness of MCD during the summer. If the children brought in dead had not been included in this study, mortality during the summer months could have been falsely lowered. This underlines the importance of case ascertainment from both post mortem as well as hospital records.

3.4.2 Group C Infections

The proportion of cases of MCD due to group C meningococci in this population was 78 (20%) of the 384 tested, however this proportion has increased significantly since 1986. This is not just a local phenomenon as the incidence of Group C disease has been reported to have increased in the rest of the north of England during the study period (Abbott et al 1985). Since 1985 there has been a rise in the incidence of cases of both group B and group C MCD in England and Wales (Jones & Kaczmarski 1991), although fewer cases were seen during 1991-2 (Jones & Kaczmarski 1993). This trend is reflected in the data presented. Eleven of the 50 deaths (22%) from MCD were due to group C infection. However since 1986 group C meningococci were responsible for 10 of the 33 deaths (30%) from MCD.

### 3.4.2.a Age profile in Group C infection.

A significant difference in age between those with groups B and C disease was not demonstrated in this study. This is contrary to the results from a hospital based study from the United States (Baker & Griffiss 1983).
In the study by Baker and Griffiss (1983) only 27% of children with group C disease were less than 2 years of age, whereas in the current study 69% were under 2. Baker found group C disease was more common in adolescents and young adults than group B disease. This does not appear to be the case in the present study.
Adolescents over 16 years of age are unlikely to be admitted to paediatric hospitals and so an increase in group C disease in these young adults could have been missed in the present study. However data from England and Wales shows that group B sulphonamide-resistant strains cause as much infection in adolescents and young adults as group C strains (Jones & Kaczmarski 1991). A genuine difference in age between those with groups B and C disease is therefore unlikely.

### 3.4.2.b Potential for a conjugate vaccine.

Current strategies for preventing MCD involve giving chemoprophylaxis to close contacts of the disease (Cooke et al 1989), and offering vaccination to contacts of cases with group C disease. In this study only 5 cases occurred in contacts, the vast majority of cases were unconnected, as in other studies (Cooke et al 1989). To prevent MCD thus requires vaccination of the whole population, not just those in contact with cases.

No vaccine is currently available for group B meningococci (Jones 1993). The currently available polysaccharide vaccine against group C meningococci, effective in children over 2 years of age, would only have prevented 24 of the 449 cases of MCD seen in RLCHs.

Techniques to improve the immunogenicity of polysaccharide vaccines have focused on the conjugation of polysaccharides to proteins such as diphtheria or tetanus toxoid (Beuvery et al 1983). This strategy has proved very successful in producing the new vaccines against *Haemophilus influenzae* type b. Widespread usage of this vaccine has led to a dramatic decrease in *H influenzae* meningitis (Peltola et al 1992).

Conjugate vaccines against group C meningococci have now been developed and are in clinical trial (Costantino et al 1992; Anderson et al 1994). If a protein-conjugated group C meningococcal vaccine, given with the routine immunisations, could protect children 4 months of age and older, then 68 cases of MCD (15%), including 11 fatal cases, could have been prevented during the period of this study. Use of such a conjugate vaccine since 1986 could have prevented 30% of the deaths in this series due to MCD. This assumes a 100% vaccine uptake, in reality uptake may be 85%-90%. However even at this level the carriage rate and epidemiology may be sufficiently altered to prevent deaths from group C meningococci.

The proportion of cases of MCD in England and Wales due to group C meningococci decreased in 1992 (Jones & Kaczmarski 1993). It is possible that group C disease only increased during the recent upsurge in cases of MCD, and may now decrease to previous levels. Further prospective, population based studies of MCD will be needed to fully assess the impact of group C vaccines.

### 3.4.3 Conclusions

In conclusion, life threatening MCD does not present as meningitis, but as septicaemia. This potentially fatal presentation has increased dramatically in the last few years. The emphasis in MCD needs to be shifted away from meningitis and towards septicaemia. Publicity about the features of septicaemia, especially the vasculitic rash, can be lifesaving (Riordan & Thomson 1993) and should replace information that refers to MCD as "meningitis".
This study also shows that an increasing proportion of cases of MCD in children over the past 17 years has been due to serogroup C. Since 1986, group C meningococci were responsible for 30% of the deaths from MCD in RLCHs.
The currently available polysaccharide vaccine would not protect the majority of children who contract group C disease, but a conjugate vaccine might decrease the mortality from MCD by up to 30%. The development and usage of such a vaccine may thus have a significant impact on the continuing high mortality from MCD.

# CHAPTER FOUR. FEATURES NOTED BY PARENTS AND DOCTORS

# CHAPTER FOUR. FEATURES NOTED BY PARENTS AND DOCTORS

## 4.1 INTRODUCTION.

Meningococcal disease can have a sudden onset and may kill within hours of the start of symptoms (Oakley & Stanton 1979). During such a rapid disease process even short delays in diagnosis and treatment may decrease the chances of survival. After reviewing all deaths from MCD during 1978, Slack (1982) concluded;

> "if the toll of deaths from this life-threatening infection is to be diminished, the only avenue is reduction of the interval between the onset of symptoms, diagnosis and treatment."

### 4.1.1 Avoidable delays in Meningococcal disease.

Slack identified several areas in which avoidable delays occurred (Table 4.1). The most frequent and lengthy delays were parents not recognising that their child was seriously ill and doctors failing to make a diagnosis of MCD.

Over a decade later Slack's recommendations do not seem to have been heeded. Children still die from MCD and delays in recognition of the disease by parents, general practitioners (GPs) and hospital doctors are still thought to be major causes for the continuing high mortality (Nadel et al 1994).

Table 4.1. Reasons for delay in diagnosis and treatment in 86 deaths from Meningococcal disease in 1978 (After Slack 1982).

| Delay in: | No. affected(%)* |
|---|---|
| Calling GP | 21 (24) |
| GP visiting after called | 11 (13) |
| Hospital admission after GP visit | 22 (26) |
| Treatment after hospital referral | 19 (22) |
| No delays | 15 (17) |

*Some patients experienced more than one delay.

### 4.1.2 Appropriate information about Meningococcal disease

Parents and doctors thus require accurate and appropriate information about MCD. However, the information commonly available may be misleading, often focusing on "meningitis" rather than the more serious septicaemia (Thomson & Hayhurst 1993).

Meningococcal disease has a spectrum of presentations (Thomson et al 1990). Giving parents and primary care doctors information about all these presentations could be confusing. To decrease mortality attention should focus on life-threatening MCD, that is septicaemia. Awareness of the features of life-threatening disease that parents notice, and seek medical advice about, could help produce appropriate information for the public. Few studies record the signs noted by parents before admission, or their reasons for seeking medical advice. Those that have been performed have found that parents noticed only non-specific signs of MCD and sought medical advice because of them (Olcén et al 1979; Tønjum et al 1983)(See Table 1.1). Pre-admission parenteral antibiotics may decrease the mortality from MCD (Cartwright et al 1992c). Knowledge of doctors' ability to diagnose MCD and give pre-admission treatment can highlight areas that need particular education.

This study aims to examine two areas of the natural history of early MCD. Firstly, the features that parents notice and why they seek medical advice. Secondly, whether doctors recognise MCD in it's early stages, and if so, whether they give pre-admission antibiotic treatment.

These questions will focus particularly on those children with life-threatening MCD.

## 4.2 METHODS

The methods have been described in chapter 2. In brief the parents of children treated for MCD at any of the 4 participating hospitals were interviewed on admission. Parents were asked about specific features they had noticed, their reasons for seeking medical advice and the outcome of any contact with a doctor. Children were later classified as MCD (Meningococcal Disease Surveillance Group 1976) or controls. Children with "other serious infections" (n=10) might have benefited from early diagnosis and treatment, and were excluded from further analysis.

## 4.3 RESULTS

One hundred and twenty six children with MCD were admitted during the study. Severe disease affected 46 children, 13 of whom died. Fifty seven children had MS, 59 had MM+MS and 10 had MM. Twelve children died from MS and one from MM+MS.

Seventy seven controls were admitted none of whom died. Table 4.2 shows the number of children admitted to each hospital. Most children were admitted to Alder Hey, including significantly more controls ($p<0.0001$). This may be due to a greater awareness of MCD at Alder Hey, so that almost all children with fever and petechiae were initially treated for MCD.

Table 4.2. Admitting hospitals for children with suspected Meningococcal disease.

Data shown as number of children (%).

| | Severe MCD (n=46) | Other MCD (n=80) | Controls (n=77) | Other[a] (n=10) |
|---|---|---|---|---|
| Alder Hey | 25(54) | 45(56) | 66(86)* | 5 |
| Arrowe Park | 4 (9) | 11(14) | 6 (8) | 2 |
| Chester | 4 (9) | 7 (9) | 1 (1) | 1 |
| Whiston | 1 (2) | 14(17) | 4 (5) | 0 |
| Other Hosp[b] | 12(26) | 3 (4) | 0 | 2 |

[a]Other=Serious infections other than MCD.

[b]Other hosp=Tertiary referral to PICU at Alder Hey.

*Difference between all MCD and controls $p<0.0001$ by $Chi^2$.

Table 4.3. Clinical data on admission of children with suspected Meningococcal disease. Statistical analysis by $Chi^2$ or Mann-Whitney U test.

| | Severe MCD (n=46) | Other MCD (n=80) | Controls (n=77) |
|---|---|---|---|
| Age in months [Median (range)] | 18(3-168) | 21(3-168) | 17(1.6-148) |
| Duration in hours of: | | | |
| Symptoms | 13(2-48) | 19(0.7-136)+ | 21(0.7-421)* |
| Symptoms before rash | 11(0-48) | 12(0-79) | 16(0-365) |
| Rash | 1.6(-8-10) | 2.1(-6-66) | 0.7(-5-168) |
| Number of children (%) with; | | | |
| Rash present: | | | |
| Maculopapular only | 1(2%) | 3(4%) | 5(7%) |
| Petechial | 43(93%) | 69(86%) | 67(87%) |
| Ecchymotic | 33(72%) | 13(16%)+ | 2 (3%)* |
| Neck stiffness | 15(32%) | 42(53%) | 12(16%)* |
| Coma[a] | 10(22%) | 8(10%) | 3 (4%)* |

*Difference between MCD and controls $p<0.02$.

+Difference between Severe MCD and less severe MCD $p<0.01$.

[a]Glasgow coma score less than 8

Children with MCD and controls were similar both in age and in the proportion of children with a petechial rash (Table 4.3). However children with MCD were significantly more likely to have ecchymotic lesions ($p<0.0001$). Children with MCD had a shorter duration of symptoms before admission than controls ($p=0.02$), and those with severe MCD also had a shorter duration than those with less severe disease. However there was no difference between those with MCD and controls in the time from the onset of a rash until admission.

### 4.3.1 Features noted by parents

The first symptoms noted in children with MCD were fever (40%), lethargy (24%) and vomiting (14%). All children with MCD, and all except three controls, developed at least one of these non-specific symptoms before admission. A petechial rash was noted in children with MCD a median of 11 hours (range 0-79) after the start of symptoms. Parents of children with MCD were significantly more likely to notice; rapid deterioration in condition (over the previous hour), vomiting and rash (Table 4.4). In severe MCD; cyanosis, deterioration, diarrhoea, vomiting and rash were all significantly more common than in controls. Parents rarely noticed headache or neck stiffness in severe MCD. One hundred and thirteen children (90%) with MCD (including all those who died), developed a rash or cyanosis, or deteriorated before admission, compared to only 55 (71%) controls. This combination of features was the most sensitive predictor of MCD (Table 4.5).

Table 4.4. Features noted by parents before admission in suspected Meningococcal disease. Data shown as number(%). Statistical analysis by $Chi^2$.

| | All MCD (n=126) | Controls (n=77) | Relative risk (95% CI) | Severe MCD (n=46) | Controls (n=77) | Relative risk (95% CI) |
|---|---|---|---|---|---|---|
| Cyanosis | 15(12) | 3 (4) | 3.06(0.91-10.2) | 11(24)# | 3 (4) | 6.14(1.81-20.9) |
| Deterioration | 46(37)* | 12(16) | 2.34(1.33-4.14) | 21(46)# | 12(16) | 2.93(1.60-5.38) |
| Headache | 41(33) | 14(18) | 1.79(1.05-3.10) | 7(15) | 14(18) | 0.84(0.36-1.92) |
| Vomiting | 96(76)* | 35(46) | 1.68(1.28-2.18) | 32(70)# | 35(46) | 1.53(1.12-2.09) |
| Diarrhoea | 31(25) | 12(16) | 1.57(0.86-2.89) | 18(39)# | 12(16) | 2.51(1.33-4.73) |
| Rash | 96(76)* | 45(58) | 1.31(1.06-1.61) | 37(80)# | 45(58) | 1.38(1.10-1.74) |
| Irritable | 80(64) | 37(48) | 1.32(1.01-1.73) | 25(54) | 37(48) | 1.13(0.80-1.61) |
| Lethargy | 112(89)* | 58(75) | 1.18(1.02-1.36) | 38(83) | 58(75) | 1.09(0.91-1.32) |
| Neck Stiffness | 24(11) | 13(17) | 1.13(0.61-2.10) | 3 (7) | 13(17) | 0.39(0.12-1.28) |
| Fever | 122(97)* | 67(87) | 1.11(1.02-1.22) | 43(93) | 67(87) | 1.07(0.96-1.20) |
| Seizures | 12(10) | 8(10) | 0.92(0.39-2.14) | 3 (7) | 8(10) | 0.63(0.18-2.25) |

* Difference between all MCD and controls $p<0.01$.

# Difference between Severe MCD and controls $p<0.01$.

Table 4.5 Predictive value for meningococcal disease of features noted by parents.

| | Sens | Spec | PPV | NPV |
|---|---|---|---|---|
| Lethargy | 88 | 25 | 66 | 42 |
| Rash | 76 | 42 | 68 | 48 |
| Vomiting | 73 | 55 | 73 | 42 |
| Fever | 65 | 29 | 97 | 13 |
| Irritable | 63 | 52 | 68 | 53 |
| Deterioration | 37 | 84 | 79 | 55 |
| Headache | 33 | 82 | 75 | 57 |
| Diarrhoea | 25 | 84 | 72 | 59 |
| Neck stiffness | 19 | 83 | 65 | 61 |
| Cyanosis | 12 | 96 | 83 | 60 |
| Rash or Cyanosis or Deterioration | 90 | 29 | 67 | 37 |
| Neck stiffness or Rash | 83 | 29 | 65 | 50 |
| Fever+Rash+Headache+ Neck stiffness | 10 | 99 | 92 | 60 |

Key

Sens=Sensitivity

Spec=Specificity

PPV=Positive predictive value

NPV=Negative predictive value

### 4.3.2 Reasons for seeking medical advice.

Rash was the commonest reason for calling a doctor in the MCD group (52%) (See Table 4.6). Advice was rarely sought for vomiting (10%), irritability (5%), headache (4%) or neck stiffness (2%). Significantly more parents of children with MCD sought advice because of a rash than controls (Relative risk 2.21 (1.39-2.25)), and this was also true in severe MCD (Relative risk 2.55 (1.63-4.00)). This may be because rash was noted more often in the MCD group. Table 4.7 therefore shows the "likelihood of seeking advice" for each feature. This is the proportion of parents who sought medical advice because of a feature, when that feature was present. The greatest likelihood of seeking advice was for seizures (83-100%). The next greatest was for rash (63-78%). Parents were still significantly more likely to seek advice because of the rash of MCD compared to controls (Relative risk 1.63 (1.13-2.35), p=0.003). Thus, the only feature that worried parents more than the rash of MCD was the occurrence of a seizure. The likelihood of seeking advice in MCD because of vomiting, headache, neck stiffness or irritability was low, especially in severe MCD.

### 4.3.3 "Who spots the spots?"

Parents were asked who was the first person to notice a petechial rash. Table 4.8 shows that parents or relatives were the first to spot a rash in 69% of all cases of MCD, and in 80% of severe MCD.

Table 4.6. Reasons for seeking medical advice in suspected Meningococcal disease. Data shown as number(%). Statistical analysis by $Chi^2$.

| | Severe MCD (n=46) | Other MCD (n=80) | Controls (n=77) |
|---|---|---|---|
| Rash | 29(63)# | 37(46) | 19(25)* |
| Fever | 14(30) | 27(34) | 26(34) |
| Lethargy | 13(28) | 19(24) | 14(18) |
| Seizure | 3 (7) | 7 (9) | 7 (9) |
| Pallor | 2 (4) | 5 (6) | 2 (3) |
| Cyanosis | 2 (4) | 0 | 1 (1) |
| Vomiting | 1 (2) | 11(14) | 5 (6) |
| Irritable | 1 (2) | 5 (6) | 9(12) |
| Headache | 1 (2) | 4 (5) | 4 (5) |
| Neck stiffness | 0 | 3 (4) | 2 (3) |
| Deterioration | 0 | 2 (3) | 2 (3) |
| Diarrhoea | 0 | 1 (1) | 0 |

*Difference between all MCD and controls $p<0.01$.

#Difference between Severe MCD and controls $p<0.01$.

Table 4.7. Likelihood of parents seeking medical advice for specific features of suspected Meningococcal disease. Data shown as percent of those seeking advice because of a feature, when that feature is present.

| | Severe MCD | Other MCD | Controls |
|---|---|---|---|
| Seizure | 100 | 78 | 88 |
| Rash | 78 | 63 | 42* |
| Lethargy | 34 | 26 | 24 |
| Fever | 33 | 34 | 39 |
| Cyanosis | 18 | 0 | 33 |
| Headache | 14 | 12 | 29 |
| Irritable | 4 | 9 | 24* |
| Vomiting | 3 | 17 | 14 |
| Neck stiffness | 0 | 14 | 15 |
| Deterioration | 0 | 8 | 17 |

*Difference between all MCD and controls $p<0.005$ by $Chi^2$.

Table 4.8. First person to notice a petechial rash in children with suspected Meningococcal disease. Data shown as number (%).

| Observer | Severe MCD (n=46) | Other MCD (n=80) | Controls (n=77) |
|---|---|---|---|
| Parent | 37(80) | 49(61) | 40(52)* |
| Other relative | 0 | 6 (8) | 3 (4) |
| Nurse | 3 (7) | 4 (5) | 7 (9) |
| General Practitioner | 0 | 1 (1) | 7 (9)* |
| Hospital Doctor | 3 (7) | 10(13) | 15(19) |
| No petechial rash | 3 (7) | 10(13) | 5 (6) |

*Difference between all MCD and controls p=0.01 by $Chi^2$.

Family members were significantly less likely to notice a rash in those who did not have MCD, this rash being first noted more frequently by doctors.

### 4.3.4 Do doctors recognise early Meningococcal disease?

Sixty children with MCD (48%) were seen, but not admitted, by a doctor in the 48 hours before admission (Table 4.9). This was significantly more than the control group (18/77, 23%; p=0.0002). Of those with MCD seen before admission, 50 were seen by a GP (3 on two occasions). On questioning the parents, 29 of these children had non-specific signs when seen, 6 had a petechial rash and 15 had a maculopapular rash. Four children subsequently died, two who had been seen with no rash and two with a maculopapular rash. Ten children were brought to Accident and Emergency departments, but not admitted. Three had no rash when first seen and seven had a maculopapular rash. None of these children died. The presence of a maculopapular rash thus led to delays in diagnosis that may have contributed to two deaths.

Sixty nine children with MCD were admitted by general practitioners, who diagnosed "Meningococcal disease"(n=22) or "meningitis"(n=15) in 54% of them. Eight children were thought to have measles or chickenpox, 6 were diagnosed "Rash" (3 petechial), and 5 were referred as "PUO". A diagnosis of "Meningococcal disease" or "meningitis" was made in 17 of the 27 (61%) GP admissions with severe MCD compared with 20 of the 42 (48%) with less severe disease.

Table 4.9. Pre-admission contact with doctor and treatment in suspected Meningococcal disease.
Data shown as number(%).

| | Severe MCD (n=46) | Other MCD (n=80) | Controls (n=77) |
|---|---|---|---|
| Seen by doctor, but not admitted: | | | |
| In previous 2 days | 19(41) | 41(51) | 18(23)* |
| Day before admitted | 5(11) | 17(21) | 4 (5)* |
| Day of admission | 14(30)# | 18(23) | 8(10)* |
| Pre-admission antibiotics: | | | |
| Oral | 5(11) | 14(18) | 15(19) |
| GP admissions: | (n=27) | (n=42) | (n=44) |
| Diagnosed MCD or meningitis by GP | 17(61)# | 20(48) | 13(30)* |
| Pre-admission antibiotics; | | | |
| IM/IV penicillin | 12(44)# | 10(24) | 8(18) |

*Difference between all MCD and controls $p<0.05$ by $Chi^2$.
#Difference between Severe MCD and controls $p<0.05$ by $Chi^2$.

This difference was not statistically significant. Five (13.5%) of the children diagnosed as "Meningococcal disease" or "meningitis" died, compared to only one (3%) of those not diagnosed.

4.3.5 Do doctors give early antibiotic treatment?

4.3.5.a Pre-admission Antibiotics.

Oral antibiotics had been given to 19 children with MCD before admission, more often to those with less severe disease (18% vs 11%)(Table 4.9). Pre-admission parenteral penicillin was given to 22 of the 69 (32%) MCD cases admitted by GPs; 12 (44%) of those with severe MCD and 10 (24%) with less severe disease (Table 4.9). This difference was not statistically significant.
All children diagnosed as MCD or given pre-admission parenteral penicillin, had a rash before admission.

4.3.5.b General Practitioners diagnosis.

Pre-admission penicillin was only given to those in whom a diagnosis of "Meningococcal disease" or "meningitis" was made, except for one child diagnosed as having chickenpox! Pre-admission penicillin was given to 21 (57%) of the children with MCD when a diagnosis of "Meningococcal disease" or "meningitis" was made. However early penicillin was given significantly more often to children who were diagnosed as "Meningococcal disease" (18/22, 82%), compared to those diagnosed as "meningitis" (3/15, 20%; $p<0.0005$, Fisher's exact test).

When "Meningococcal disease" was diagnosed pre-admission penicillin was not given before admission because either the GP had none (n=3), or it was out of date (n=1). GPs thus often gave early parenteral antibiotics when they diagnosed "Meningococcal disease" but rarely when they diagnosed "meningitis".

Four children (18%) given pre-admission penicillin died, compared with two (4%) not given antibiotics by their admitting GP. This difference was not statistically significant.

### 4.3.6 Treatment delays after admission to hospital.

Appropriate antibiotic therapy was commenced prior to, or on admission in 111 (88%) children with MCD. In 15 children treatment was delayed. Ten of these children presented with a maculopapular rash which was attributed to a viral infection or a reaction to MMR immunisation (Riordan et al 1995b). One of these children died. Three children developed a petechial rash after admission and one of these children also died. Five of these children with maculopapular or late developing rashes also presented with febrile convulsions. Two children never developed a rash and the diagnosis of MM was only made when they had a lumbar puncture some time after admission. Delays in diagnosis on admission thus occurred in those with either a maculopapular rash or no rash, and/or a febrile convulsion.

## 4.4 DISCUSSION

This study shows that the features of life-threatening MCD which parents notice are; rash, cyanosis, deterioration, diarrhoea and vomiting. Ninety percent of children with MCD develop either a rash, cyanosis or deterioration before admission. Parents commonly notice and seek medical advice because of these features of septicaemia, but not because of features of meningitis. Delays in treatment occur when doctors do not recognise the rash of septicaemia, especially if it is maculopapular. Pre-admission antibiotics are given if the doctor makes a diagnosis of MCD rather than meningitis.

If the mortality from MCD is to be decreased by earlier treatment, then parents need to know the early signs of life-threatening MCD. Parents are currently advised to watch for the following features of MCD; headache, fever, vomiting, neck stiffness, coma, photophobia and lastly, rash (Department of Health & National Meningitis Trust 1994). This study shows that these may not be the most useful signs of life-threatening MCD in children.

### 4.4.1 Rash.

A vasculitic rash is a common feature of MCD (Thomson & Hayhurst 1993). The current study shows that parents are often the first to notice a petechial rash and that a rash is also the commonest reason for seeking medical advice in MCD. This differs from previous studies where advice was sought more often because of non-specific features of MCD (Olcén et al 1979; Tønjum et al 1983).

This may be because of an increased awareness of MCD in Merseyside, due to its high incidence (Abbott et al 1985).

The current study involved a highly selected population with fever and petechiae who were all initially treated for MCD. The proportion of children with a petechial rash was similar in the MCD and control groups. However parents were still significantly more likely to notice a rash, and to seek medical advice because of it, in MCD compared to controls. This may be because of the higher incidence of ecchymoses in children with MCD (Gedde-Dahl et al 1990c) making the rash different from other petechial rashes. Parents sought advice more often because of the rash of MCD than for any other feature except seizures.

The goal of early treatment in MCD should be to give antibiotics as soon as the diagnosis is made. The diagnostic sign in severe MCD is often the vasculitic rash, although this appeared on average 11 hours after more non-specific features. However the median time a rash was present before admission in severe MCD was only 1.6 hours. To shorten this time, treatment needs to be given practically as soon as the rash appears. Information about the significance of a vasculitic rash in a child with fever, lethargy or vomiting, and the need for urgent treatment, should therefore be given to those most likely to first notice the rash; that is to parents.

### 4.4.2 Non-specific signs.

All children with MCD and most controls had fever, lethargy or vomiting. These features thus might alert parents to the possibility of MCD, but are poor discriminators between MCD and less serious disease.
Fever and lethargy were commonly noted by parents in children with MCD. These non-specific signs were also common reasons for seeking medical advice as in previous studies (Olcén et al 1979; Tønjum et al 1983). However these symptoms, though seen significantly more often in MCD discriminated poorly between MCD and controls (relative risk 1.1). Vomiting was noted by 70% of parents of children with severe MCD, but parents rarely sought medical advice because of it. This contrasts with the findings of Olcén et al (1979) where 61% of patients sought advice because of vomiting. In the current study parents sought advice because of vomiting significantly more often in less severe MCD ($p=0.05$).

### 4.4.3 Signs of meningitis.

Headache and neck stiffness were rarely noticed by parents in severe MCD, even though neck stiffness was present on admission in one third of these children. This confirms parent's inability to detect or appreciate the significance of these features (Valmari et al 1987). Both these features were significantly more common amongst children with less severe MCD. Neither headache nor neck stiffness are therefore useful signs of life-threatening MCD and information advising parents to look for these features is inappropriate.

### 4.4.4 Signs of life-threatening disease.

In severe MCD cyanosis, deterioration and diarrhoea were all significantly more common, confirming their association with a poor prognosis (Gedde-Dahl et al 1990c). Parents however rarely sought advice because of these features.

To allow early diagnosis of severe MCD parents should be told to seek medical advice for any child who is febrile, lethargic or vomiting who then develops a rash or cyanosis, or who deteriorates rapidly.

### 4.4.5 Delays in diagnosis.

Nearly half the children with MCD had been seen by a doctor, but not admitted, in the 48 hours before admission as in other studies (Sørensen et al 1992a; Nadel et al 1994). Thirty percent of parents of children with severe MCD sought medical advice twice on the day of admission. Parents therefore do appreciate that their child requires medical attention, and will persist until they receive it. When a doctor was first called, the child often had non-specific signs (fever, lethargy, vomiting), making the diagnosis of MCD extremely difficult. However 22 children (37%) who were seen and not admitted had a maculopapular rash, and 6 (10%) had a petechial rash. None of those seen with a petechial rash died, implying these children may have had less severe disease making the doctor less likely to make the correct diagnosis. However 2 children seen, but not admitted, with a maculopapular rash later died.

Treatment was also delayed following admission in 10 children with maculopapular rashes, one of whom also died. Avoidable delays in treatment leading to death thus occurred when a maculopapular rash was present. This confirms previous studies from our hospitals and elsewhere (Marzouk et al 1991a; Oakley & Stanton 1979; Slack 1982). When called to a child who is non-specifically unwell with fever, lethargy or vomiting, doctors could ask parents to call them again if the child then develops a rash or cyanosis, or deteriorates rapidly. If such a child develops a maculopapular rash, MCD needs to be considered.

4.4.6 Delays in treatment.

Pre-admission parenteral penicillin was given to less than a third of children with MCD admitted by GPs. This is a similar proportion to previous studies (Gossain et al 1992; Cartwright et al 1992b), despite most patients being less than 2 years old, the age group where diagnosis is most difficult (Mathiassen et al 1989). However unlike most other studies only 55% of children were referred to hospital by a GP, the rest being self referrals to A&E. Parenteral penicillin was only given to children with a rash. This is in contrast to a recent study of meningitis in the UK where pre-admission antibiotics were given more often to those without a rash (Research Committee of the BSSI 1995)

Parenteral penicillin is carried by most GPs (Ong & Dunbar 1988; Colbridge et al 1995), but may not be to hand when needed (Stevenson 1992). However the most important factor affecting whether children received pre-admission penicillin was the GP's diagnosis.

When a diagnosis of MCD was made, 18 out of 22 children (82%) were given penicillin. In the other cases no penicillin was available. In the 15 children diagnosed as having "meningitis", only 3 (20%) were given penicillin. Where another diagnosis, or no diagnosis was made penicillin was given to 1 child, thought to have chickenpox. All children diagnosed as MCD or given pre-admission penicillin had a petechial rash, as did 33 other children in whom meningitis or another diagnosis was made. To increase the numbers of children given pre-admission penicillin attention should focus on septicaemia, identified by the rash, and not "meningitis".

Advising GPs to give pre-admission penicillin for cases of suspected "meningitis" has lead to confusion about its value in MCD (Nanayakkara & Cox 1994). Only 1 in 10 children referred by GPs as "meningitis" actually have it (Nielsen et al 1988), and there is little evidence that children with bacterial meningitis benefit from pre-admission antibiotics (Talan et al 1988). Children with bacterial meningitis may however benefit from dexamethasone given with, or before, the first dose of antibiotic (Schaad et al 1993). Pre-admission antibiotics for bacterial meningitis should thus either be combined with dexamethasone, or deferred till admission if steroids are unavailable.

Life threatening MCD does not present as meningitis, but as septicaemia (Thomson & Hayhurst 1993). The proportion of cases with this life-threatening presentation is increasing (Jones & Kaczmarski 1994; Riordan et al 1995a).

Pre-admission penicillin should thus be recommended for cases of MCD presenting as septicaemia, with a petechial or purpuric rash. Such "on the spot" treatment may help reduce the mortality from this devastating infection.

In conclusion, parents of children with MCD often recognise that their child is ill and seek medical advice during the early stages of the illness. Children with MCD initially have non-specific symptoms (fever, lethargy, vomiting), but then develop rash, cyanosis or deterioration in 90% of cases. Parents are often the first to notice the petechial rash and commonly seek medical advice because of it, especially in severe MCD. They do not notice or seek advice about the features of meningitis. Information about MCD for parents should thus focus on signs of septicaemia (rash, cyanosis and deterioration) and not on signs of meningitis. Avoidable delays occur when doctors do not recognise the rash of MCD, particularly if the child has a maculopapular rash. The first doctor to see a child with MCD needs "knowledge out of proportion to their previous experience" (Welsby & Golledge 1990). MCD presenting with a maculopapular rash therefore needs to be highlighted for GPs, casualty officers and junior hospital doctors. Pre-admission penicillin is often given if MCD is diagnosed, but not if a diagnosis of "meningitis" is made. GPs and casualty officers need education to recognise the rash of MCD and to give "on the spot" treatment before admission, and not to delay by looking for signs of meningitis (Farmer 1993).

# CHAPTER FIVE. CYTOKINE RESPONSES.

# CHAPTER FIVE. CYTOKINE RESPONSES.

## 5.1 INTRODUCTION

The pro-inflammatory cytokines, TNF-α and IL-1β, either singly or together may be responsible for many of the harmful sequelae of MCD. High levels of TNF-α, IL-1β and IL-6 are associated with septic shock and death in MCD (Girardin et al 1988; Waage et al 1987; Waage et al 1989b). IL-10, an anti-inflammatory cytokine produced by T cells and macrophages, is an important regulator of immune function (Fiorentino et al 1989). IL-10 can inhibit the production of TNF-α, IL-1ß and IL-6 (de Waal Malefyt et al 1991b, Fiorentino et al 1991) and increase production of their natural inhibitors (de Waal Malefyt et al 1993, Joyce et al 1994). IL-10 also inhibits macrophage procoagulant activity (Pradier et al 1993; Ramani et al 1993) and the production of toxic oxygen radicals (Bogdan et al 1991). IL-10 production may thus influence the progression of septic shock and survival in MCD. IL-10 can protect animals after an endotoxic challenge, by decreasing the amount of TNF-α produced (Howard et al 1993). Adults in septic shock have high levels of IL-10 (Marchant et al 1994a; Gómez-Jiménez 1995), as do children with meningococcal septic shock (Derkx et al 1995). No studies correlating IL-10 levels with other cytokines have been performed in children or in MCD.

This study sought to measure levels of IL-10 in children with MCD, and to compare them with levels of the pro-inflammatory cytokines (TNF-α, IL-6) in those who died and in survivors.

## 5.2 MATERIALS AND METHODS.

### Cytokine Assays.

### 5.2.1 Tumour Necrosis Factor-α

#### 5.2.1.a Materials

Microtitre plate with 96 anti-TNF-α coated wells.
TNF-α standards (0,15,50,150,500,1500 pg/ml):
each reconstituted with 1ml distilled water.
Controls: reconstituted with specified amount of distilled water.
Washing solution (Tween 20): 2ml diluted in 400 ml distilled water.
Borate solution ready for use.
Conjugate solution: 0.6 ml anti-TNF-α-horse radish peroxidase (HRP) conjugate, added to 6 ml conjugate buffer.
Revelation solution: 0.2ml of chromogen tetramethylbenzidine (TMB), added to 21 ml substrate buffer.
Stopping reagent ($H_2SO_4$ 1.8 N).

#### 5.2.1.b Method.

Standards, controls and washing, revelation and conjugation solutions were made up as indicated above.

Tumour necrosis factor-α was measured by an immunoenzymatic assay (Medgenix Diagnostics, Brussels) (Figure 5.1).

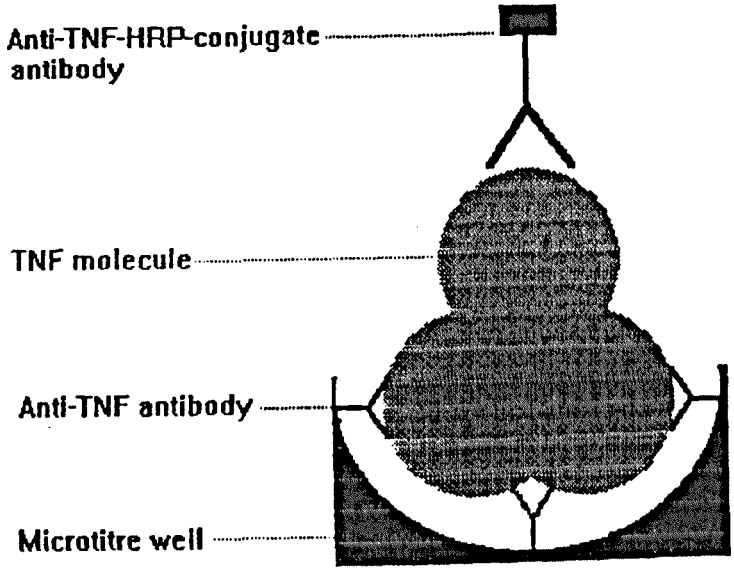



Figure 5.1 Schematic diagram of immunoenzymatic assay. The TNF-α molecule is "sandwiched" between the antibody-coated well and an antibody conjugated to HRP. When the revelation solution is added, it reacts with the conjugated antibody to produce a measured colour change.

A 96-well microtitre plate is coated with several monoclonal antibodies against different epitopes of TNF-α. Any TNF-α in the sample added to each well was bound by these monoclonal antibodies. The remaining sample was removed by washing and anti-TNF-α antibodies conjugated to HRP were added. These conjugated antibodies bound to any TNF-α in the well and the excess was then washed away. A substrate was added which was hydrolysed by the HRP on the conjugated antibodies. The change in colour was thus proportional to the amount of TNF-α in the well. The result was measured by the absorbance of light at 450 and 490 nm. The absorbance was plotted against the standards and sample concentrations were then calculated from this calibration curve. The assay was performed according to the manufacturers instructions, with no modifications. The sensitivity of the assay was 10pg/ml.

## 5.2.2 Interleukin-6

### 5.2.2.a Materials

Microtitre plate with 96 anti-IL-6 coated wells.
IL-6 standards (0,20,50,150,500,1000,2000 pg/ml):
each reconstituted with 1ml distilled water.
Controls: reconstituted with 1 ml distilled water.
Washing solution (Tween 20): 2ml diluted in 400 ml distilled water.
Borate solution ready for use.
Conjugate solution (anti-IL-6-HRP) ready for use.
Revelation solution: 0.2ml of chromogen TMB added to 21 ml substrate buffer.
Stopping reagent ($H_2SO_4$).

5.2.2.b Method

Interleukin-6 was measured by an immunoenzymatic assay (Medgenix Diagnostics, Brussels), similar to that described for TNF-α. The assay was performed according to the manufacturers instructions, with no modifications. The sensitivity of the assay was 1pg/ml.

5.2.3 Interleukin-10

5.2.3.a Materials

Microtitre plate with 96 anti-IL-10 coated wells.
IL10 standards (0,11,40,120,480,1335 pg/ml):
each reconstituted with 1ml distilled water.
Controls: reconstituted with 1 ml distilled water.
Washing solution (Tween 20): 2ml diluted in 400 ml distilled water.
Borate solution ready for use.
Conjugate solution (anti-IL-10-HRP) ready for use.
Revelation solution: 0.2ml of chromogen TMB added to 21 ml substrate buffer.
Stopping reagent ($H_2SO_4$ 1.8N).

5.2.3.b Method.

Interleukin 10 was measured by an immunoenzymatic assay (Medgenix Diagnostics, Brussels), similar to that described for TNF-α. The assay was performed according to the manufacturers instructions, with no modifications. The sensitivity of the assay was 1pg/ml, and the coefficient of variation was 2.8%.

### 5.2.4 Sample collection

One millilitre of blood was collected on admission, or at the next time blood was taken after admission. Blood was taken using sterile technique (or from an indwelling arterial catheter), kept on ice and then separated as soon as possible after collection. Serum was stored at -70°C until assayed. Samples were assayed blind, in batches at the end of the study by a single technician. Cytokine levels were only measured in children with MCD on PICU.

## 5.3 RESULTS.

### 5.3.1 Interleukin 10.

IL-10 was measured in 53 children with MCD, 9 of whom died. Levels were significantly higher in those who died (1434pg/ml(range 549-1905) compared to survivors (143 pg/ml(range 1-1995); $p<0.005$) (Table 5.1). Levels were also significantly higher in septic shock, severe disease and MS (See Tables 5.1 and 5.2). IL-10 levels correlated strongly with many prognostic factors (GMSPS, WBC, PT, APPT), as well as with the length of time from the start of symptoms (See Table 5.3). To study the association between IL-10 and the duration of symptoms, IL-10 levels were plotted against the length of time from the first symptom until the sample was taken. IL-10 was not detected in children who's symptoms began more than 30 hours before admission. Raised levels were mostly found in the first 24 hours after the onset of symptoms (Figure 5.2).

Table 5.1. Cytokine levels (pg/ml) on admission in children with Meningococcal disease. Data shown as median (range).

| TNF-α (n=42) | Feature Present | Absent | P* |
|---|---|---|---|
| Died (n=7) | 1660(831-2291) | 64(10-1603) | 0.0001 |
| Septic shock (n=20) | 568(18-2291) | 53(10-479) | 0.00001 |
| GMSPS≥8 (n=23) | 501(37-2291) | 45(10-479) | 0.00001 |
| DIC (n=15) | 832(281-2291) | 58(10-1972) | 0.00001 |
| IL-6 (n=47) | | | |
| Died (n=8) | 2630(2137-3180) | 1447(1-3983) | 0.008 |
| Septic shock (n=22) | 2707(131-3981) | 457(1-3020) | 0.00001 |
| GMSPS≥8 (n=26) | 2630(63-3180) | 524(1-3981) | 0.0003 |
| DIC (n=16) | 2834(2089-3180) | 525(1-3981) | 0.00001 |
| IL-10 (n=53) | | | |
| Died (n=10) | 1434(549-1905) | 14.3(1-1995) | 0.004 |
| Septic shock (n=25) | 1429(1.2-1906) | 1.2(1-1995) | 0.00001 |
| GMSPS≥8 (n=29) | 1412(1.2-1995) | 1.2(1-1737) | 0.00001 |
| DIC (n=18) | 1435(1405-1905) | 5.1(1-1995) | 0.00001 |

*Difference by Mann-Whitney U test.

Table 5.2. Cytokine levels (pg/ml) on admission across the spectrum of Meningococcal disease. Data shown as median (range).

| | MS | MM+MS | MM |
|---|---|---|---|
| TNF-α | (n=20) | (n=21) | (n=1) |
| | 531(36-2291)* | 60(10-1972) | 53 |
| IL-6 | (n=22) | (n=24) | (n=1) |
| | 2571(25-3180)* | 976(1-3981) | 1 |
| IL-10 | (n=24) | (n=27) | (n=2) |
| | 1421(1-1995)* | 4(1-1737) | 1.2(1-1.3) |

*Difference between MS and MM+MS, $p<0.005$.

Table 5.3. Correlations of cytokine levels with prognostic factors in children with Meningococcal disease.

| | TNF-α | | IL-6 | | IL-10 | |
|---|---|---|---|---|---|---|
| | r | p | r | p | r | p |
| GMSPS | 0.66 | 0.0001 | 0.65 | 0.0001 | 0.60 | 0.0001 |
| White cell count | -0.64 | 0.0001 | -0.56 | 0.0001 | -0.61 | 0.0001 |
| Platelets | -0.50 | 0.001 | -0.53 | 0.0001 | -0.41 | 0.003 |
| PT | 0.84 | 0.0001 | 0.45 | 0.003 | 0.45 | 0.002 |
| APPT | 0.67 | 0.0001 | 0.37 | 0.013 | 0.45 | 0.001 |
| Fibrinogen | -0.57 | 0.0001 | -0.62 | 0.0001 | -0.62 | 0.0001 |
| Creatinine | 0.67 | 0.0001 | 0.44 | 0.004 | 0.42 | 0.004 |
| C-reactive protein | -0.51 | 0.006 | -0.63 | 0.0001 | -0.68 | 0.0001 |
| TNF-α | - | - | 0.58 | 0.0001 | 0.74 | 0.0001 |
| IL-6 | 0.58 | 0.0001 | - | - | 0.81 | 0.0001 |
| IL-10 | 0.74 | 0.0001 | 0.81 | 0.0001 | - | - |
| Time since onset of symptoms. | -0.40 | 0.009 | -0.54 | 0.0001 | -0.58 | 0.0001 |

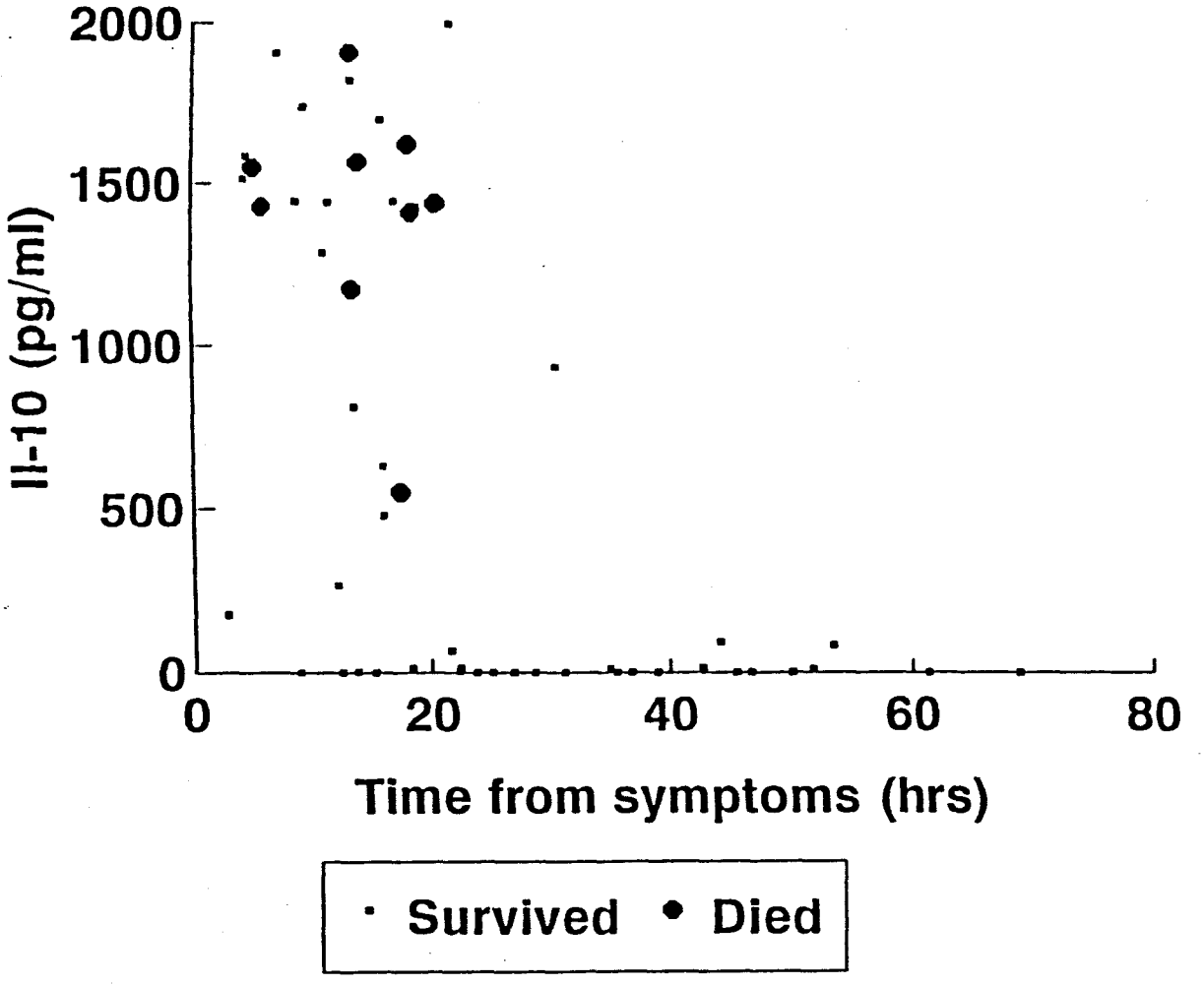



Figure 5.2. Admission interleukin-10 levels in 53 children with Meningococcal disease against time from onset of symptoms. Only one measurement per child.

### 5.3.2 Pro-Inflammatory cytokines.

TNF-α and IL-6 were measured in 42 and 47 children respectively. Levels were significantly raised in those who died, as well as in those with septic shock, severe disease, DIC and MS (Tables 5.1 and 5.2). There was a marked difference in survival with TNF-α levels above or below 800pg/ml. Only two of the nine children with levels above 800pg/ml survived (1 neurologically handicapped), compared to all those with levels below 800pg/ml surviving. Strong correlations were again seen with prognostic factors and with the length of symptoms before admission (Table 5.3 and Figures 5.3 and 5.4).

### 5.3.3 Interaction of cytokines.

Both TNF-α and IL-6 had very strong correlations with IL-10 levels (r=0.74 and 0.81 respectively), as well as with each other (r=0.58) (Table 5.3 and Figures 5.5, 5.6 and 5.7).

Survivors had higher IL-10 levels for a given level of TNF-α, than those who died. The ratio of IL-10 to TNF-α was calculated and plotted against TNF-α (Figure 5.8). All those with an IL-10:TNF-α ratio above 1.7 survived. However these children all had levels of TNF-α below 800pg/ml. Two children with TNF-α levels above 800 pg/ml had IL-10:TNF-α ratios above 1. One of these survived intact and the other died from cerebral haemorrhage more than one month after admission. Those who died acutely all had IL-10:TNF-α ratios below 1.

5.3.3.a Effect of duration of symptoms on cytokines.

All those who died had symptoms for less than 24 hours before admission. Those with longer periods of symptoms, who all survived, had lower cytokine levels. The duration of symptoms may thus be a confounding variable when examining the association between cytokines and mortality. Cytokine levels were thus compared between those who died and survivors with symptoms for 24 hours or less.

Interleukin-10 levels remained higher in those who died (1434 pg/ml (range 549-1905) compared with survivors with a similar length of symptoms (722(1-1995), but this was not statistically significant ($p=0.12$). However TNF-$\alpha$ levels remained significantly higher in those who died (1660(831-2291) vs 269 (18-1603); $p=0.0004$), but IL-6 levels were not significantly higher (2630(2137-3180) vs 2239(25-3981); $p=0.10$).

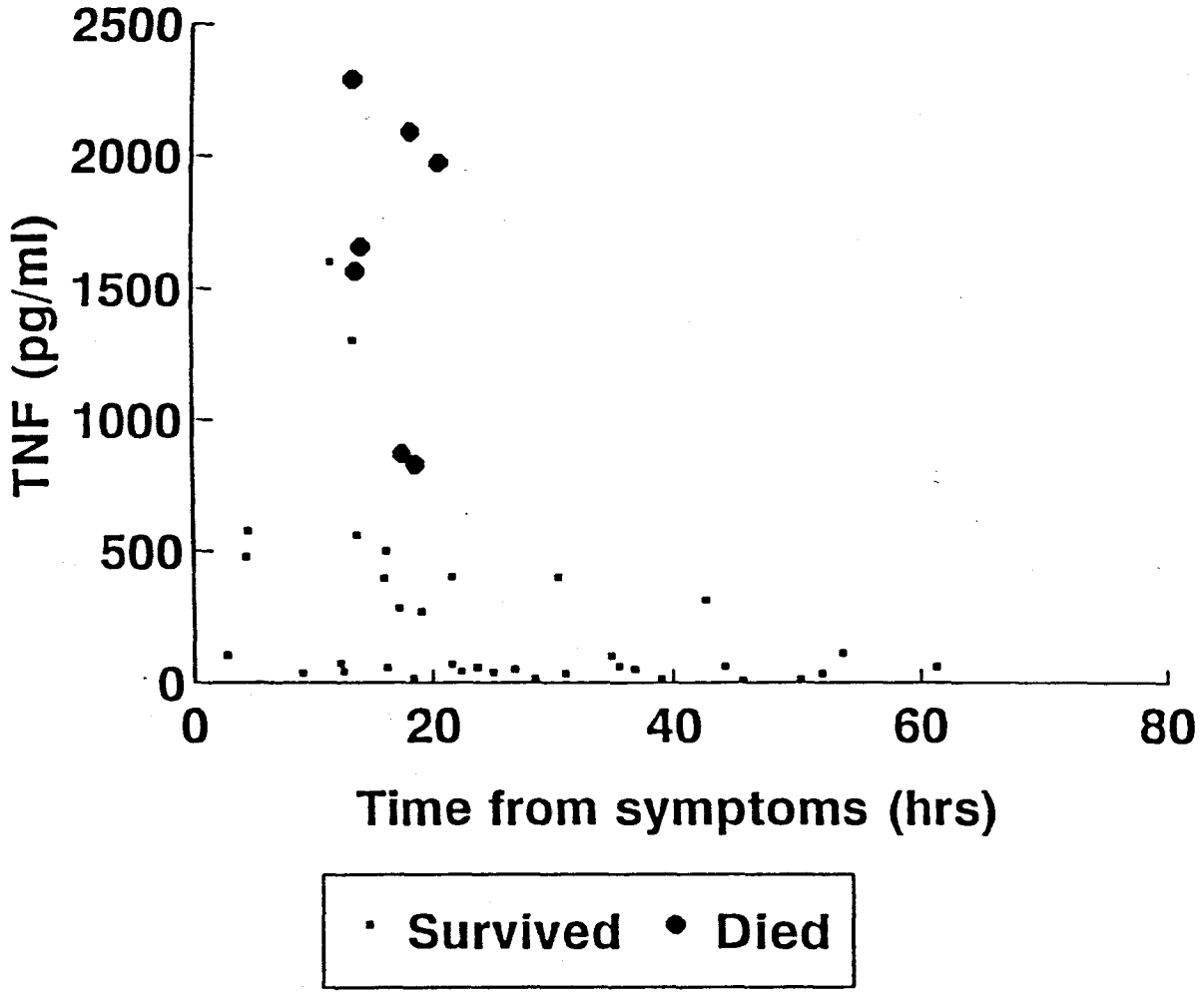



Figure 5.3. Admission Tumour Necrosis Factor levels in 42 children with Meningococcal disease against time from onset of symptoms. Only one sample per child.

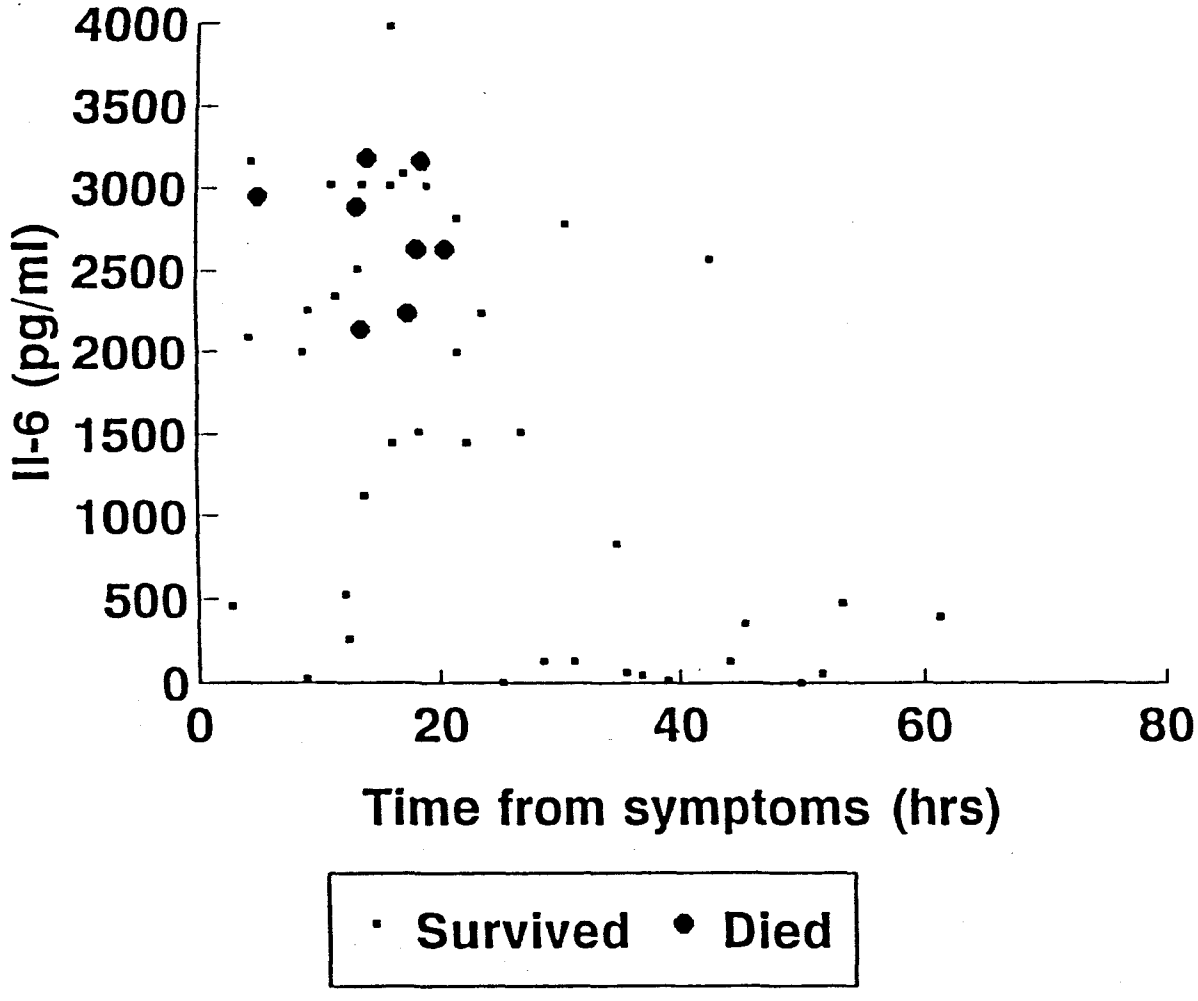



Figure 5.4. Admission interleukin-6 levels in 47 children with Meningococcal disease against time from onset of symptoms. Only one sample per child.

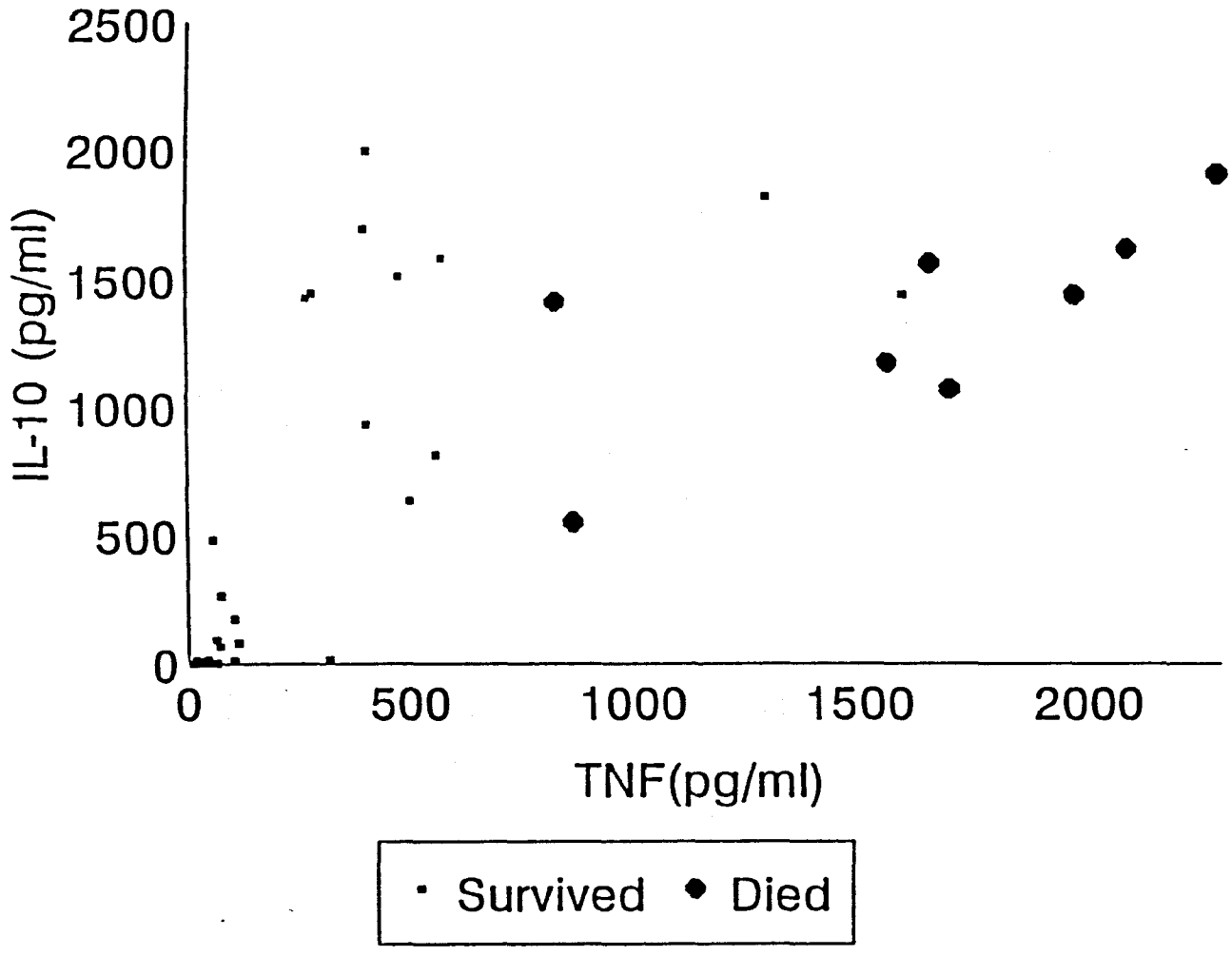



Figure 5.5. Admission interleukin-10 levels against admission Tumour necrosis factor-α levels in 42 children with Meningococcal disease.

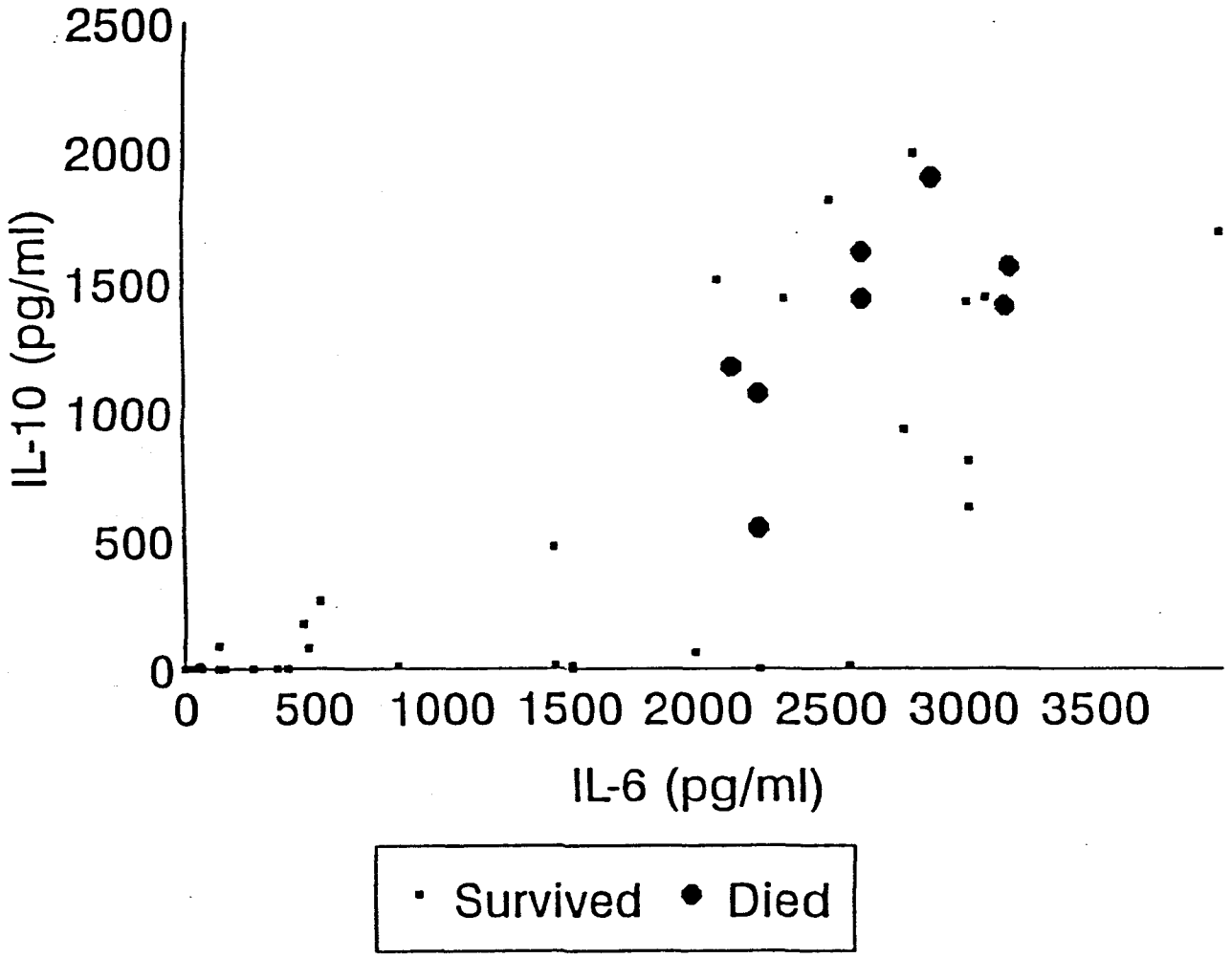



Figure 5.6. Admission interleukin-10 levels against admission interleukin-6 levels in 47 children with Meningococcal disease.

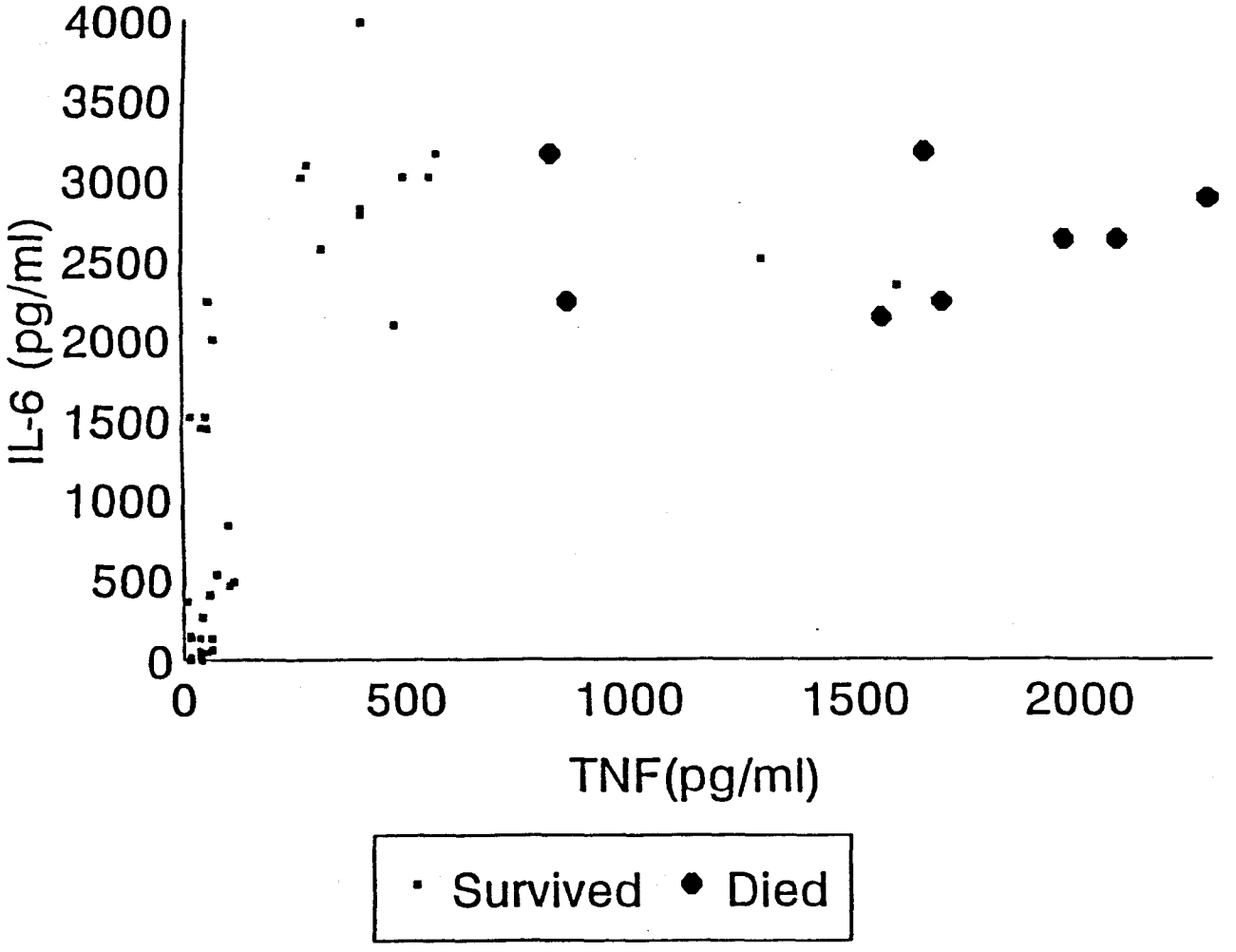



Figure 5.7. Admission interleukin-6 levels against admission Tumour necrosis factor-α levels in 42 children with Meningococcal disease.

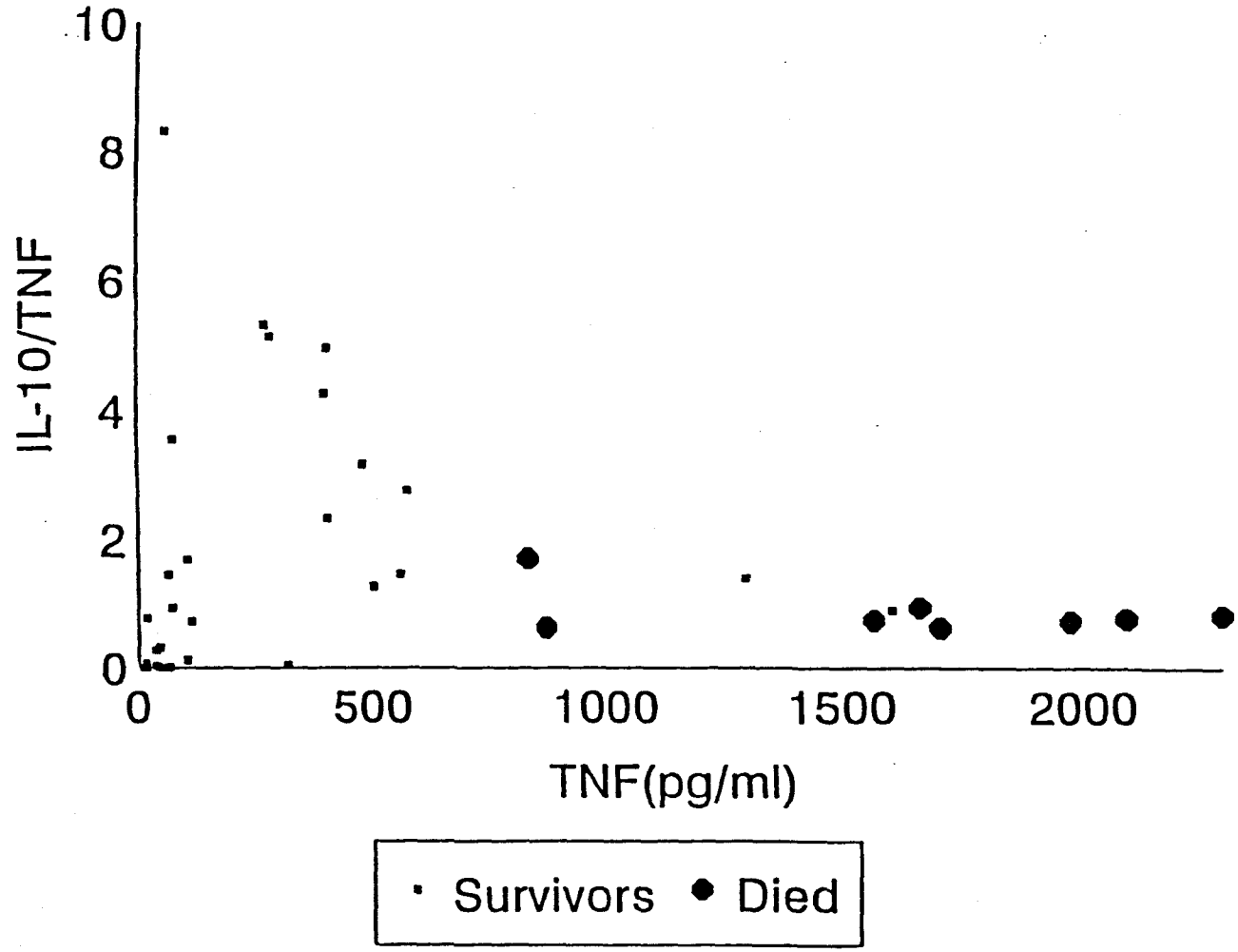



Figure 5.8. Ratio of admission interleukin-10 levels to Tumour necrosis factor-α levels against admission Tumour necrosis factor-α levels in 42 children with Meningococcal disease.

The relationship between TNF-α and IL-10 did not appear to be linear. At TNF-α concentrations below 800pg/ml there was a steep rise in IL-10 for an increase in TNF-α. Above this concentration the relationship reached a plateau.

## 5.4 DISCUSSION.

### 5.4.1 Interleukin-10

In this study levels of IL-10, a cytokine synthesis inhibitor, were significantly correlated with levels of cytokines known to be associated with septic shock and death; TNF-α and IL-6. IL-10 levels were also significantly associated with disease severity, septic shock and death.

Raised levels of IL-10 have previously been found in 25 children with meningococcal septic shock (Derkx et al 1995). High levels were associated with a high GMSPS but not with mortality, probably due to the small number studied. The current study confirms these findings and also confirms that higher levels are found in those with septic shock than those with septicaemia (Marchant et al 1994a).

### 5.4.2 Tumour Necrosis Factor-α and Interleukin-6.

Direct comparison with previously published work on TNF-α and IL-6 is difficult due to the different cytokine assay methods used. Waage et al (1987; 1989b) used a bioassay which detects only active cytokines, unbound by natural inhibitors like sTNFr.

Girardin et al (1988) used a radio-immunoassay which detects both bound and unbound cytokines. The ELISA method used in the current study measures both bound and unbound cytokines. Whilst this may not reflect the bio-activity of the cytokines (Duncombe & Brenner 1988), it does give information on the levels of cytokines released. The sensitivity of some ELISA kits is now very similar to that of bioassay (Bienvenu et al 1993).

However the current study does confirm previous findings that high levels of TNF-α and IL-6 are associated with death in MCD; all those who died had TNF-α levels above 800pg/ml. The study also confirms that TNF-α and IL-6 levels correlate strongly with each other and that cytokine levels also correlate with disease severity (Waage et al 1987; Waage et al 1989b; Girardin et al 1988; Marzouk 1995).

### 5.4.3 Time course of cytokines.

All the cytokines showed a significant correlation with the duration of illness before admission, as in previous work (Marzouk 1995). High levels of TNF-α or IL-10 were not found 30 hours after the onset of symptoms (Figures 5.2 & 5.3). TNF-α levels were still significantly higher in those who died even when compared to survivors with symptoms for 24 hours or less, but IL-6 and IL-10 levels were not. TNF-α is cleared quickly from the circulation (Waage et al 1989b). The low levels found in those with a longer duration of symptoms may thus be due to the transient rise in TNF-α being cleared before admission. Alternatively, those with the highest levels may have the most severe disease, and may therefore be admitted sooner. IL-6 has multiple routes of induction (via endotoxin or other cytokines) and is detectable up to 36 hours after admission (Waage et al 1989b).

The low levels of IL-6 found in those with a longer duration of symptoms implies lower cytokine activation in this group, rather than high levels being rapidly cleared. This also links with the finding that those with severe disease had a shorter history (See Chapter 4). The fact that some children can respond to meningococcaemia with a minimal activation of cytokines, implies some natural anti-inflammatory response controlling cytokine activity. IL-10 is suggested as a mediator of this response (Marchant et al 1994b). Does the current study support this?

### 5.4.4 Interaction of Interleukin-10 and Tumour Necrosis Factor-α.

Interleukin-10 levels correlated strongly with TNF-α levels ($r=0.74$, $p=0.0001$) as in a study of adult septic shock (Gómez-Jiménez et al 1995). Interleukin-10 release matched the pattern of TNF-α release. Raised IL-10 levels were only found when TNF-α levels were also raised (Figure 5.5), and raised levels of both cytokines were only found up to 30 hours after the onset of symptoms. These findings suggests that TNF-α and IL-10 share a common stimulus, or that one directly stimulates release of the other. This supports data that IL-10 is rapidly released by macrophages after stimulation with endotoxin (Marchant et al 1994b), similar to TNF-α (Cannon et al 1990). It also supports the suggestion that IL-10 release is mediated by TNF-α (Wanidworanun & Strober 1993).

Survivors had higher levels of IL-10 for a given level of TNF-α than those who died, with all those having an IL-10:TNF-α ratio above 1.7 surviving (Figure 5.8).

IL-10 levels did not appear to increase once TNF-α levels were greater than 800 pg/ml (Figure 5.5). It is tempting to speculate that once above this threshold level, IL-10 could not further inhibit TNF-α synthesis in these children. An imbalance between TNF-α and IL-10 production could therefore influence the development of shock and death. A similar imbalance between TNF-α and it's antagonist sTNFr, has previously been seen in children with MCD (Girardin et al 1992). This imbalance is likely to be due to IL-10 production since IL-10 stimulates sTNFr release (Joyce et al 1994). Only admission cytokine levels were measured in this study. Serial levels may provide more information on the interactions between IL-10, TNF-α and sTNFr.

IL-10 may be a candidate for treatment of sepsis (Howard et al 1993). The current study suggests that the level of IL-10 required to improve survival may be dependent on the systemic levels of TNF-α. Alternatively enough TNF-α may already have been secreted before admission for any subsequent inhibition with IL-10 to be of no benefit. Further studies of the interactions between pro- and anti-inflammatory cytokines in MCD are required, before IL-10 can be used as a possible treatment.

### 5.4.5 Conclusions

In conclusion this study has confirmed that high levels of the cytokines, TNF-α and IL-6 are associated with septic shock and death in MCD. The study has also shown that levels of IL-10, an anti-inflammatory cytokine, are also raised in those who die from MCD and correlated strongly with levels of the pro-inflammatory cytokines.

Similarities in the levels of TNF-α and IL-10 suggest a common stimulus. Children who died had TNF-α levels above 800pg/ml and an IL-10:TNF-α ratio below 1.7. High levels of IL-10 may thus inhibit the development of lethal levels of TNF-α. The administration of IL-10 to children with fulminant MCD could therefore be beneficial and further studies are required.

# CHAPTER SIX. ADRENAL CORTICAL FUNCTION

# CHAPTER SIX. ADRENAL CORTICAL FUNCTION

## 6.1 INTRODUCTION

A fulminating, rapidly fatal syndrome with a purpuric rash, circulatory collapse and bilateral adrenal haemorrhage was first described a century ago (Voelcker 1894-5;Quoted in Waterhouse 1911). The early reports were reviewed by Waterhouse (1911) and Friderichsen (1918) and the syndrome now bears their name, although neither identified the organism most commonly responsible; *N meningitidis* (Andrewes 1906; Maclagan & Cooke 1916). The circulatory collapse was presumed to be due to adrenal insufficiency (Nelson & Goldstein 1951; Lanman 1955), although others thought the adrenal haemorrhage was purely coincidental (Morrison 1943; Kass & Finland 1958; May 1960).

### 6.1.1 Relative adrenal insufficiency

Bacterial infection leads to an increased cortisol production rate (Bassøe et al 1965; Midgeon et al 1967; Cornil et al 1968) and host survival requires an intact adrenal cortex (Bertini et al 1988). Plasma cortisol levels are markedly raised in patients with septic shock (Schein et al 1990) and especially in those who are dying (Sandberg et al 1956). Despite the stress of shock or impending death a few patients have plasma cortisol levels within the normal range. These levels may represent "relative adrenal insufficiency" (Sibbald et al 1977).

In MCD a subgroup of children with a septicaemic component to their illness have inappropriately low plasma cortisol levels (<800nmol/l)(Midgeon et al 1967). The mortality in this group is high and post mortem examination confirms them as true cases of the Waterhouse-Friderichsen syndrome. Steroid replacement in this sub-group may be beneficial (Bosworth 1979) and is recommended by some (Leclerc et al 1988), but not others (May 1960). However the use of corticosteroids in those children with meningococcal septic shock with already raised plasma cortisol levels seems inappropriate, and may be harmful (Bone et al 1987). Methods for rapidly identifying those children with relative adrenal insufficiency are thus required.

### 6.1.2 Cytokines and Cortisol.

The pro-inflammatory cytokines, TNF-α and IL-1β, are major mediators of MCD, high levels being associated with septic shock and death (Waage et al 1987; Waage et al 1989b; Girardin et al 1988). ACTH and cortisol release are directly stimulated by IL-1β, and to a lesser extent by TNF-α and IL-6 (Besedovsky et al 1986; Marinkovic et al 1989). The production of both IL-1β and TNF-α is down regulated by glucocorticoids (Sáez-Llorens et al 1990) and steroid production is thus part of the regulation of cytokine secretion. Adrenalectomy increases the toxicity of these cytokines (Bertini et al 1988). TNF-α in large doses can cause adrenal haemorrhage (Tracey et al 1986) and cytokines may also inhibit cortisol production in sepsis (Catalano et al 1984). The balance between cytokines and cortisol may thus influence survival in MCD.

This study will determine the initial cortisol levels in children with MCD to establish whether children who die have low, or inappropriately low levels. The value of methods for identifying such a sub-group, if one is found, will then be determined. The pattern of steroid levels in children with severe MCD during the early phase of treatment will also be determined. Cortisol levels will also be compared with levels of the pro-inflammatory cytokines.

## 6.2 METHODS.

The methods have been fully described in chapter 2. In summary blood samples were taken on admission from all children. Additional samples were taken at 12 hourly intervals over the next 2 days from children admitted to PICU. Children enrolled in the Centoxin study were seen 8 weeks after discharge and a random cortisol was taken at that time.

Plasma cortisol levels were measured by radio-immunoassay by the Department of Clinical Chemistry, University of Liverpool.

Any steroid therapy, given at the discretion of the supervising clinician, was recorded.

### 6.2.1 Definitions.

Oliguria was defined as a urine output of less than 0.5 ml/kg/hr.

Hypoglycaemia was defined as blood glucose less than 2.4 mmol/l at any time during the first 48 hours of treatment.

## 6.3 RESULTS.

### 6.3.1 Initial Cortisol Levels.

Admission samples were obtained from 96 children with MCD (40 with severe disease) and from 45 controls. Samples were also obtained from 3 children with other bacterial infections (Pneumococcal septicaemia 2, bacterial meningitis 1) one of whom died. Septic shock was present in 29 children with MCD, 11 of whom died. No post mortem examinations were carried out on those who died.

Compared to controls, plasma cortisol levels were markedly increased in children with MCD (median 1210 nmol/l (range 430-5124) vs 797 (201-1835), p <0.0001). However children who died from MCD had significantly lower initial cortisol levels than survivors (see Table 6.1). Ninety children with MCD were over 6 months old, and should have established a diurnal variation in cortisol levels (Onisihi et al 1983). However there was no significant difference in the time of admission between those who died and those who survived (14.15 hrs vs 14.05 hrs; p=0.70 Smirnov two sample test).

Initial plasma cortisol levels differed significantly across the spectrum of MCD (See Figure 6.1). Children with MS had significantly lower levels (1185nmol/l (430-4410)) than those with MM+MS (1288 nmol/l (447-5124); p=0.04). This difference disappeared if only the survivors were included in the analysis (MS 1249 nmol/l (447-4410) vs MM+MS 1261 nmol/l (447-5124); p=>0.05). The high number of deaths in the MS group were thus responsible for the lower cortisol levels with this presentation.

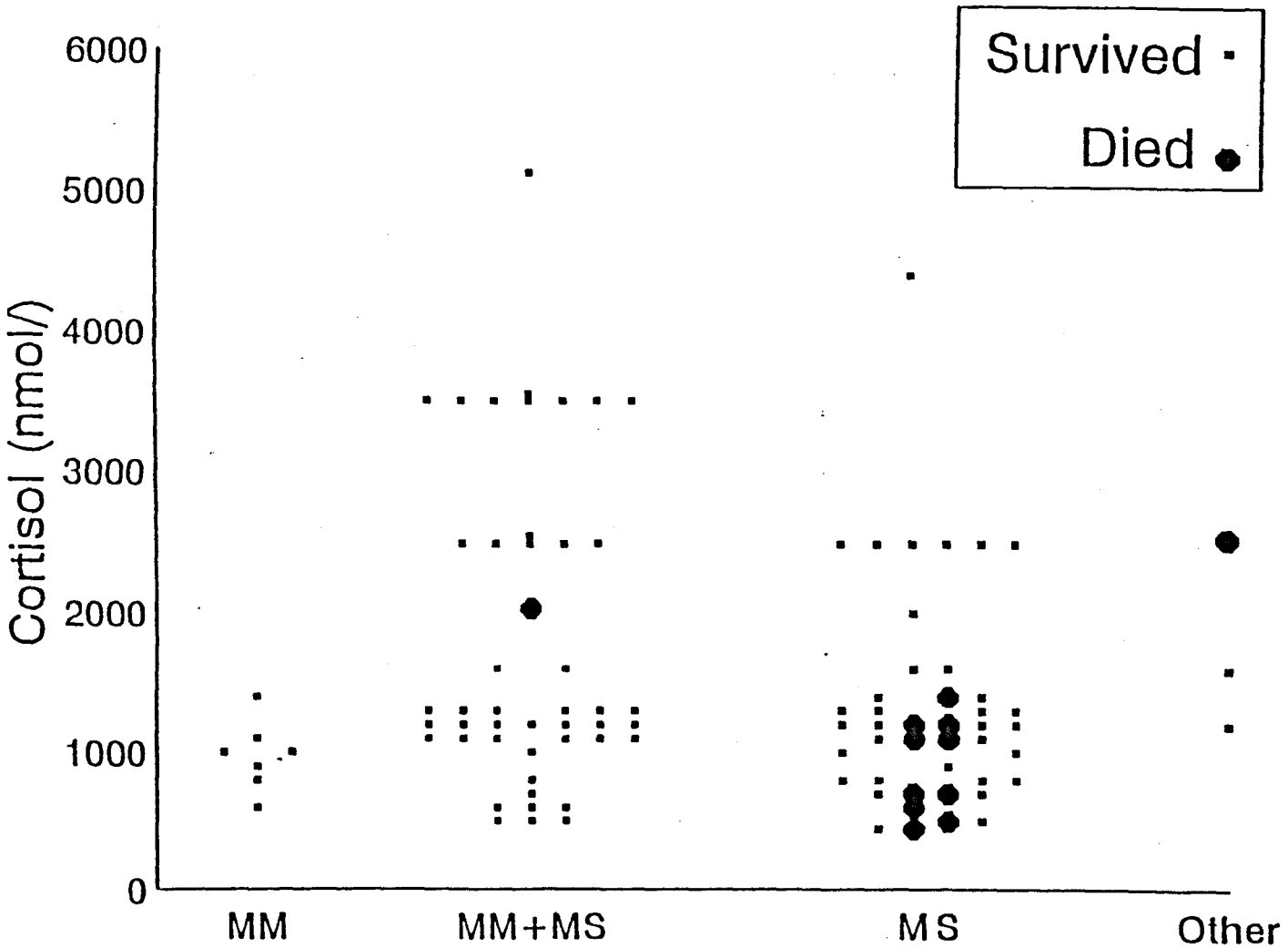



Figure 6.1. Initial plasma cortisol levels across the clinical spectrum of Meningococcal disease.

Table 6.1. Initial plasma cortisol(nmol/l) in 96 children with Meningococcal disease by clinical features. Results shown as median (range).

| Feature | n | Present | Absent |
|---|---|---|---|
| Died | 10 | 1063(430-2030)* | 1249(447-5124) |
| Septic Shock | 29 | 1161(430-4410) | 1249(447-5124) |
| GMSPS≥8 | 40 | 1207(430-4410) | 1212(447-5124) |
| DIC | 22 | 1205(430-4410) | 1209(497-5124) |
| Oliguria | 15 | 1051(430-2030)* | 1249(447-5124) |
| Hypoglycaemia | 9 | 723(521-4410) | 1243(430-5124) |

*$p \leq 0.05$ by Mann Whitney U test.

No case of true adrenal insufficiency was seen, all children had levels above 400nmol/l (0%; 95%CI 0-3.8%). However ten children with severe MCD had levels below 800nmol/l, suggesting relative adrenal insufficiency. Five of these children died.

6.3.2 Factors predicting initial cortisol <800nmol/l and death.

There were no statistically significantly differences in initial cortisol levels in children with severe MCD, septic shock or DIC. However children with MCD who developed oliguria had significantly lower cortisol levels, and those with hypoglycaemia showed a trend towards lower levels (p=0.055) (see Table 6.1).
Initial cortisol levels did not correlate with blood glucose, sodium, potassium, bicarbonate, urea, eosinophil count or the with the length of symptoms before the sample was taken.

Tables 6.2 and 6.3 show which features best predicted initial cortisol <800nmol/l and death in children with severe MCD. Creatinine >65 μmol/l, white cell count <5 x $10^9$, neutrophil count <2.5 x $10^9$ and fibrinogen <2 g/l were all present in the 5 children who died and had cortisol levels below 800nmol/l. However only creatinine >65 μmol/l identified all 11 children who died, as well as 8 out of 10 children with severe MCD and initial cortisol levels below 800 nmol/l.

Table 6.2. Features predicting low initial plasma cortisol (<800nmol/l) in 40 children with severe Meningococcal disease. Data shown as %.

| Feature | Sensitivity | Specificity | PPV |
|---|---|---|---|
| Creatinine >65$\mu$mol/l | 80 | 50 | 35 |
| Oliguria (<0.5ml/kg/hr) | 70 | 73 | 47 |
| WBC <5x10$^9$ | 50 | 67 | 33 |
| Neutrophils <2.5 x 10$^9$ | 50 | 63 | 31 |
| Fibrinogen <2 g/l | 50 | 87 | 56 |
| Hypoglycaemia (<2.4mmol/l) | 50 | 84 | 56 |
| Eosinophils ≥0.05 x 10$^9$ | 71 | 25 | 36 |

Key

PPV=positive predictive value for cortisol <800nmol/l.

Table 6.3. Features predicting death in 40 children with severe Meningococcal disease. Data shown as %.

| Feature | Sensitivity | Specificity | PPV |
|---|---|---|---|
| Creatinine >65μmol/l | 100 | 59 | 48 |
| Oliguria (<0.5ml/kg/hr) | 91 | 83 | 67 |
| WBC $<5x10^9$ | 91 | 83 | 67 |
| Neutrophils $<2.5 \times 10^9$ | 73 | 83 | 62 |
| Fibrinogen <2 g/l | 64 | 93 | 78 |
| Hypoglycaemia (<2.4mmol/l) | 83 | 44 | 44 |
| Eosinophils $\geq 0.05 \times 10^9$ | 38 | 8 | 21 |

Key

PPV=positive predictive value for death.

6.2.3 Steroid treatment.

Hydrocortisone was given to 8 children, only one of whom survived. Hydrocortisone was given to two children before the initial cortisol sample was taken, both of whom died shortly after arrival. Initial cortisol levels in non-survivors remained significantly lower, even including these 2 post treatment levels. Figure 6.2 shows the cortisol profiles of 7 children who received hydrocortisone. (One further child received hydrocortisone after all samples were taken.) Rises in plasma cortisol far above the naturally produced levels occurred after treatment, especially in the one child who survived. This child however was left with neuro-developmental delay and a hemiplegia.

Dexamethasone was given to 5 children, all of whom had initial samples taken within 6 hours of treatment. Subsequent cortisol levels decreased rapidly (Figure 6.3)

6.3.4 Cortisol profiles in Meningococcal disease.

Cortisol profiles were obtained from 26 children who survived MCD without steroid treatment (See Table 6.4 & Figure 6.4). Cortisol levels decreased significantly after 12 hours of treatment and again by 24 hours. Further non-significant deceases occurred over the next 24 hours. By 48 hours cortisol levels were not significantly different from a random sample taken 8 weeks after discharge.

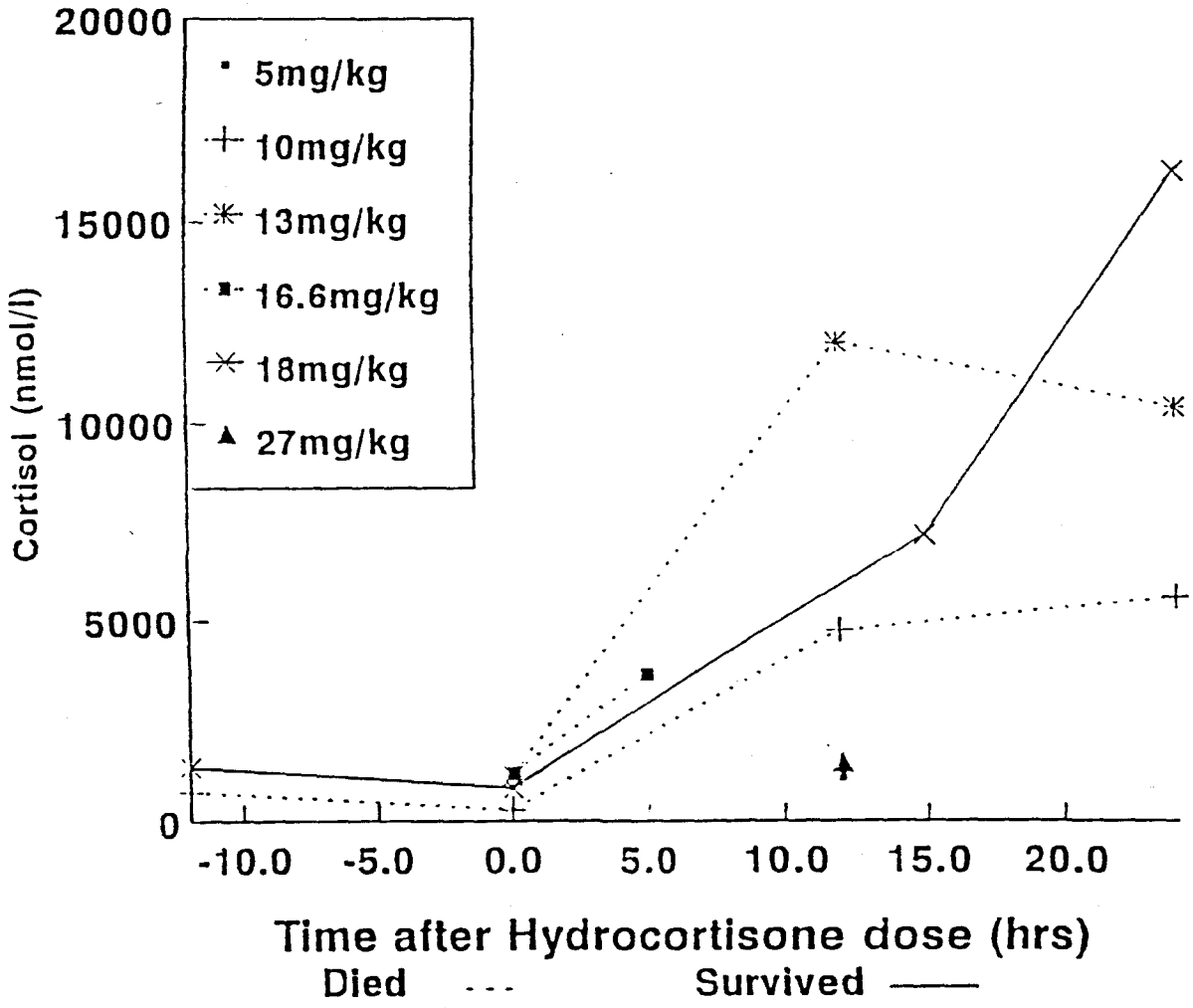



Figure 6.2. Cortisol profiles in 7 children with Meningococcal disease treated with hydrocortisone.

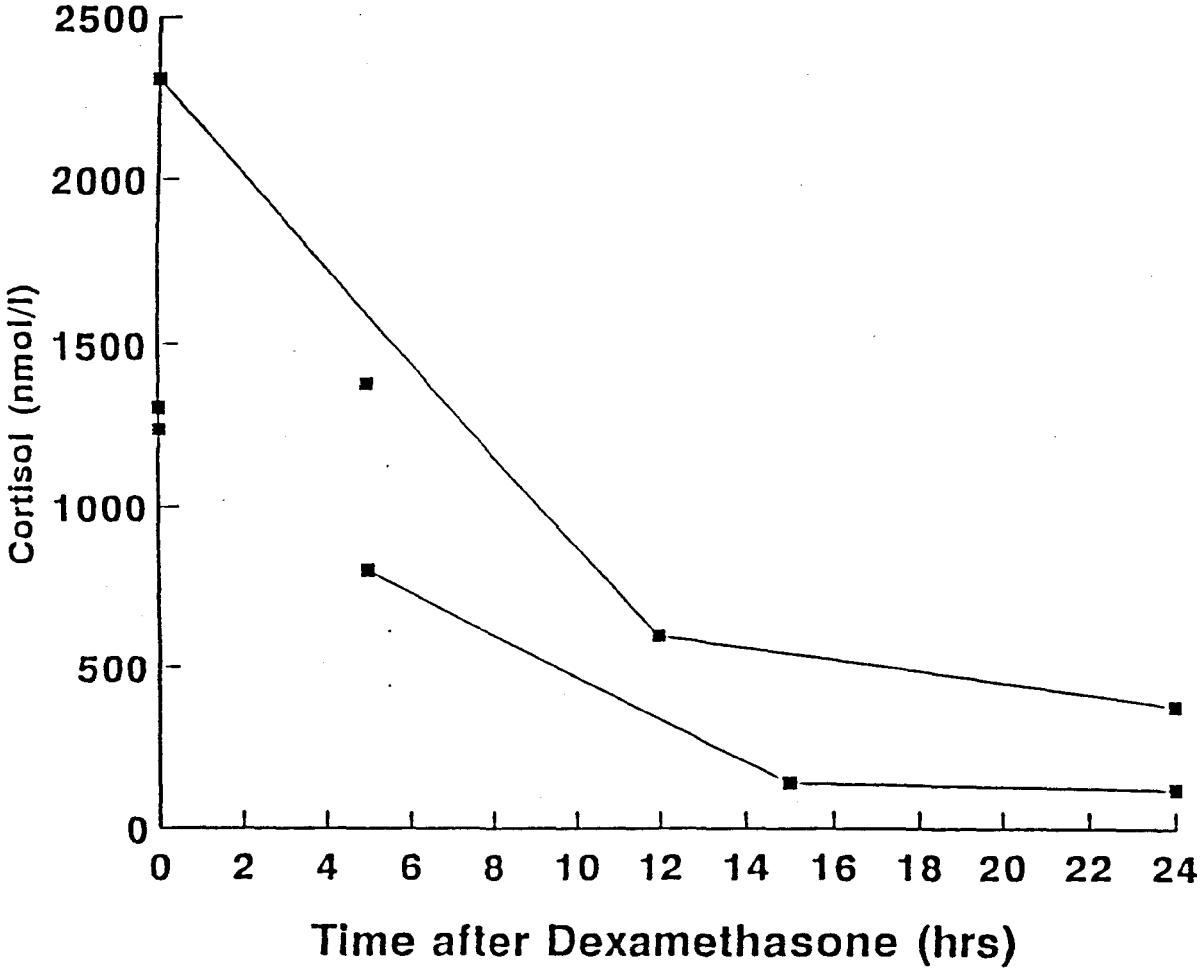



Figure 6.3. Cortisol profiles in 5 children with Meningococcal disease treated with dexamethasone.

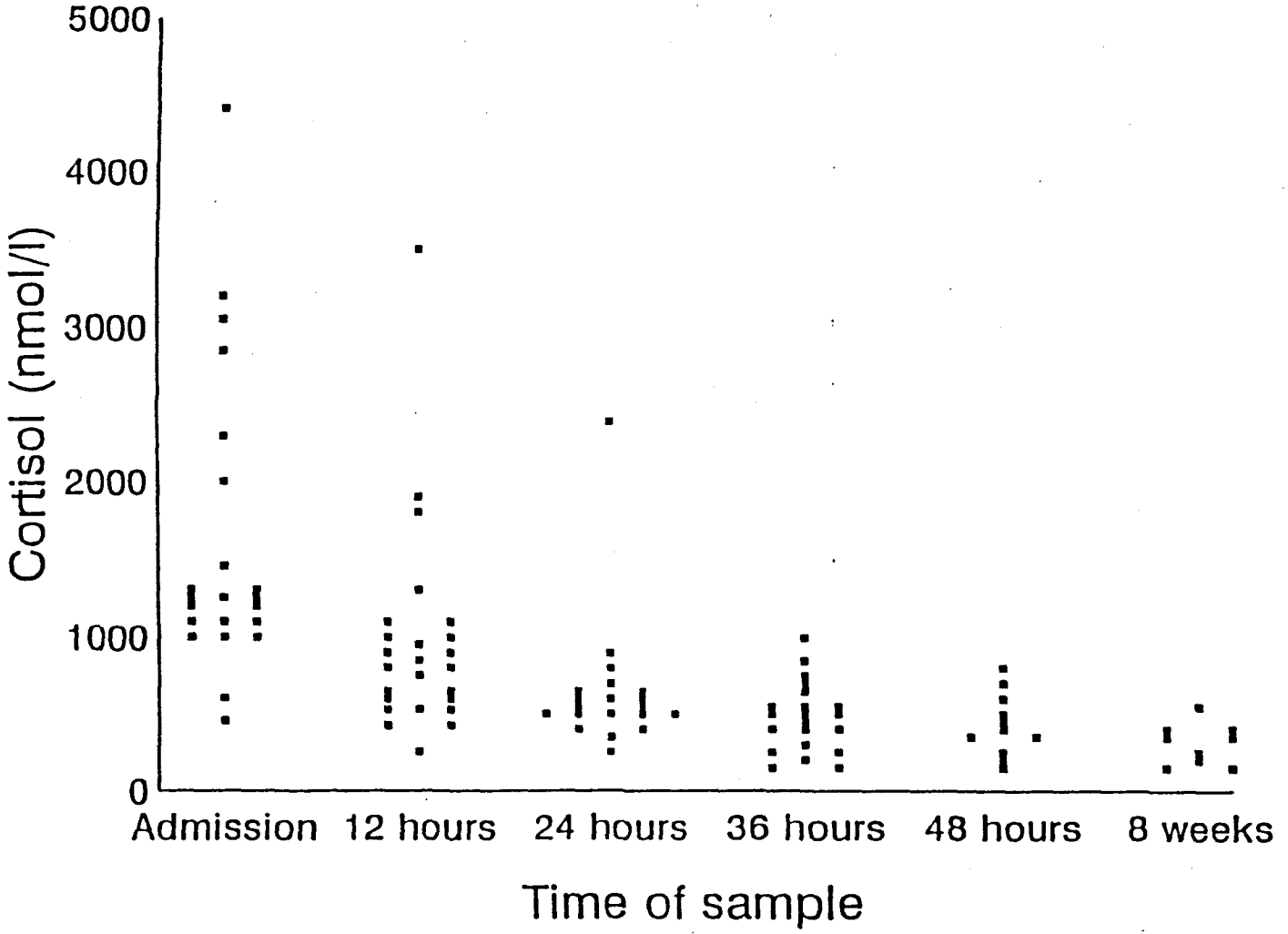



Figure 6.4. Cortisol profiles in children admitted to PICU who survived Meningococcal disease without steroid treatment

Table 6.4. Cortisol profiles in children admitted to PICU who survived Meningococcal disease without steroid treatment. Results shown as median(range). Statistical significance by Mann Whitney U test.

| Time since treatment started | Cortisol (nmol/l) |
|---|---|
| 0 hours (n=21) | 1283(447-4410)* |
| 12 hours (n=26) | 850(394-3980)*# |
| 24 hours (n=22) | 573(245-2408)# |
| 36 hours (n=20) | 489(155-996)+ |
| 48 hours (n=13) | 418(121-780) |
| 8 weeks (n=9) | 367(154-530)+ |

* Difference between 0 and 12 hours $p<0.0005$.

# Difference between 12 and 24 hours $p<0.005$.

+ Difference between 36 hours and 8 weeks $p<0.05$.

6.3.5 Cytokine and Steroid levels.

Initial cytokine levels were measured in 47 children with MCD on PICU. Cortisol levels did not correlate significantly with TNF-α ($r=-0.23$, $p=0.16$) or IL-6 ($r=-0.07$, $p=0.65$).

## 6.4 DISCUSSION.

This study shows that children with MCD have raised cortisol levels compared to children with less severe infections. However children who died from MCD had significantly lower levels than those who survived. Seven children who died had initial levels above 800nmol/l, but two of these had received hydrocortisone prior to sampling, and a further two died more than one month after admission. Thus five out of eight children who died acutely and had not received prior steroids, had levels below 800nmol/l. In the face of overwhelming infection and impending death, these levels are inappropriately low (Sandberg et al 1956; Schein et al 1990) and imply a relative adrenal insufficiency consistent with Waterhouse-Friderichsen syndrome. Unfortunately no autopsy studies were carried out to confirm the presence of adrenal haemorrhage in these cases. However unlike other studies or case reports (Midgeon et al 1967; Bosworth 1979; McWhinney et al 1989; Enriquez et al 1990), no cases of true adrenal insufficiency were found, all children having cortisol levels above 400 nmol/l.

Cortisol secretion has a diurnal variation. Lower levels might be found in those who died if they were admitted more often overnight. This is unlikely to be true for two reasons. Firstly there was no difference in the time of admission of survivors and those who died. Secondly severely ill patients often lose the normal diurnal variation in plasma cortisol levels (Sainsbury et al 1981). Lower levels in those who died is likely to be a real finding and confirms previous studies (Midgeon et al 1967; Zachmann et al 1974).

### 6.4.1 Causes of relatively low cortisol levels

The mechanism of relative adrenal insufficiency in meningococcal septic shock may not be due solely to adrenal haemorrhage. Corticosteroid production is inhibited by plasma from septic animals (Catalano et al 1984). This inhibition is not caused by endotoxin alone (Rosenfeld 1955) and is thus due to other mediators, possibly cytokines. The decreased blood flow in severe shock may also cause inadequate perfusion of the adrenal cortex and decrease secretory function (Herman et al 1969). The adrenal shares some of its blood supply with the kidney and oliguria is associated with decreased renal blood flow in MCD (M Alwadiah, personal communication). This may explain the association found between low cortisol levels and oliguria found in this study.
Brainstem dysfunction can also lead to low cortisol levels (Feibel et al 1983). Ischaemia of the brainstem due to hypotension, vasculitis and thrombosis of cerebral vessels may also contribute to decreased cortisol levels.

Studies of cortisol levels in adults with septic shock have found a small group of patients with low cortisol levels and no response to corticotrophin. Patients in this group died, unless treated with steroids (Sibbald et al 1977; Jacobs & Nabiro 1969). This group with relative adrenal insufficiency has not been found in all studies (Schein et al 1990), and its existence is questioned. However children with MCD may behave differently to adults with a variety of different infections.

### 6.4.2 Previous studies

Previous studies of cortisol levels in children with MCD have been performed on small numbers of children (Table 1.2) and none used the modern, precise radio-immunoassay techniques used in the current study.

Midgeon et al (1967) studied 26 children with MCD using an isotope dilution technique. Their study found significantly lower initial cortisol levels in children who died from MCD. Zachmann et al (1974) determined cortisol levels fluorimetrically in 25 children with fulminant MCD. They also found significantly lower levels in those who died.

Lewis (1979) measured cortisol levels by competitive protein binding, in 30 children with acute meningococcaemia in Nigeria. He stated that all cases had high cortisol levels, with no significant difference between those who died and survivors. However, 7 of the 12 children who died had cortisol levels below 800nmol/l, an unexpected finding in view of the previously demonstrated high agonal levels (Sandberg et al 1956).

A few case reports of low serum cortisol levels in fulminant MCD have appeared (Bosworth 1979; McWhinney et al 1989; Enriquez et al 1990), but no other study of cortisol levels across the spectrum of MCD has been published.

The current study shows that absolute adrenal insufficiency is rare in MCD (95% CI 0-3.8%). However a subgroup of children with MS do have inappropriately low plasma cortisol levels, and a high mortality. Steroid replacement in this group may be beneficial. How can these children be identified? Moreover how can they be identified in time to make steroid replacement worthwhile?

### 6.4.3 Identifying children at risk of relative adrenal insufficiency.

Adrenal insufficiency can be confirmed by a lack of rise in plasma cortisol in response to corticotrophin (Wood et al 1965), even in those in septic shock (Rothwell et al 1991). In the only study of corticotrophin in MCD, adults with MM showed a rise in cortisol but those with MM+MS showed no response (Wajchenberg et al 1978). No patients in this study had severe disease, further studies of the response to corticotrophin in MCD are required.

However there are difficulties in interpreting the response to corticotrophin when basal cortisol levels are already elevated above normal (Martinez & Marcos 1991) and others have suggested measuring endogenous corticotrophin (Reincke et al 1991). Cortisol or corticotrophin levels are unlikely to be available immediately in a clinical setting. Other markers are required for urgently diagnosing relative adrenal insufficiency in MCD.

Haemodynamic improvement brought about by the administration of glucocorticoids may indicate relative adrenal insufficiency (Varma & Park 1991; Schneider & Voerman 1991; Baldwin & Allo 1993), but this is disputed (Robinson et al 1962).

Abdominal ultrasound can detect adrenal haemorrhage in children with MCD (Sarnaik et al 1988), and these children may have low cortisol levels (Enriquez et al 1990). However screening all children with fulminant MCD for adrenal haemorrhage with ultrasound would be costly and time consuming and the reliability of this test has been questioned (Heyderman et al 1993).

Adrenal insufficiency leads to hypoglycaemia, hyponatraemia, hyperkalaemia and hypotension (Rao et al 1989; Burke 1992). Children with hypoglycaemia did have lower cortisol levels ($p=0.055$; Table 6.1), but hypoglycaemia was an insensitive marker for cortisol <800 nmol/l (Table 6.2). None of the other features correlated with cortisol levels in the present study, as found in previous studies (Nelson and Goldstein 1951). Blood eosinophil count has been said to be a reliable index of adrenal function, and has previously been used to monitor steroid therapy in MCD (Hodes et al 1952). However it was neither a sensitive, nor a specific marker of inappropriately low cortisol levels in this or other recent studies (Rao et al 1989). Cortisol deficiency is accompanied by impaired free water clearance (Burke 1992) and children with oliguria in this study did have significantly lower cortisol levels. Oliguria was thus a marker for relative adrenal insufficiency, but was relatively insensitive.

Serum creatinine correlates with endotoxin levels in MCD (Brantzaeg et al 1989; O Marzouk personal communication), and is a commonly available urgent investigation. An admission creatinine above 65μmol/l identified 80% of children with severe MCD and an initial cortisol below 800nmol/l, as well as all children who died, irrespective of their cortisol levels. Creatinine above 65 μmol/l was thus the most sensitive marker for low cortisol in children with severe MCD and for mortality. Steroid therapy could thus be considered in children with severe MCD who have a serum creatinine above 65 μmol/l. What regimen of steroid treatment should be used in these children?

### 6.4.4 Treatment for relative adrenal insufficiency

In this study treatment with commonly used doses of hydrocortisone (Heyderman et al 1993), produced large rises in plasma cortisol levels, as seen in a previous study (Sainsbury et al 1981). In contrast children who survived MCD without steroid treatment, had cortisol levels which consistently decreased during the first 48 hours of treatment, as seen with other infections (Beisel et al 1967).

Patients in septic shock with adrenal insufficiency may benefit from physiological doses of glucocorticoids (McKee & Finlay 1983; Schneider & Voerman 1991), but higher doses may increase mortality (Kass & Finland 1958) or morbidity (Bone et al 1987). In the current study initial plasma cortisol levels in severe MCD varied from "high normal" to ten times this value.

An individual child's cortisol response may be precisely sufficient for their needs or a non-specific stress response in excess of requirements (Nickels & Moore 1989). However for treatment purposes it seems wise to reproduce the cortisol response seen in survivors. Cortisol secretion increases four-fold in children with MCD (Midgeon et al 1967), so to mimic the body's response to MCD a dose of hydrocortisone equivalent to four times the normal cortisol secretion (i.e. 40-50 $mg/m^2/day$) is recommended (Zachmann et al 1974). This dose could be decreased as the clinical condition improved, so that a dose equivalent to the normal cortisol secretion (i.e. 12.5 $mg/M^2/day$) is then given (Burstein & New 1989).
In survivors cortisol levels decreased rapidly over the first 48 hours of treatment. Reports of "inappropriately low levels" taken more than 12 hours after admission (Midgeon et al 1967) should thus be viewed with caution.

### 6.4.5 Cytokine and Steroid levels.

Cortisol levels did not correlate with TNF-$\alpha$ or IL-6 levels, despite the fact that both these cytokines stimulate ACTH secretion (Tracey et al 1987b; Marinkovic et al 1989). However in critically ill patients cortisol levels do not correlate with ACTH levels (Duker & McLaughlin 1986). The interaction between cortisol, ACTH and the cytokines is dynamic. Comparison of cortisol and cytokine levels taken at a single time point may not reflect this interaction. Further studies of cortisol, ACTH and cytokines in patients with MCD are required.

### 6.4.6 Conclusion.

In conclusion, this study found a wide range of initial plasma cortisol levels in children with MCD, although none were truly deficient. However significantly lower levels were found in those who died. These children may have relative adrenal insufficiency, consistent with Waterhouse-Friderichsen syndrome, and might benefit from replacement corticosteroids. A trial of hydrocortisone in children with severe MCD and a serum creatinine above 65 μmol/l should therefore be considered.

# CHAPTER SEVEN. FIBRONECTIN

# CHAPTER SEVEN. FIBRONECTIN

## 7.1 INTRODUCTION

Plasma fibronectin may have a vital role in modulating the host inflammatory response by increasing phagocytosis without excessive tissue damage (Yang et al 1993) and by improving the endothelial clearance of tissue debris (Snyder et al 1981; Saba 1986). Adequate levels may lessen the capillary leak seen in sepsis (Wheatley et al 1993) and reduce mortality, particularly in combination with specific immunoglobulins (Hill et al 1984). Clinical studies have shown that plasma fibronectin is decreased in critically ill adults, especially those with sepsis and/or disseminated intravascular coagulation (Coulaud et al 1982; Mansberger et al 1989). However fibronectin shares this pattern with a number of other plasma proteins (Coulaud et al 1982; Rubli et al 1983). Fibronectin levels may be especially low in patients who die (O'Connell et al 1984). Decreased levels may also be a marker for infection (Gerdes et al 1983).

However most studies have included either adults or neonates with a wide variety of infections and other factors that can affect plasma fibronectin levels (eg liver dysfunction and the use of blood products). These studies may not be relevant to children with MCD.

Children with MCD have been shown to have decreased plasma fibronectin levels in a small study (Blanco et al 1990), but levels did not correlate with mortality. No comparison of plasma fibronectin levels with disease severity or between those with meningitis or septicaemia was performed and no assessment of decreased plasma fibronectin as a marker of MCD was made. If plasma fibronectin levels are confirmed to be decreased in children with MCD, then a trial of fibronectin therapy (perhaps combined with a monoclonal antibody) may be justified.

This study seeks to confirm that plasma fibronectin levels are low in children with MCD. The study will also compare initial plasma fibronectin levels between survivors and non-survivors. Fibronectin levels will also be studied in severe MCD, across the clinical spectrum of disease and over time. Finally an evaluation of measurement of initial plasma fibronectin concentration as an aid to diagnosis in early MCD will be made.

## 7.2 METHODS.

The full details of the methods have been described previously (See chapter 2). Briefly blood samples were taken on admission, with additional samples on day 5-7 if other routine samples were taken. Fibronectin levels were measured by turbidimetric immunoassay.

## 7.3 RESULTS

### 7.3.1 Initial fibronectin levels.

Admission samples were collected from 150 children; 101 with MCD and 49 controls. Forty five children had MS, 49 had MM+MS and 7 had MM. Severe MCD affected 42 children, 12 of whom died. Two children, both of whom died, received FFP or cryoprecipitate before the admission sample was taken. Further samples were collected from 23 children with MCD on day 5-7. The median age of the MCD group was not significantly different from the control group (20 months range 3-168 vs 17 months (2-148)). Twenty five children with MCD (25%) were less than 1 year of age compared to 18 (37%) of controls ($p>0.05$).

Median fibronectin on admission in children with MCD was 57 $\mu$g/ml (range 4-800) and this was significantly lower than controls (105 $\mu$g/ml (range 5-260);$p<0.005$) (Figure 7.1). Children who died from MCD had significantly lower admission fibronectin levels as did children with severe MCD, DIC and septic shock (Table 7.1). In children with MCD, fibronectin on admission showed a significant (although weak) negative correlation with IL6 ($r=-0.3$, $p=0.05$).(Figure 7.2) There was no significant correlation with CRP level or with the length of symptoms before admission.

Children with MS showed a trend toward lower levels than those with MM+MS or MM, but this was not statistically significant (See Table 7.2).


Figure 7.1. Admission fibronectin levels in children with Meningococcal disease and controls.

Table 7.1. Associations of plasma fibronectin levels (μg/ml) on admission in 101 children with Meningococcal disease with disease severity. Data shown as median (range). Statistical analysis by Mann-Whitney U test.

| | Feature | | |
|---|---|---|---|
| | Present | Absent | P |
| Died (n=12) | 29(5-150) | 62(4-800) | 0.01 |
| DIC (n=23) | 27(5-167) | 66(4-800) | 0.0005 |
| Septic shock (n=31) | 30(5-165) | 75(4-800) | 0.00001 |
| GMSPS≥8 (n=42) | 39(5-800) | 75(4-650) | 0.003 |

Table 7.2. Plasma fibronectin and C-reactive protein across the spectrum of Meningococcal disease. Data shown as median (range). Statistical analysis by Mann-Whitney U test.

| | MS (n=45) | MM+MS (n=49) | MM (n=7) | Controls (n=49) |
|---|---|---|---|---|
| Fibronectin (μg/ml) | 45(5-650)* | 57(4-280)+ | 72(40-800) | 105(5-260) |
| C-reactive protein (mg/l) | 54(4-279)*† | 129(4-420)+ | 208(51-321)‡ | 11(3-283) |

* Difference between MS and controls $p<0.01$.

\+ Difference between MM+MS and controls $p<0.05$.

† Difference between MS and MM+MS $p<0.005$.

‡ Difference between MS and MM $p<0.005$.

7.3.2 Subsequent fibronectin levels.

In MCD fibronectin levels rose significantly by day 5-7 (See Table 7.3). Children who received FFP or cryoprecipitate had significantly lower fibronectin levels on admission than those who received neither ($p<0.005$). However the rise in fibronectin levels was significantly greater in those who received FFP or cryoprecipitate ($p<0.05$). In fact the rise in levels in those who did not recieve FFP or cryoprecipitate was not statistically significant. The highest fibronectin levels found in the children who died were in the two children who received FFP or cryoprecipitate before samples were taken (80 and 150 $\mu$g/ml). Excluding these values however did not significantly alter the results.

The wide range of results meant that fibronectin levels did not discriminate well between survivors and those who died. All children who died had levels before treatment below 60 $\mu$g/ml, but 54% of those who survived also had levels below this value.

7.3.3 Markers for Meningococcal disease

C-reactive protein was measured on admission in 121 children; 79 with MCD and 42 controls. Median CRP in MCD was 77 mg/l(range 4-420), significantly higher than in controls (11 mg/l(3-283); $p<0.0001$). Table 7.4 lists the sensitivity, specificity, positive and negative predictive values for fibronectin, CRP and white cell count on admission for differentiating between MCD and controls.

Table 7.3. Sequential plasma fibronectin (μg/ml) in children with Meningococcal disease on PICU, in those who received fresh frozen plasma or cryoprecipitate (FFP/Cryo) and those who did not. Data shown as median (range). Statistical analysis by Mann-Whitney U test.

| | Admission | Day 5-7 |
|---|---|---|
| All MCD (n=23) | 33(5-280) | 101(51-317)* |
| FFP/Cryo (n=12) | 27(5-75)+ | 98(51-317)* |
| No FFP/Cryo (n=11) | 53(15-280)+ | 105(60-240) |

* Difference between admission and day 5-7 $p<0.005$ by Mann-Whitney U test.

+ Difference between FFP/Cryo and No FFP/Cryo $p<0.005$ by Mann-Whitney U test.

Table 7.4. Sensitivity, specificity, positive and negative predictive values of tests for Meningococcal disease.

| | Sens (%) | Spec (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|
| WBC >10 X $10^9$ | 67 | 30 | 61 | 64 |
| Fibronectin <100μg/ml | 70 | 53 | 76 | 54 |
| CRP >10 mg/l | 97 | 48 | 78 | 9 |
| CRP + Fibronectin | 99 | 17 | 69 | 13 |
| CRP + WBC | 100 | 26 | 72 | 0 |
| CRP + WBC + Fibronectin | 100 | 17 | 69 | 0 |

Key

Sens=sensitivity

Spec=specificity

PPV=positive predictive value

NPV=negative predictive value

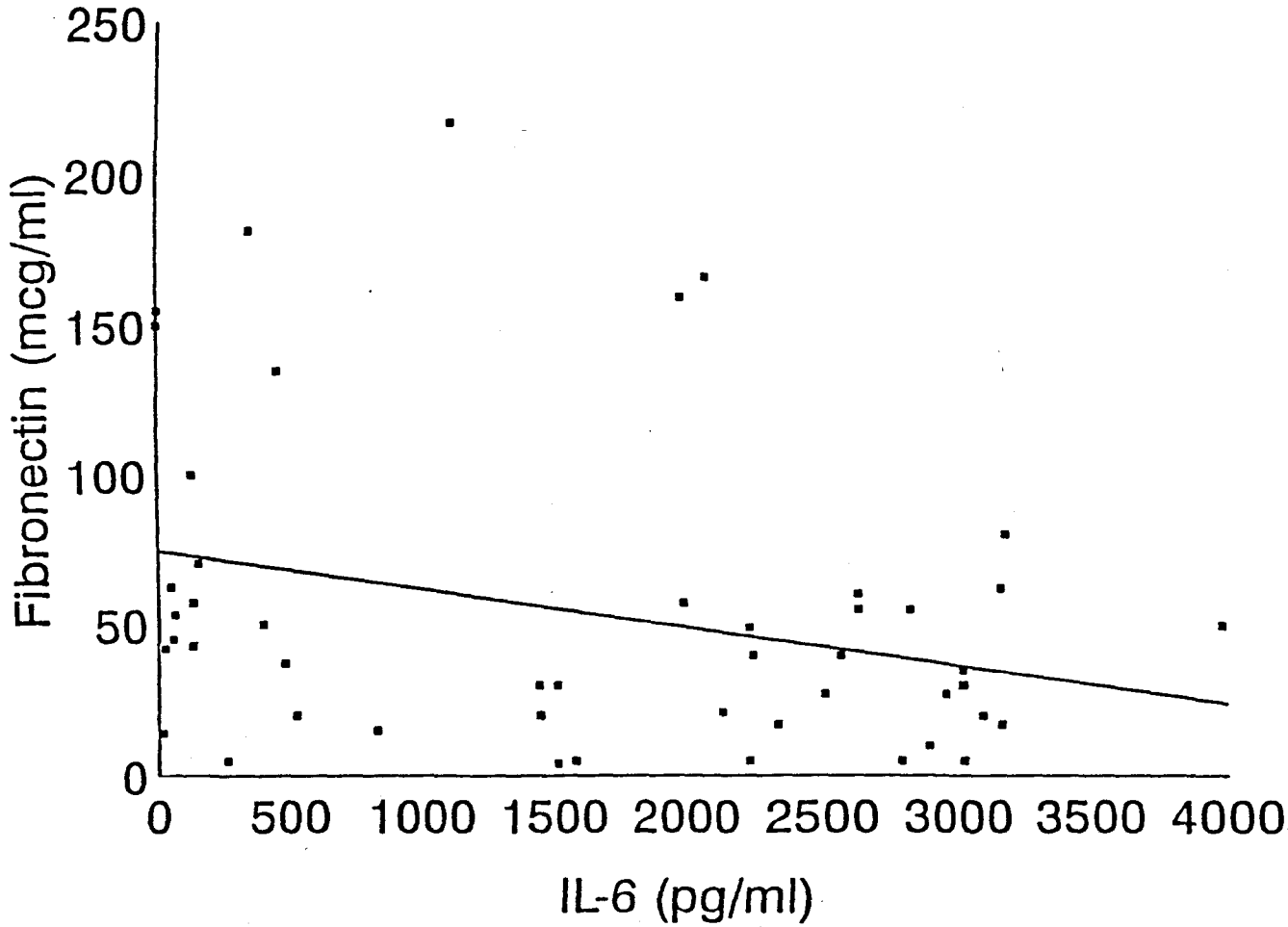



Figure 7.2 Admission fibronectin levels vs IL-6 levels in 47 children with Meningococcal disease.

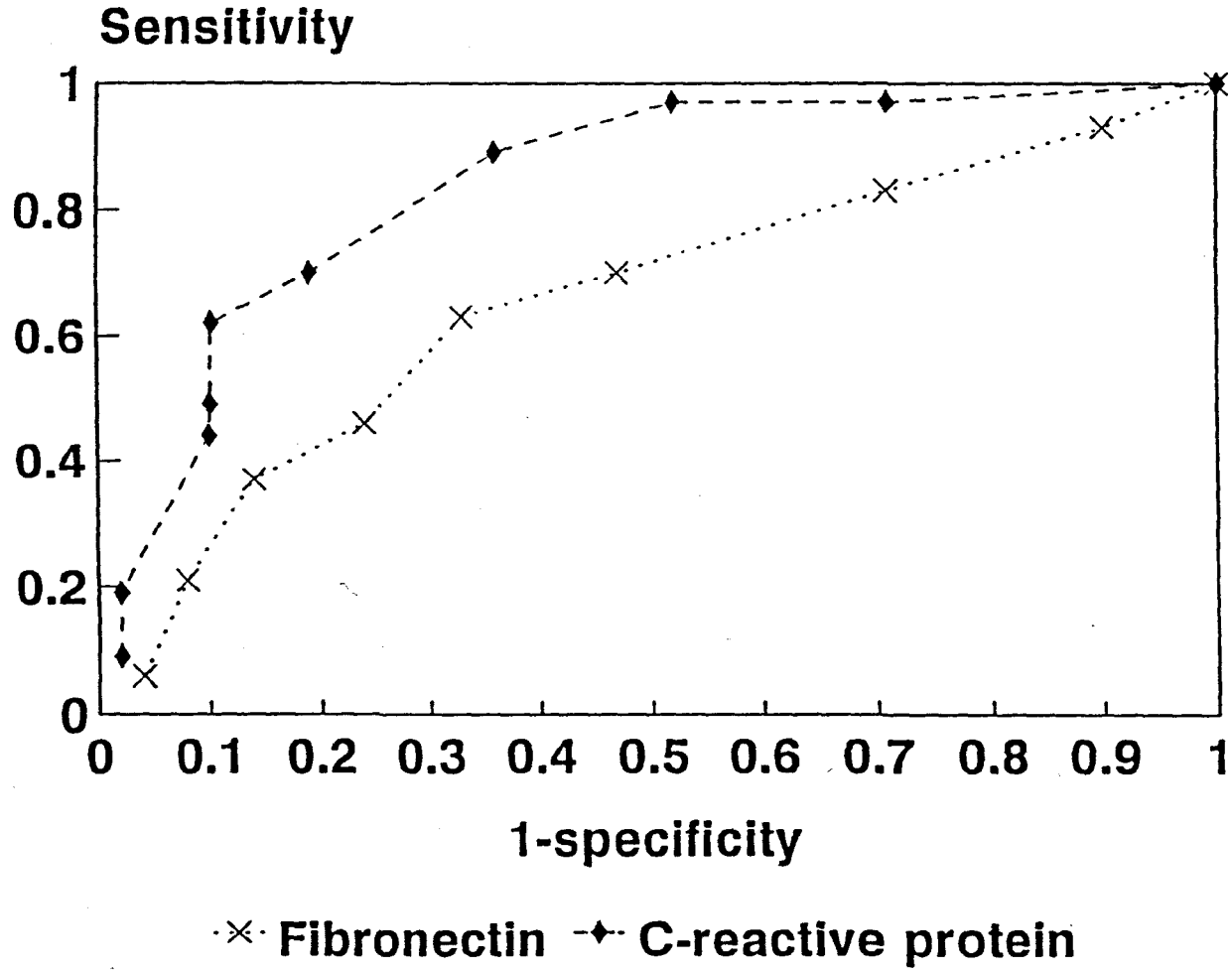



Figure 7.3 Receiver-operating characteristic curves for CRP and fibronectin levels predicting Meningococcal disease.

The combination of CRP >10mg/l and white cell count > 10x $10^9$ identified all cases of MCD. The addition of fibronectin to this combination decreased both the specificity and the positive predictive value.
Receiver-operating characteristic curves were plotted for CRP and fibronectin. This graphical comparison of diagnostic tests plots the sensitivity against 1-specificity for a variety of cut offs. A test that perfectly discriminates between two groups would give a curve that co-incides with the left and top of the plot. A completely useless test gives a straight line from the origin to the top right hand corner (Altman & Bland 1994). At all levels, CRP had better characteristics for detecting MCD than fibronectin (Figure 7.3).

## 7.4 DISCUSSION.

This study confirms low initial levels of plasma fibronectin in children with MCD (Blanco et al 1990). Fibronectin levels were significantly lower in severe disease, septic shock, DIC and in those who died. Levels increased by day 5-7, especially in those children who received FFP or cryoprecipitate. However, low fibronectin levels were poor predictors of MCD or death.
This study has avoided some of the confounders of earlier studies in that a single infection was studied, samples were taken at a similar time point and before the infusion of blood products in all but two cases. Samples were taken from children with a wide range of ages, but these were similar in the MCD and control groups. However it was not possible to control for DIC or the severity of illness.

The control group did not contain normal healthy children, but those initially thought to have MCD. This is the ideal group in which to assess fibronectin as an aid to diagnosis. However it is unlikely that the control group had normal fibronectin levels, as respiratory viral illness (the main diagnosis in the control group) can also cause decreased levels (Anokin et al 1990). Despite this fibronectin levels were still significantly lower in children with MCD compared to controls.

### 7.4.1 Previous studies.

Plasma fibronectin levels are low in adults and neonates with sepsis (Ahlgren et al 1985; Gerdes et al 1983; Barnard & Arthur 1983), and the low concentration is related to the severity of the disease (Brodin et al 1986), as in our study.

Disseminated intravascular coagulation also leads to abnormalities in fibronectin (Bone 1992). In MCD the reduction in fibronectin level correlated with protein C and antithrombin III levels (Blanco et al 1990), as in other forms of sepsis (Mansberger et al 1989; Coulaud et al 1982; Rubli et al 1983). Fibronectin opsonises fibrin-fibrinogen complexes (Saba 1986) and may be involved in maintenance of microvascular integrity at the tissue level (Mosher 1984). The decreased levels in sepsis and DIC may thus be due to its binding to bacteria (Sorvillo & Pearlstein 1985), consumption by phagocytes (Saba et al 1980) or incorporation into the tissue pool of fibronectin (Jin et al 1991).

However plasma fibronectin levels in sepsis also correlate significantly with transferrin, $C_3$ and pre-albumin levels (Coulaud et al 1982; Rubli et al 1983). Low fibronectin levels in sepsis may thus be part of a broader pattern of protein depletion. Studies in adults with sepsis show that the decrease in fibronectin is not due to increased consumption, but to decreased synthesis (Pussell et al 1985; Hesselvik 1987). These changes may be linked with the acute phase reaction regulated by IL-6, since Il-6 causes decreased secretion of fibronectin by hepatocytes (Castell et al 1989). This is supported by the current study where plasma fibronectin levels correlated negatively with IL-6 levels.

Fibronectin levels in septic adults return to normal without supplementation within 2 weeks (Ahlgren et al 1985). The present study found a significant rise by day 5-7, similar to studies in neonates (Gerdes et al 1983), although the rise in fibronectin levels in those children who did not receive FFP or cryoprecipitate was non-significant. However those who were given FFP or cryoprecipitate had significantly lower initial fibronectin levels than those who did not receive blood products, giving a greater range over which they could rise. Plasma fibronectin levels increase after cryoprecipitate infusion (Saba et al 1978), but the half life is only 12 hours. The rise in fibronectin by day 5-7 is thus unlikely to be due to blood products, but to the return of normal endogenous production.

### 7.4.2 Effect on mortality

Blanco et al (1990) did not find lower fibronectin levels in children who died from MCD, like some studies in adults with sepsis (Reviewed by Grossman 1987). Others have found lower fibronectin levels in patients dying from sepsis (Coulaud et al 1982; O'Connell et al 1984), as in our study. Only 4 children died in Blanco's study, this number may be too small to detect a significantly lower level in those who died. Low levels however were poor predictors of prognosis in individual patients in this study, as in others (O'Connell et al 1984).

### 7.4.3 Fibronectin as an aid to diagnosis.

Meningococcal disease may be difficult to diagnose even after admission to hospital (Borchsenius et al 1991; Olcén et al 1979). An acute reduction in plasma fibronectin has been suggested as an early indicator of infection (Koenig et al 1988). This has a sensitivity of 53-75% (Koenig et al 1988; Gerdes and Polin 1987; Edwards et al 1993), similar to that seen in our study. To help decrease mortality from MCD a test, or combination of tests is needed that would correctly identify all cases. If these tests also excluded some without MCD who were initially treated for it, this would also be beneficial. The combination of CRP >10 mg/l and peripheral white cell count > 10 x10, gave 100% sensitivity in our series. An abnormal plasma fibronectin was not a useful additional marker of infection, as in other studies (Gerdes & Polin 1987).

The confirmation of low plasma fibronectin levels in those with MCD, especially in those who died, suggests a possible role for fibronectin therapy in MCD.

### 7.4.4 Fibronectin therapy.

Cryoprecipitate is rich in fibronectin and can increase plasma fibronectin levels. In a series of uncontrolled trials of cryoprecipitate, Saba and co-workers (Saba et al 1978; Scovill et al 1978; Scovill et al 1979) showed improvements in cardiovascular, pulmonary and renal function in critically ill adults.

However controlled trials of fibronectin in septic adults have produced contradictory, though mostly negative results (Grossman et al 1983; Hesselvik et al 1989; Todd et al 1984; Lundsgaard-Hansen et al 1985; Stevens et al 1986; Mansberger et al 1989). These trials contain a very mixed group of small numbers of adults with a variety of infections. There are no trials of fibronectin in MCD. The survival of severely malnourished children however was significantly improved by fibronectin (Sandberg et al 1990).

Fibronectin may enhance the protective effect of monoclonal antibodies in sepsis (Hill et al 1984). The disappointing results of anti-endotoxin antibodies in MCD (J5 Study Group 1992; Marzouk 1995) may be due to the low levels of fibronectin in severe MCD. Trials of immunotherapy combined with fibronectin should be considered in MCD.

### 7.4.5 Conclusions

In conclusion, initial plasma fibronectin levels are decreased in children with life threatening MCD. Low levels however are poor predictors of MCD or death. Levels rise by one week, especially in those given cryoprecipitate. Trials of fibronectin, in combination with monoclonal antibodies may help decrease the mortality from MCD.

# CHAPTER EIGHT. VITAMIN A AND NUTRITIONAL STATUS

# CHAPTER EIGHT. VITAMIN A AND NUTRITIONAL STATUS

## 8.1 INTRODUCTION.

Infection and nutrition often interact. Nutritional status can affect susceptibility to infection (Berkowitz 1992) and infection can affect nutritional status (Scrimshaw et al 1968). Meningococcal disease may have its disease process hindered by poor nutrition, with better nourished children being more likely to die (Neveling & Kaschula 1993). Meningococcal disease may also have an adverse nutritional impact on the child. These changes in nutritional status may be influenced by catabolic hormones like cortisol. The impact of nutritional status on infection and the impact of infection on nutritional status has rarely been studied in developed countries.
Vitamin A levels decrease during febrile illnesses (Thurnham 1989; Arroyave & Calcano 1979). The decrease may be due to the acute phase response to infection (Thurnham & Singkamani 1991; Louw et al 1992; Filteau et al 1993), and correlates with disease severity (Frieden et al 1992; Butler et al 1993; Neuzil et al 1994). Low vitamin A levels are also associated with mortality in measles (Markowitz et al 1989). Supplementation with vitamin A in measles decreases mortality by 60-66% (Glasziou & Mackerras 1993, Fawzi et al 1993), as well as decreasing morbidity (Coutsoudis et al 1991).This protection against death from measles is found even in populations without overt vitamin A deficiency (Ellison 1932; Hussey & Klein 1990).

There are no studies of vitamin A levels in MCD. "It would be of great interest and importance to know whether vitamin A status has an effect on morbidity and mortality from meningitis" (Keusch 1990).

This study seeks to determine whether an association exists between nutritional status and disease presentation or severity of MCD. Changes in nutritional status during the acute phase of treatment will also be measured. Vitamin A levels will be measured in children with MCD to test whether levels correlate with clinical presentation, disease severity or death. If low levels are associated with severity, then vitamin A supplements might decrease mortality from MCD.

## 8.2 METHODS.

Full details of methods have been given in Chapter 2. Briefly children were weighed and had their mid-arm circumference (MAC) and triceps skinfold thickness (TSF) measured on admission. Weight-for-age z scores were then calculated. Measurements were repeated on children with MCD 5-7 days later.

Blood for retinol levels was collected on admission. Retinol levels were measured in a sub group of those with MS, MM+MS and controls, by high performance liquid chromatography (HPLC).

## 8.3 RESULTS.

### 8.3.1 Nutritional status.

Measurements were taken on admission from 117 children with MCD and 60 controls. Mean weight-for-age z score was significantly lower in those with MM+MS compared to MS, MM (Table 8.1) and controls (p=0.024). The nutritional status of the control group (as measured by weight, weight-for-age, MAC and TSF) was not significantly different from that of the MS or MM groups. Children with severe MCD also did not differ in their nutritional status from those with less severe disease (Table 8.2). Weight-for-age was significantly negatively correlated with IL-6 in children with MCD (r=-0.46, p=0.013), but did not correlate with levels of TNF-α. Weight-for-age z scores were only measured in 6 children who died (mean (SD)= 0.17(1.34)), and were not significantly different from survivors (0.28(1.36)).

Repeat measurements were made on 84 children with MCD after 5-7 days of treatment. Weight did not alter significantly over this time, but there was a small but highly significant decrease in MAC and TSF (Table 8.3). Changes in MAC or TSF did not correlate with disease severity (as measured by GMSPS or TNF-α), or catabolic response (as measured by IL-6, cortisol or CRP levels).

Table 8.1. Nutritional status and initial plasma retinol levels across the spectrum of Meningococcal disease. Data shown as median (range), except z score [mean(SD)].

| | MS | MM+MS | MM |
|---|---|---|---|
| Weight | (n=42) | (n=41) | (n=8) |
| (kg) | 12(6-29) | 11(6-32) | 12(9-68) |
| Weight for age | | | |
| z score | 0.67(1.16)* | -0.28(1.39)*# | 1.06(0.94)# |
| MAC | (n=51) | (n=57) | (n=9) |
| (cm) | 17.0(14-30) | 16.3(14-23) | 17.5(15-28) |
| TSF | (n=50) | (n=56) | (n=9) |
| (mm) | 9.0(5-14) | 8.4(5-17) | 9.0(5-16) |
| Retinol | (n=23) | (n=21) | |
| (μg/ml) | 0.14(0-6.64) | 0.11(0-3.93) | |
| Creatinine | | | |
| (μmol/l) | 77(38-228)† | 63(37-157)† | 59(36-88) |
| Length of symptoms | | | |
| (hrs) | 12(2-76)* | 19(1-106)*# | 40(8-137)# |

* Difference between MS and MM+MS p=0.001 by Students t test.

# Difference between MM and MM+MS p=0.01 by Students t test.

† Difference between MS and MM+MS p=0.02 by Mann-Whitney U test.

Table 8.2. Nutritional status and plasma retinol levels of children on admission. Data shown as median (range), except z score [mean(SD)].

| | Severe MCD | Mild MCD | Controls |
|---|---|---|---|
| Weight (kg) | (n=30) | (n=61) | (n=60) |
| | 11(9-19) | 12(6-68) | 11(3-53) |
| Weight for age z score | 0.24(1.32) | 0.35(1.37) | 0.27(1.29) |
| MAC(cm) | (n=42) | (n=75) | (n=58) |
| | 16.5(14-28) | 16.3(14-30) | 16.8(10-28) |
| TSF(mm) | (n=41) | (n=74) | (n=54) |
| | 8.6(5-14) | 8.7(5-17) | 9.0(4-18) |
| Retinol ($\mu$g/ml) | (n=17) | (n=27) | (n=22) |
| | 0.14(0-6.6) | 0.11(0-5.0) | 0.15(0-2.78) |

Table 8.3. Changes in nutritional measurements during treatment of children with Meningococcal disease.

| | Admission | Day 5-7 | P* |
|---|---|---|---|
| Weight (kg) (n=40) | 12(6-32) | 12(6-35) | 0.44 |
| MAC(cm) (n=84) | 16.4(14-28) | 16.0(13-27) | 0.0001 |
| TSF(mm) (n=82) | 8.6(5-17) | 8.0(5-18) | 0.006 |

* Comparison by Wilcoxon Signed Ranks Test.

8.3.2 Vitamin A levels.

Initial plasma retinol levels were measured in 44 children with MCD; 17 with severe disease, 4 of whom died. Levels were also measured in 22 controls, all initially treated for MCD but later found to have less serious illnesses; viral infection 8, respiratory infection 7, vasculitis 4, asthma 1, febrile convulsion 1, abscess 1.

Retinol levels in children with MCD were not significantly different from controls (0.13 μg/ml (0-6.64) vs 0.15 μg/ml (0-2.78); p=0.76), but there was a trend towards lower levels in those with a meningitic component to their illness (Table 8.1). Ten of 21 children (48%) with MM+MS had retinol levels indicating deficiency (below 0.1 μg/ml), compared with 8 of 23 (35%) with MS and 5 of 22 (23%) controls, but this did not reach statistical significance.

Plasma retinol levels were not significantly different in children with severe MCD (Table 8.2) or in those who died (0.39 μg/ml(0.07-6.6) vs 0.12 μg/ml(0-5.0); p=0.17). One of the four children who died had deficient levels. No children were given vitamin A supplements, but 8 children had markedly raised retinol levels (>1.8 μg/ml), 3 controls and 5 with MCD. The child with the highest level died. Retinol levels showed non-significant correlations with CRP (r=-0.19; p=0.21) and IL-6 levels (r=0.34; p=0.15). Samples stored in light protected bottles did not give significantly different results from samples stored in clear tubes (0.14 μg/ml vs 0.14 μg/ml; p=0.53). This implied that the clear tubes had been well protected from light during storage, preventing retinol degradation.

## 8.4 DISCUSSION.

This study shows that nutritional status in MCD may be associated with disease presentation. Children with MM+MS had significantly lower weight-for-age z scores than other presentations and controls. However nutritional status did not appear to be associated with disease severity or mortality. A pilot study of plasma retinol levels shows that some children with MCD have low levels on admission. Low levels were found more often in those with a meningitic component to their illness.

### 8.4.1 Nutritional status

Children with MM+MS had significantly lower weight-for-age z scores than those with MS or controls. This may be a spurious result but did remain significant even after correcting for multiple comparisons ($p<0.05$ by Bonferroni method). Poor nutrition impairs cytokine production and overnutrition may allow overproduction of cytokines (Grimble 1990). Better nutrition may thus lead to higher cytokine levels during the initial bacteraemia of MCD, and the development of MS. Children with lower weight-for-age z scores may produce a lower cytokine response, and develop the less fulminant presentation, MM+MS. However this theory is not supported by the current study, as higher IL-6 levels were found in those with lower weight-for-age.

The lower weight-for-age in the MM+MS group may be due to the longer period of symptoms before admission. The greater length of anorexia and vomiting may have lead to a lower weight on admission in this group. However the lower weight-for-age is unlikely to be due to greater dehydration in this group, since those with MS had significantly greater creatinine levels on arrival (Table 8.1). Further evidence that nutrition does not influence cytokine levels comes from the finding that the severity of MCD was not associated with nutritional status in this study. This confirms one previous study of disease severity and nutritional status (Ryder et al 1987), but contradicts another (Neveling & Kaschula 1993). Both these previous studies were from a hospital in Cape Town, South Africa where malnutrition is common, and these studies may not be relevant to Merseyside. The median weight-for-age z score in the current study was above 0 (ie. above the 50th centile), implying the population (both MCD and controls) were well nourished, except the MM+MS group whose mean was below 0. The control group however were also hospitalised with infection (although most were viral), and may not be the most appropriate group for comparison. Further comparisons of nutritional status of those with MCD with non-hospitalised healthy controls will be required to assess if MS is more likely in better nourished children.

A small (2%) but significant decrease in MAC and TSF occurred in children with MCD during treatment. Similar changes have been found in children with *Haemophilus influenzae* meningitis (Sherry et al 1989). These changes did not correlate with markers of catabolism; cortisol and CRP levels. The clinical significance of these decreases, if any, remains to be established.

These results suggest that children acquiring MCD are adequately nourished, except for those with MM+MS. However disease severity is not associated with nutritional status and the disease itself has a limited impact on nutritional status during treatment.

### 8.4.2 Retinol Levels

Low retinol levels were found in those with MCD and those with less serious infections. Decreased retinol levels have been found in children with acute infections both in the developing world (Thurnham 1989; Arroyave & Calcano 1979) and in the United States (Frieden et al 1992; Butler et al 1993; Arrieta et al 1992; Neuzil et al 1994). These studies found that mortality and disease severity were associated with low retinol levels (Markowitz et al 1989; Butler et al 1993; Frieden et al 1992; Neuzil et al 1994), unlike the present study. In the current study deficient levels were found more often in those with MM+MS, the group with lower weight-for-age z scores. The lower levels may have been influenced by the child's nutritional status.

A sample size calculation shows that a study of 180 cases of MS and 180 with MM+MS would be needed to demonstrate a significant difference in initial retinol levels. In the UK this would require a multicenter study and since mortality was not associated with low retinol levels, such a trial would be of questionable value. However in the developing world where vitamin A deficiency is common and the mortality from meningitis is high (Baraff et al 1993), vitamin A status may be associated with mortality from meningitis (Keusch 1990). Further studies in the developing world might be warranted.

8.4.2.a Low retinol levels in infection.

Follow up studies have found that retinol levels return to normal without supplementation within 8 weeks of infection (Bhaskaram 1985). This implies that these low levels are not true deficiency, but a self correcting, transient adaptation to infection. However infection may produce an accelerated depletion of liver retinol stores (Campos et al 1987).

The cause of this transient decrease is unknown; impaired retinol absorption (Sivakumar & Reddy 1972), inadequate mobilisation of liver stores (Hussey & Klein 1990), redistribution (Vitale 1977) or leakage through the vascular endothelium (Thurnham 1989) have all been suggested. Infection also leads to increased urinary excretion of retinol (Stephensen et al 1994). In other studies retinol levels correlate negatively with IL-6 levels (Tabone et al 1992) and the acute phase reactant, α-1-acid glycoprotein (Filteau et al 1993). Retinol levels also mirror CRP levels (Louw et al 1992).

The decrease in plasma levels may thus be part of the acute phase response (Rosales & Kjohede 1992). The current study however did not find any significant correlation between retinol and CRP or IL-6, probably due to the small numbers involved.

8.4.2.b Vitamin A supplementation

The changes that accompany the acute phase response are normally protective. There may thus be some survival advantage, in certain circumstances, in lower plasma retinol levels during infection. Alternatively the low plasma levels may reflect increased retinol delivery to tissues (Thurnham & Singkamani 1991; Tabone et al 1992). It is suggested that in children with initially low vitamin A levels, the very low levels produced by infections like measles, may impair recovery (Thurnham 1989). Vitamin A supplements improve both the morbidity and mortality from measles (Glasziou & Mackerras 1993; Fawzi et al 1993; Coutsoudis et al 1991). Hence vitamin A supplements may be beneficial in other infections that decrease plasma vitamin A levels.

The finding of markedly raised retinol levels in 8 children was unexpected. No children were given vitamin A supplements after admission, although they may have received them at home. The highest retinol level was found in a child with fulminant disease who developed renal failure. Vitamin A is excreted in the urine (Tomkins & Hussey 1989), and renal compromise may explain the raised levels.

Vitamin A supplementation to these children with high levels may produce toxic effects, such as raised intracranial pressure (de Francisco et al 1993). Further studies of retinol levels in MCD would be required to assess potential toxicity, before vitamin A supplementation could be recommended.

8.4.2.c True vitamin A status in infection

The low plasma retinol levels found in this study may be co-incidental to the acute phase response and raised levels may be due to renal failure. Plasma retinol levels may thus not reflect vitamin A status (Pitt 1981), particularly during infection. A better assessment of vitamin A status is the relative dose response test(RDR).

Relative Dose Response

This test is based on the fact that if the body stores of vitamin A are low then plasma retinol will increase after a dose of vitamin A, peaking at 5 hours. If body stores are high the plasma level will be unaffected by an oral dose. However this test requires two blood samples 5 hours apart, as well as the administration of vitamin A.
A modified relative dose response (MRDR) measuring the increase in plasma of 3,4,didehydroretinol, an isomer of retinol, 5 hours after oral dosage requires only one blood sample (Tanumihardjo et al 1990). However dehydroretinol is not commercially available and requires HPLC for measurement of plasma levels (Underwood 1990). The reliability of this method is now being assessed (Tanumihardjo et al 1994).

Using the RDR or MRDR during acute infection, may define vitamin A status better than plasma retinol levels. Preliminary studies suggest however that infection also affects RDR by decreasing the release of retinol binding protein (Filteau & Tomkins 1995).

### 8.4.3. Conclusion

In conclusion the nutritional status of children with MM+MS was significantly different from those with MS and controls. This undernutrition may protect against the development of MS or may simply be due to a longer period of symptoms before admission. Nutritional status was not associated with disease severity and MCD had only a minor impact on nutritional status during the acute illness. Low plasma retinol levels were found in some children with MCD, especially those with meningitis. Other children had markedly raised levels. On the basis of these findings vitamin A supplements cannot be recommended in MCD. Assessment of true vitamin A status during infection may require a measure of liver stores, such as the RDR, rather than plasma retinol levels.

# CHAPTER NINE. POLYMERASE CHAIN REACTION TECHNIQUES.

# CHAPTER NINE. POLYMERASE CHAIN REACTION TECHNIQUES.

## 9.1 INTRODUCTION

There is a need in MCD for a rapid, highly sensitive test that can confirm the diagnosis, especially at low bacterial concentrations and when prior antibiotics have been given.

The ability of PCR to detect minute amounts of specific DNA in clinical samples makes it a potentially useful method for detecting meningococci in patients with suspected MCD. Three reports of it's use in diagnosing MCD have been published so far (Kristiansen et al 1991; Ni et al 1992a; Saunders et al 1993). All used PCR to detect meningococci in CSF. As discussed in Chapter 3, lumbar puncture may now be undertaken less frequently, so that CSF is not often available to perform PCR on. The previous studies recognise this and the need to develop PCR for use with other specimens in MCD, the most obvious candidate being blood.

PCR for meningococci should ideally be able to detect less than $10^4$ cfu/ml in CSF (since these samples are likely to be negative on microscopy and antigen studies) and 10-100 cfu/ml in blood samples.

This study will seek to confirm the work of Ni et al (1992a) by using PCR to detect meningococci in the CSF of suspected cases of MCD, using primers to the IS*1106* insertion sequence. The technique will then be used to try to detect meningococcal DNA in the blood of these children. If a more sensitive method is needed a "hot-start nested PCR" as described by Saunders et al (1993) could be used.

## 9.2 MATERIALS AND METHODS

### 9.2.1 Materials

-*Taq* DNA Polymerase 5U/ml

-10X PCR Buffer 200 mM Tris-HCL (pH 8.4)
500 mM KCl

-Magnesium Chloride 50mM

-deoxyNucleotide Tri-phosphate 200 μM of each nucleotide (dNTPs). All the above supplied by GibcoBRL.

-Primers (See table 9.1). Department of Molecular Medicine, King's College, London.

-Tris Acetate EDTA Buffer (TAE);
10mM Tris-HCl, pH8.0
1mM ethylenediamine tetra-acetic acid (EDTA)

-Phosphate Buffer solution (PBS)

-Gel Loading Buffer (Blue/Orange, Promega, USA)

-Agarose Sigma Chemical Co., St Louis, USA

-DNA Marker (Promega, USA)

-Sterilised, distilled water (micropure)

-Mineral Oil

-Ethidium Bromide 10mg/ml

-Wizard PCR Preps DNA purification system (Promega Corps, USA).
Direct purification buffer;
50 mM potassium chloride
10 mM Tris HCl (pH8.8 @ 25°C)
1.5mM magnesium chloride
Purification resin

-80% isopropanol BDH Laboratory Supplies, Poole, UK.

Table 9.1 Sequences of primers used for PCR of meningococci by Ni et al (1992a) and Saunders et al (1993).

| Primer type | | Primer sequence | Description |
|---|---|---|---|
| *IS1106* gene | | | |
| Primer 1 | (A521) | ATT ATT CAG ACC GCC GGC AG | Forward |
| Primer 2 | (A522) | CCG ATA ATC AGG CAT CCG | Reverse |
| *por A* gene | | | |
| Primer 1 | (AR03) | GCG GCC GTT GCC GAT GTC AGC C | Outer forward |
| Primer 2 | (AR04) | GCG GCA TTA ATT TGA GTG TAG TTG CC | Outer reverse |
| Primer 3 | (AR05) | CAA AGC CGG CGT GGA AG | Inner forward |
| Primer 4 | (AR06) | GAT CGT AGC TGG TAT TTT CGC C | Inner reverse |
| Primer S | (AR07) | TGA TTT TCG TCC TGA TGC GGC | Sequencing primer |

9.2.2 Methods

9.2.2.a Determining concentrations of suspensions of meningococci

An isolate of *N meningitidis* B15 P1.16,7 was cultured on chocolate agar plates overnight at 37°C in an atmosphere of 5% $CO_2$. Approximately half the colonies were removed on a swab which was then eluted in 10 ml sterile PBS. Doubling dilutions of this suspension were made and the optical density at 540 nm of each was measured. Serial one in ten dilutions of each doubling dilution were made. Fifty $\mu$l of each was plated on chocolate agar in duplicate and incubated overnight as before.
The number of colonies on each plate was counted the following day.

Results

Accurate colony counts were able to be made of the $10^{-4}$ and $10^{-6}$ dilutions.
A plot of optical density vs colony forming units gave a correlation coefficient of 0.998 (Figure 9.1). The equation of the curve was;
meningococcal cfu/ml = $4.5 \times 10^{8} \times$ (O/D @ 450 nm) + 4153869.
The concentration of subsequent suspensions of meningococci was derived from their optical density using the above formula. These dilute suspensions of meningococci were then used to test the sensitivity of PCR.

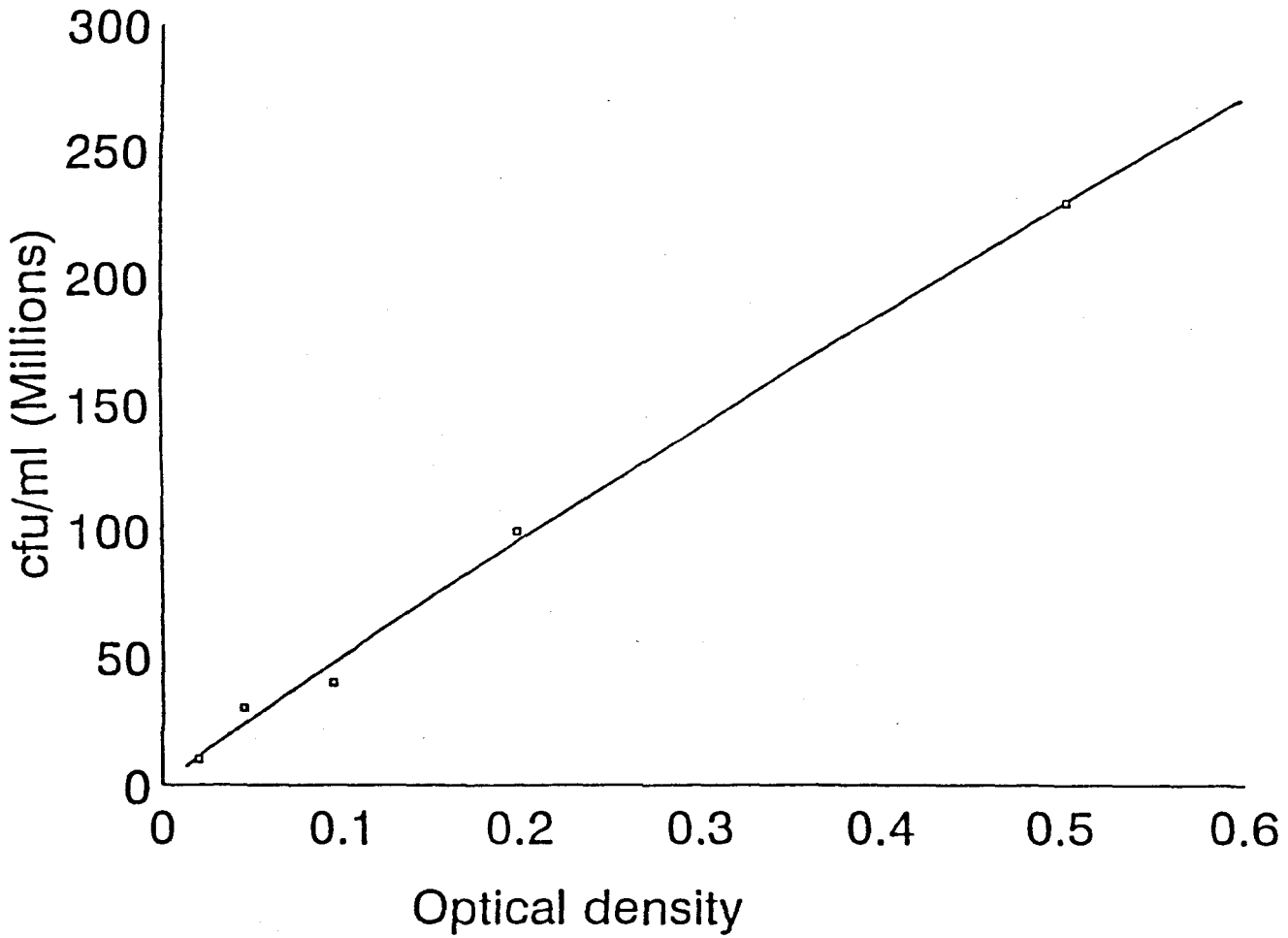



Figure 9.1 Optical density at 450 nM of suspensions of *N meningitidis* against colony forming units per millilitre (cfu/ml).

9.2.2.b PCR for Meningococcal IS*1106* gene.

A "Master-mix" solution was made up on a clean bench as follows;

| | | |
|---|---|---|
| Sterile Water | 29 µl | |
| 10 x *Taq* Buffer | 5 µl | |
| Magnesium Chloride (50mM) | 2 µl | |
| 100 x dNTPs | 0.5µl | |
| Primer 1 (A521) | 5 µl | (300ng) |
| Primer 2 (A522) | 5 µl | (300ng) |
| *Taq* polymerase | 0.5µl | (2.5U) |
| Total | 47.0µl | |

Forty seven microlitres of mastermix was made for each sample and positive control, plus 47 µl extra for a reagent control. The mastermix was then placed on ice.

Three microlitres of each sample and positive control were incubated at 95°C for 5 minutes to lyse cells and release DNA. These samples were then each added to 47 µl of mastermix. The solutions were overlaid with 50µl mineral oil, to prevent evaporation, and placed in a thermal cycler.

Initial denaturation was at 95°C for two minutes, followed by;

32 cycles of;

94°C for 25 seconds - denaturation,

59°C for 40 seconds - annealing

72°C for 60 seconds - extension.

When completed the samples were then stored at -20°C.

A 1.2% agarose electrophoresis gel was made by dissolving 0.96g agarose in 80 ml Tris-Acetate-EDTA (TAE) buffer in a water bath at 95°C. This was allowed to cool and when "hand hot", 3 μl Ethidium bromide (10mg/ml) was added. The agarose was then poured into a plate, fitted with a comb to create wells. When set the comb was removed and the gel placed in a fridge until used.

PCR products were removed from the freezer and the mineral oil aspirated and discarded. When thawed 10 μl of each PCR product was mixed with 2 μl Gel loading buffer and then carefully loaded into a separate well in the Gel. Seven microlitres of DNA marker was also loaded with 2 μl Gel loading buffer. The remainder of the PCR products were stored in a freezer at -20°C.
The gel was then run in an electrophoresis bath, containing TAE buffer, at 100V for 2 hours. DNA was then visualised and photographed on the gel under ultraviolet light.

Testing the sensitivity of the method of Ni et al.

Ni et al (1992a) calculated their method could detect the DNA contained in 10 organisms in a 3 μl sample, equivalent to 3.3 $x10^3$ organism/ml.
Meningococcal suspensions between 1 x $10^9$ and 2 x $10^2$ cfu/ml were tested by this method. PCR products were visualised only for samples containing greater than 1 x $10^7$ cfu/ml. To improve sensitivity the stringency of the reaction was varied.

Varying the reaction stringency.

The stringency of PCR can be altered by varying the magnesium concentration or the denaturation time.
PCR was done using a meningococcal suspension of 1 x $10^9$ cfu/ml. The magnesium concentration in the reaction was varied from 0 to 4 mmol. The clearest band was obtained by using a final magnesium concentration of 2 mmol, confirming this to be the best concentration to use.
To ensure lysis of the organisms and release of DNA the sample denaturation time was increased from 5 to 10 minutes. To ensure denaturation of the DNA to allow primer to bind to it, the denaturation time in the PCR cycle was also increased to 1 minute.
Despite these measures only samples with greater than 1 x $10^8$ cfu/ml were detected.

An experiment performing PCR on two different strains of meningococci (B15 P1.16,7 or Bnt P1.4) and two different suspension fluids (PBS or water) was performed. There was no difference in the sensitivity of the PCR for either organism or suspension fluid. Only suspensions of 1 x $10^8$ cfu/ml or greater were detected.

9.2.2.c Nested PCR.

The method by Ni et al (1992a) proved too insensitive, in our hands, to detect meningococci in clinical samples. Sensitivity could not be improved by altering the stringency of the reaction or the samples used. Nested PCR can increase sensitivity, so the method described by Saunders et al (1993) was used.

"Hot-start" nested PCR for meningococcal *por A* gene.

First Round.

A "Master-mix" solution was made up on a clean bench as follows;

| | | |
|---|---|---|
| Sterile Water | 31 µl | |
| 10 x *Taq* Buffer | 10 µl | |
| Magnesium Chloride (50mM) | 4 µl | |
| Diluted dNTPs | 10 µl | (20µmol) |
| Diluted Primer 1 (AR03) | 5 µl | (20pmol) |
| Diluted Primer 2 (AR04) | 5 µl | (20pmol) |
| Total | 65 µl | |

Sixty five microlitres of mastermix was made for each sample and positive control, plus 65µl extra for a reagent control. The mastermix was placed on ice.

Sample preparation.

One hundred microlitres of each sample and positive control was centrifuged at 13,000 rpm for 10 minutes. The supernatant was aspirated and discarded. The pellet was resuspended in 25µl sterile water and then denatured in a water bath at 95°C for 10 minutes. Samples were then centrifuged and placed on ice.
Sixty five µl of master-mix was added to each sample and overlaid with 75µl mineral oil. Tubes were placed in a thermal cycler and heated to 90°C for 5 minutes.

*Taq* DNA polymerase (2.5 units in 0.5 µl) was diluted 1 in 20 with sterile water, and 10 µl was added to each sample after they had been heated for 5 minutes ("Hot-start").

This was followed by 15 cycles of;

95°C for 1.5 minutes - denaturation,

70°C for 3 minutes - annealing & extension.

then 15 cycles of;

95°C for 1.5 minutes - denaturation,

70°C for 4 minutes - annealing & extension.

Samples were then stored at -20°C.

Second Round.

A "Master-mix" solution was made up on a clean bench as follows;

| | | |
|---|---|---|
| Sterile Water | 46 µl | |
| 10 x *Taq* Buffer | 10 µl | |
| Magnesium Chloride (50mM) | 4 µl | |
| Diluted dNTPs | 10 µl | (20µmol) |
| Diluted Primer 3 (AR05) | 5 µl | (20pmol) |
| Diluted Primer 4 (AR06) | 5 µl | (20pmol) |
| Total | 80 µl | |

Eighty microlitres of master-mix was placed in each sample tube and stored on ice.

The products from the first round were removed from the freezer and the mineral oil was aspirated and discarded. Once thawed 10 µl of first round product was added to the mastermix in each sample tube and overlaid with 75µl mineral oil.

Tubes were placed in a thermal cycler and heated to 90°C for 5 minutes and then had a "Hot-Start" with the addition of diluted *Taq* DNA polymerase, as before.

This was followed by 3 cycles of;

95°C for 3 minutes - denaturation,
50°C for 2 minutes - annealing
72°C for 2 minutes - extension.
then 27 cycles of;
95°C for 3 minutes - denaturation,
50°C for 2 minutes - annealing
72°C for 2 minutes - extension.

Samples were analysed by electrophoresis on a 1.2% agarose gel, stained with ethidium bromide, and visualised and photographed under ultraviolet light as before.

Results with "Hot-Start" nested PCR.

Using this technique samples with a concentration of 1 x $10^2$ cfu/ml were detected. However samples containing only 1-10 cfu/sample produced a smaller band of DNA than samples with higher concentrations (See figure 9.2). This could be due to mispriming in the PCR because of a low target DNA or poor amplification in the second round. Higher concentrations gave a product similar in size to that seen after the first round, suggesting poor amplification during the second round.

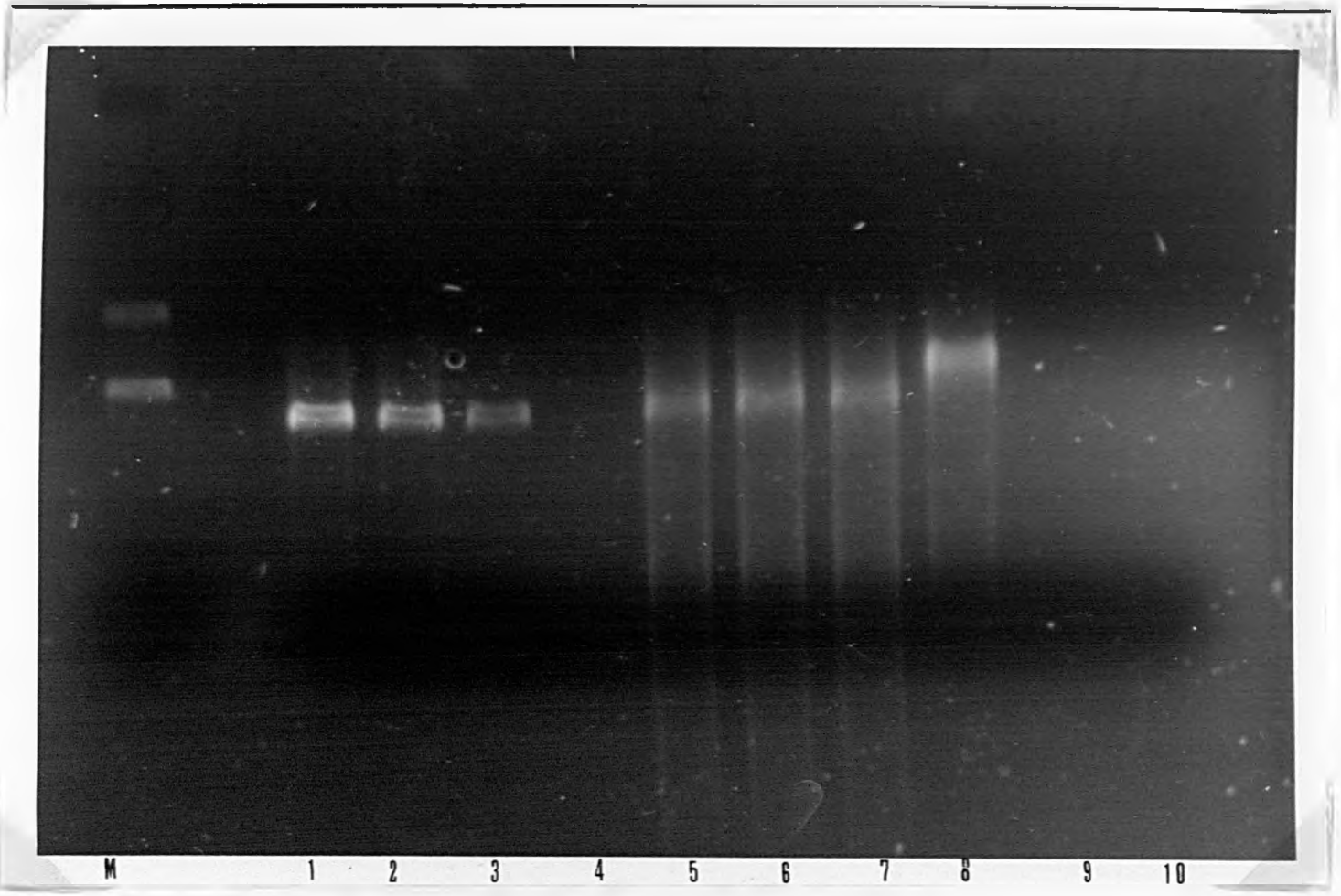



Figure 9.2 Electropheresis gel of products from nested PCR.

Lane M=DNA mass marker. Lanes 1-4=First round products from suspensions of $10^9$, $10^7$, $10^6$ and $10^3$ respectively. Lanes 5-8=Second round products from suspensions of $10^9$, $10^7$, $10^6$ and $10^3$ respectively. Lanes 9 and 10=negative controls. All products slightly larger than 1 kbp, except lane 8.

Altering the stringency of the PCR.

The magnesium concentration was varied to alter the stringency and reduce possible mispriming. However with samples below 1 x $10^2$ cfu/ml the smaller band was still seen at magnesium concentrations of 1, 1.5 and 2 mmol. At the lower magnesium concentrations only faint bands were obtained.

Altering Primers.

The second round produced less product than the first round, rather than more. This suggested problems with the second round primers, failing to amplify first round product. A "mock second round", using the first round primers again was performed. This produced larger amounts of product, much more as expected.
Second round primers were therefore not able to amplify the section of *por A* gene produced by the first round. The primer sequences were checked against the known *por A* gene sequence, and matched exactly (Barlow et al 1989). Class 1 proteins vary between subtypes of meningococci, so the second round primers may not match the sequence in the organism tested. A range of group B subtypes and group A meningococci were therefore compared in the PCR using either first or second round primers in the second round. For every group B subtype tested, and for group A, clearer bands were obtained using first round primers in both rounds, implying mismatching of one of the second round primers (Figure 9.3).

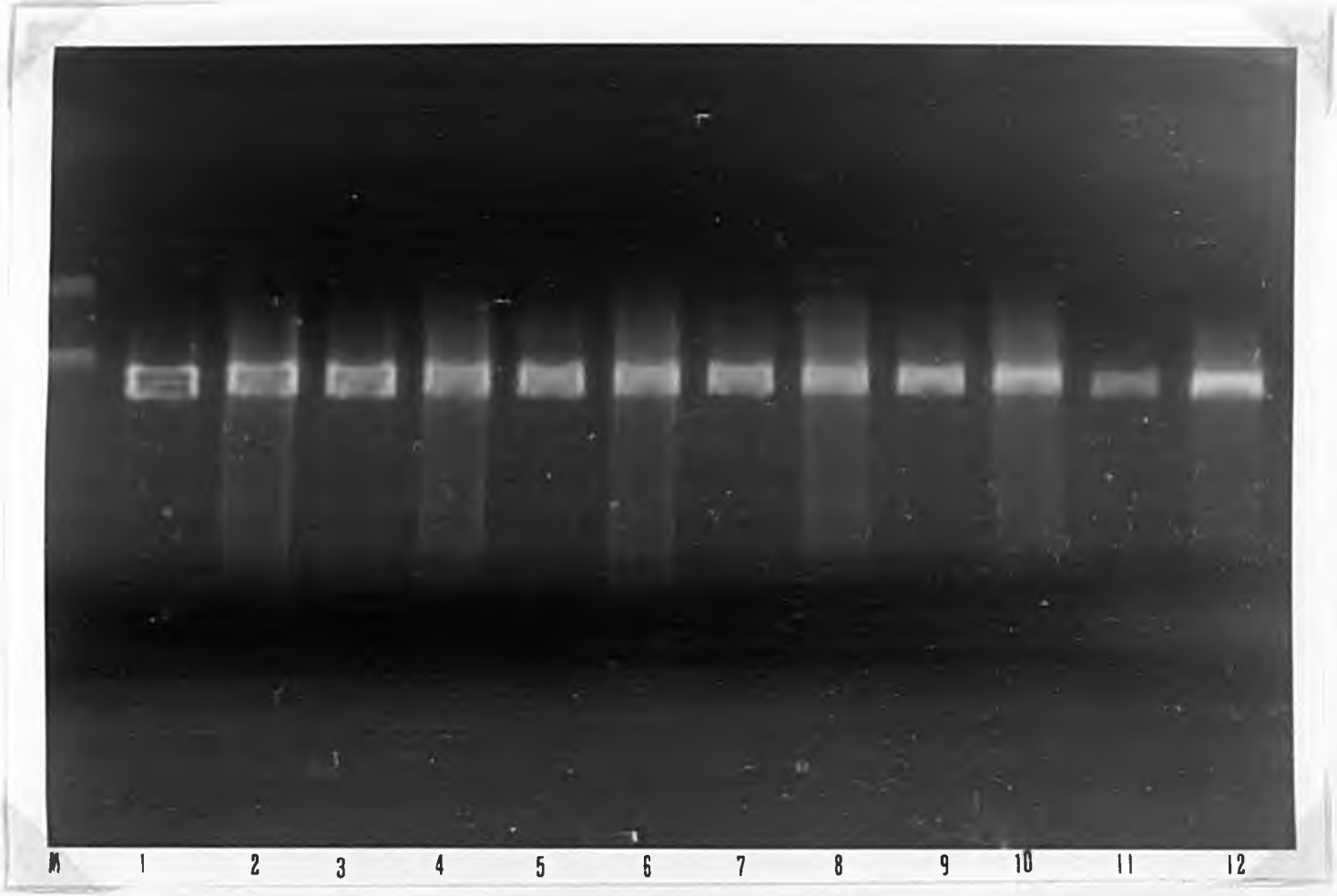



Figure 9.3 Electrophoresis gel of PCR products from a variety of meningococci. First and second round products for each strain tested are shown, first round products on the left. First round primers were used for both first and second rounds of PCR. Lane M DNA mass marker. Lanes 1 & 2 BntP1.4, lanes 3 & 4 Bnt, lanes 5 & 6 non-groupable, lanes 7 & 8 B P1.10, lanes 9 & 10 B P1.14, lanes 11 & 12 B P1.16.

To determine which second round primer may be mismatched, PCR was done on two meningococcal subtypes using 4 combinations of primer in the second round; 1 and 2, 3 and 4, 1 and 4 or 2 and 3. Primer 3 and 4 gave only slightly more product than that generated from the first round. Primers 1 and 2 gave by far the largest amount of product. Primers 2 and 3 produced slightly more than 1 and 4, but neither gave as much as primers 1 and 2. It was therefore decided to use primers 1 and 2 for both rounds of the PCR, and to confirm the product they made by sequencing it.

9.2.2.d PCR of clinical samples.

PCR was used to try to detect meningococci in CSF samples collected from patients. Samples from culture positive MM, clinically suspected MM (culture negative after prior antibiotics), and culture negative from a child with a viral illness were examined by PCR. Meningococci were not detected in any samples. This implied poor sensitivity of the PCR, possibly due to inhibition by components of CSF.

Sensitivity of PCR on CSF samples.

CSF from children known not to have meningitis was "spiked" with varying concentrations of meningococci (1 x $10^5$ cfu to 1 cfu per sample). PCR was unable to detect meningococci in any of these spiked samples. This implied CSF inhibited the PCR. To confirm this water was spiked in the same way as the CSF. The method was unable to detect meningococci in concentrations below 1 x $10^4$ cfu/ml.

The decrease in the method's sensitivity was investigated. Despite using new reagents and freshly prepared suspensions of *meningococci*, sensitivity did not improve beyond 1 x $10^4$ cfu/ml.
This level of sensitivity would detect meningococci in CSF, but mostly those that could be seen on microscopy, but would not detect the levels of meningococci found in blood. The analysis of further samples was therefore not undertaken.

9.2.2.e Specificity of PCR.

For PCR to be useful it needs to be highly specific, detecting all meningococci, but not giving false positive results with other *Neisseria* species or other pathogens. Suspensions of a variety of organisms were made (Table 9.2). These included pathogens likely to be found in CSF or blood. These suspensions contained high numbers of organisms, likely to be in excess of those found in clinical samples. Suspensions of a number of other *Neisseria and related* species were also tested with PCR (see Table 9.2). Only *N meningitidis* gave positive results, except for one other *Neisseria* species.
This organism had been identified as *N flava*, but on subsequent culture it displayed some properties of both *N flava* and *N meningitidis*. It did not give the typical yellow colonies seen with *N flava* although it did utilise sucrose, unlike meningococci. It did not agglutinate with meningococcal grouping sera.

Table 9.2. Organisms used to test specificity of PCR.

| Pathogens; | Other *Neisseria* and related species; |
|---|---|
| *Haemophilus influenzae* | *N flava* |
| *Streptococcus pneumoniae* | *N gonorrhoea* |
| Group A *streptococcus* | *N lactamica* |
| *Staphylococcus aureus* | *N pharyngis* |
| *Staphylococcus epidermidis* | *N sicca* |
| *Enterobacter cloacae* | *Acinetobacter calcaoaccticus* |
| *Burkholderia cepacia* | *Moraxella catarhalis* |
| *Corynebacterium* | |

A different isolate of *N flava*, which displayed the typical properties of *N flava*, did not give a positive result with PCR. It therefore seemed that the first isolate was a hybrid between *N flava* and *N meningitidis*. Further identification of this organism showed the presence of pili on electron microscopy. These pili did not react with antibodies against Type 1 meningococci pili. The sequence of the PCR product from this organism was determined.

9.2.2.f Sequencing of PCR product.

To confirm that PCR did produce copies of the *por A* gene, the PCR product was sequenced. The Wizard™ PCR Preps DNA purification system (Promega Corps) was used to remove primers, primer dimers and other impurities. Three hundred microlitres of PCR product from *N meningitidis* Bnt P1.4 was mixed with 100μl of direct purification buffer, and then 1 ml of resin was added. The resin/DNA mix was pushed through the supplied column and washed with 2 ml 80% isopropanol. The resin was dried and the DNA was then eluted off with 30 μl of water. Doubling dilutions of the purified DNA were run on an electrophoretic gel to estimate the DNA concentration. The amount of product was compared with the known concentration of the DNA marker on the gel. This gave an estimated amount of purified DNA as 400ng/μl.

This product was then sequenced using an Applied Biosystems automatic DNA sequencer (Figure 9.4). Sequencing was performed by the Department of Genetics and Microbiology, Liverpool University. The outer primers (1 and 2) were used as sequencing primers.

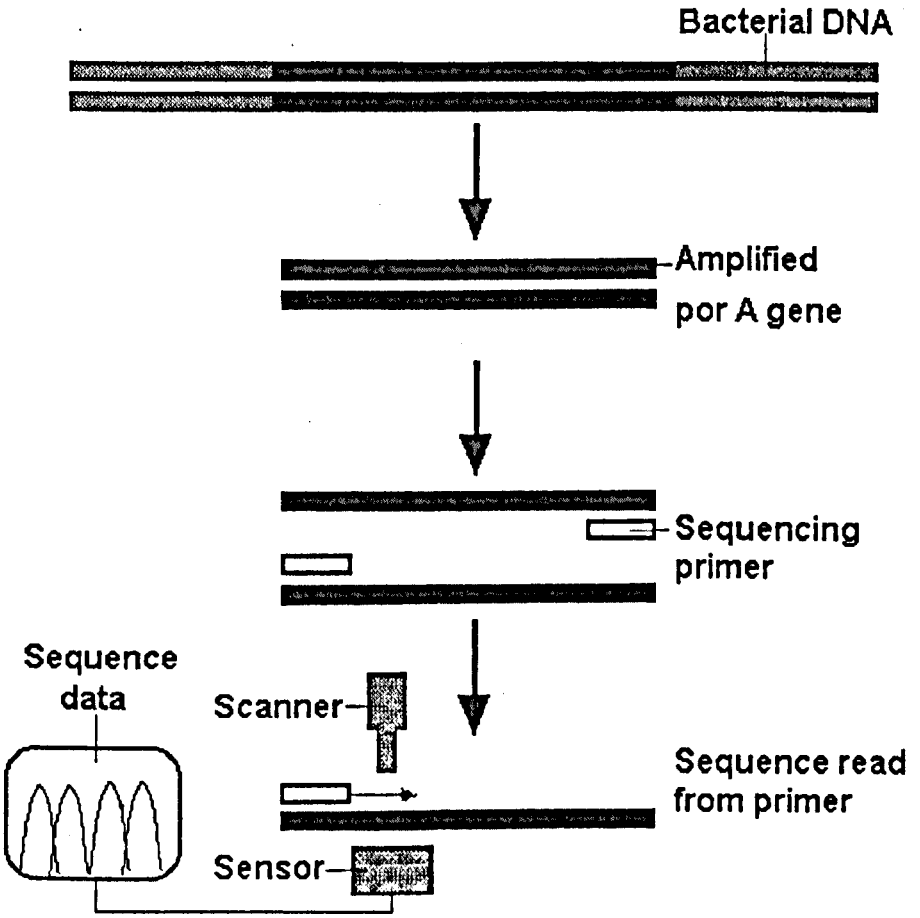



Figure 9.4 Diagram of DNA sequencing. A sequencing primer anneals to the ampified portion of DNA (PCR product). The sequence is read from the primer.

Sequence of meningococcal DNA.

The sequencer was able to read 489 bases from primer 1 and 635 bases from primer 2. The entire 1.1 kbase product was thus sequenced from either end with some overlap in the middle.

A sequence of 1065 bases was read and differed from that published by Barlow et al (1989) for P1.16 by 110 bases (10.3%) (Figure 9.5). The sequencing method is least accurate at the beginning of sequencing and after 450 bases, and sporadic differences were mostly found at the start and end of the determined sequences. Two areas had marked differences from the published sequence. These both occurred in the two regions where major variation in other Class 1 proteins have been described; variable regions 1 and 2 (McGuinness et al 1990). A further difference was a two amino acid deletion corresponding to the sixth outer membrane loop of protein 1, one of two semivariable regions (McGuinness et al 1993)(Figure 9.6).

The sequence in the second variable region was compared with that for other P1.4 serosubtypes (Maiden et al 1992). The sequence differed by 5 out of 39 bases (See Figure 9.7)

A mismatch was found at the site of primer 3 where two guanosines replaced two adenosines. This might explain the poor results with the nested primers, but was close to the start of sequencing and could also be due to misreading. To confirm this sequence a sequencing primer (AR07) was designed to bind 100 bases upstream from the region of primer 3, so that an accurate sequence of this area could be obtained.

Sequencing with this primer confirmed the variation seen in the first variable region, but found the site of primer 3 to match the published sequence exactly (Figure 9.8). The sequence in the first variable region was compared with that for other serosubtypes (Maiden et al 1991). The sequence exactly matched that for the P1.7 subtype, except for a two amino acid deletion in the centre of the region (See Figure 9.7).

Sequence of *N flava* DNA.

PCR product from the strain of *N flava* found to be positive by PCR was purified and sequenced as above. The sequence showed great homology to that of the meningococcal *por A* gene with 174 out of 1082 bases (16%) mismatched (Figure 9.9). The greatest variation was again in the two variable regions. The deduced amino acid sequence of the first variable region was similar to that described for non-subtypable meningococci (McGuinness et al 1993). Amino acid deletions were also seen in the semivariable regions, as with the previous sequence.

1 3 gg
GCG GCC GTT GCC GAT GTC AGC CTG TAC GGC GAA ATC AAA GCC GGC GTG
x n t t t t a xx ax cc
GAA GGC AGG AAC AT-C CAG GCG CAA TT-G ACC GAG **CAG CCC CAA GTA**
g ga gcg agc gg
**ACT AAC GGT GTG CAA GGC AAT CAG GTA AAA** GTT ACT AAG GCC AAA
S
AGC CGC ATC AGG ACG AAA ATC AGC GAT TTC GGC TCG TTT ATC GGC
g t a
TTT AAG GGG AGC- GAG GAT TTG GGC GAA GGG CTG AAG GCT GTT TGG
g t a tc a
CAG CTT G-AG CAA GA-C GTA TCC GTT GCC GGC GGC GGC GCG TCC CAG
n tc ag c
TGG GGC AAC AGG- GAA TCC TTT ATC G-GC TTG GCA GGC- GAA TTC GG-T
x tgg g c
ACG CTG CGC GCC GGT CGC GTT GCA- AAT CAG TTT -GAC GAT GCC AGC-
c g c
CAA GCC ATT AAT CCT TGG GAC AGC AAT AAT GAT GTG GCT TCG CAA TTG
c g t
GGT ATT TTC AA-A CGC CAC GAC G-AT ATG CCG GTT TCC GTA CGC TAC
c c x c x
GAT TCT CCG GAA TTT TCC GGT TTC AGC GGC AGC GTC CAA- TTC GTT CCG
ac x x x c x xx gtt gtt
GCT CAA AAC AGC AAG TCC GCC TAT AAG- CCG **GCT TAT TAT ACT AAG**
tg aat c g gt ca t a c c
**GAT ACA AA-C AAT AAT CTT ACT CTC GTT** CCG G-CT GTT GTC GGC- AAG
t t c
CCC GGA TCG GAT- GTG TAT TAT GCC GGT CTG AAT TAC- AAA AAT GGC
t
GG-T TTT GCC GGG AAC TAT GCC TTT AAA TAT GCG AGA CAC GCC AAT GTC

GGA CGT AAT GCT TTT GAG TTG TTC TTG ATC GGC AGC GCG ACG AGT
c
GAT GAA GCC AAA GGT ACC GAT CCC TTG AAA AAC CAT CAG GTA CAC CGC

CTG ACG GGC GGC TAT GAG GAA GGC GGC TTG AAT CTC GCC TTG GCG
t ag xxx xxx c
GCC CAG TTG GAT TTG TCT GAA AAT GGC GAC AAA GCC AAA ACC AAA AAC

AGT ACG ACC GAA ATT GCC GCG ACT GCT TCC TAC CGC TTC GGT AAT GCA

GTT CCA CGC ATC AGC TAT GCC CAT GGT TTC GAC TTG ATC GAA CGC GGT
4
AAA AAA GGC GAA AAT ACC AGC TAC GAT CAA ATC ATC GCC GGC GTT GAT
tx xxa cac a
TAT GAT TTT TCC AAA CGC ACT TCC GCC ATC GTG TCT GGC GCT TGG CTG
2
AAA CGC AAT ACC GGC ATC GGC AAC TAC ACT CAA ATT AAT GCC GCC

Figure 9.5 Nucleotide sequence of *por A* gene from Barlow et al (1989) shown in upper case. Differences from the sequence derived from P1.4 in the current study are shown; base substitutions in lower case and deletions as x. Binding sites for primers are numbered and shown as; CCC. Variable regions are shown in bold; **CCC**, semivariable regions underlined; CCC.

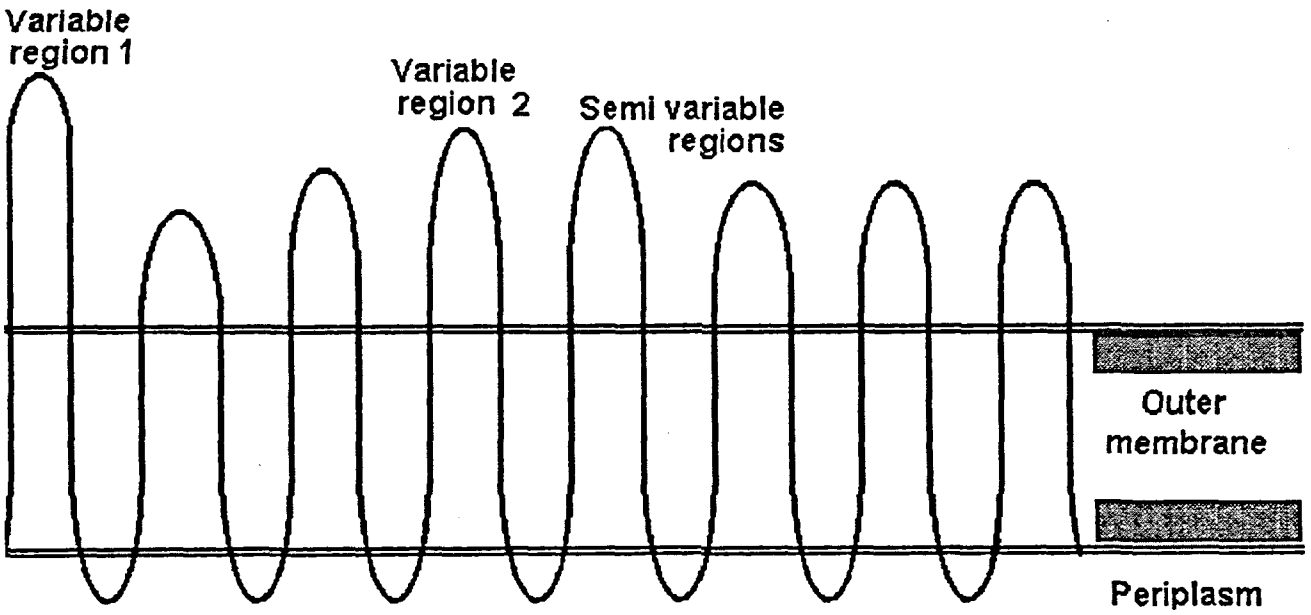



Figure 9.6 Proposed structure of meningococcal class 1 outer membrane protein (*por A*). Variable and semivariable regions are shown on the tips of the outer membranous loops. After van der Ley et al (1991) and Maiden et al (1991).

Variable region 1:

xxx xxx

GCA CAA GCC GCT AAC GGT GGA GCG GGA **GCG AGC GGT CAG** GTA AAA

Ala Gln Ala Ala Asn Gly Gly Ala Gly Ala Ser Gly Gln Val Lys

Variable region 2:

Tyr Pro Ile

t c c a t

CAT GTT GTT **GTG AAT AAC-** **AAG GTT** GCT ACT CAC GTT CCG

His Val Val Val Asn Asn Lys Val Ala Thr His Val Pro

Figure 9.7 Nucleotide sequence of Variable region 1 of *por A* gene from P1.7 meningococcus (Maiden et al 1991) and Variable region 2 of P1.4 meningococcus (Maiden et al 1992) shown in upper case. Deduced amino acid sequence shown below. Differences from sequence obtained in the current study are shown; base substitutions in lower case and deletions as x. Epitope recognised by sub-typing antibodies shown in bold; **CCC**.

```
1                                   a                   3
GCG GCC GTT GCC GAT GTC AGC CTG TAC GGC GAA ATC AAA GCC GGC GTG GAA GGC
        ta      ct              t   a xx    x       cc g            ga
AGG AAC ATC CAG GCG CAA TTG ACC GAG CAG CCC CAA GTA ACT AAC GGT GTG
gcg a   gg                      cca         S
CAA GGC AAT CAG GTA AAA GTT ACT AAG GCC AAA AGC CGC ATC AGG ACG AAA ATC A
```

Figure 9.8 Nucleotide sequence of *por A* gene from Barlow et al (1989) shown in upper case. Differences from the sequence derived from P1.4 using sequencing primer (S) are shown; base substitutions in lower case and deletions as x. Binding sites for primers are numbered and shown as; CCC. Variable region 1 shown in bold; **CCC**

1 c xx x x 3 gg
GCG GCC GTT GCC GAT GTC AGC CTG TAC GGC GAA ATC AAA GCC GGC GTG
ct g a c t
GTG GAA GGC AGG AAC ATC CAG GCG CAA TTG ACC GAG **CAG CCC CAA**
xxx xxx t at xxx gcg xxx cc xxx xxx cg
**GTA ACT AAC GGT GTG CAA GGC AAT CAG GTA AAA** GTT ACT AAG GCC AAA

AGC CGC ATC AGG ACG AAA ATC AGC GAT TTC GGC TCG TTT ATC GGC TTT
g g
AAG GGG AGC- GAG GAT TTG GGC GAA GGG CTG AAG GCT GTT TGG CAG
t a
CTT GAG CAA GA-C GTA TCC GTT GCC GGC GGC GGC GCG TCC CAG TGG
n a cx
GGC AAC AGG- GAA TCC TTT ATC GGC TTG GCA GGC GAA TTC GG-T ACG
gg c
CTG CGC GCC GGT CGC GTT GCA- AAT CAG TTT GAC GAT GCC AGC CAA-
g t c
GCC ATT- AAT CCT TGG GAC AGC AAT AAT GAT GT-G GCT TC-G CAA TTG
t n g tt t cncnn
GGT ATT TT-C AA-A CGC CAC GAC GAT ATG- CCG GTT T-CC -GTA- CGC TAC
c cc a n g n c
GAT TCT CCG GAA TTT TCC-- GGT TTC- AGC- GGC AGC G-TC CAA TTC GTT
n c n ccc a a a c a g t aa
-CCG -GCT-- CAA AAC AGC AAG- TCC GCC TAT AAG CCG **GCT TAT- TAT ACT**
a ncc cc a t g xxx ca t t x t t c
**AAG --- GAT ACA AAC AAT AAT CTT ACT CTC GTT** CCG GCT GTT GTC GGC-
x g g gtn gg antn a t t g a c c
AAG CCC GGA TCG GAT GTG- TAT TAT GCC GGT C-TG AAT TAC- AAA AAT
g gg c cca ana a a
GGC GGT TTT GCC-- GGG AAC TAT GCC TTT AAA TAT --- GCG AGA CAC GCC
g cc g c a g xxx
AAT GTC GGA CGT AAT GCT TTT GAG TTG TTC TTG ATC GGC- AGC GCG ACG
g c c c
AGT GAT- GAA GCC- AAA GGT ACC- GAT CCC TTG AAA AAC- CAT CAG GTA
c
CAC- CGC CTG ACG GGC GGC TAT GAG GAA GGC GGC TTG AAT CTC GCC TTG
t xxx xxx ca n
GCG GCC CAG TTG GAT TTG TCT GAA AAT GGC GAC AAA GCC AAA AC-C AAA
c
AAC AGT ACG ACC GAA ATT GCC GCG ACT GCT TCC TAC CGC TTC GGT AAT
t
GCA GTT CCA CGC ATC AGC TAT GCC CAT GGT TTC GAC TTG ATC GAA CGC
4
GGT AAA AAA GGC GAA AAT ACC AGC TAC GAT CAA ATC ATC GCC GGC GTT
c
GAT TAT GAT TTT TCC AAA CGC ACT TCC- GCC ATC GTG TCT GGC GCT TGG
c 2
CTG AAA CGC AAT ACC GGC ATC GGC AAC TAC ACT CAA ATT AAT GCC GCC

Figure 9.9 Nucleotide sequence of *por A* gene from Barlow et al (1989) shown in upper case. Differences from the sequence derived from *N flava* variant are shown; base substitutions in lower case and deletions as x. Binding sites for primers are numbered and shown as; CCC. Variable regions are shown in bold; **CCC**, semivariable regions underlined; CCC.

## 9.3 DISCUSSION.

The methods of PCR used were too insensitive to detect meningococcal DNA in clinical samples. Bacterial culture is currently the most sensitive method of detecting meningococci, with 80% sensitivity in CSF (Bohr et al 1983). However pre-treatment with antibiotics decreases the sensitivity to 50% in CSF and less than 10% in blood (Cartwright et al 1992b). CSF microscopy and antigen detection are insensitive if less than $10^3$ - $10^5$ cfu/ml are present. However in this study neither method of PCR that was used could consistently detect concentrations lower than this.

### 9.3.1 Sensitivity of PCR.

The sensitivity of PCR depends on the number of target DNA molecules present in the sample and the complexity of the non-target sequence. If only a few copies of the target are present in a sample, they will require a large number of cycles to amplify them sufficiently to be detected. This however will give more opportunity for amplifying the non-target area. This mispriming occurs during the initial boiling, cooling and denaturation, as does the annealing of the primers to each other (primer-dimers).

9.3.1.a The method of Ni et al.

The method of Ni et al (1992a), had a calculated sensitivity of 3.3 x $10^3$ cfu/ml. However this estimation was made from the minimum amount of purified bacterial DNA detected by PCR. In reality the lysis of organisms in clinical samples may lead to the release of inhibitors (Lee 1994). PCR on clinical samples may be much less sensitive than with purified DNA, although Ni and co-workers state that less than 5 $\mu$l of CSF did not inhibit their method. Our study later found that the sensitivity of PCR of "spiked "CSF was less than that of "spiked" water.
This does not fully explain the lack of sensitivity in the current study. Ni et al were able to detect meningococci in 10 of 11 CSF samples, 5 of which were negative on microscopy (antigen studies were not performed). The sensitivity in our study ($10^8$) was unlikely to give positive results with any clinical samples. A more sensitive method was sought.

9.3.1.b The method of Saunders et al.

Saunders et al (1993) state that many of their initial attempts at identifying low concentrations of meningococci were unsuccessful. A smear rather than a distinct band was seen on the gel, suggesting mispriming. When a sample contains less than 10 copies of the target DNA, PCR gives inconsistent results (Varas et al 1991). This is often due to mispriming of other DNA sites, since there is so little target DNA for the primers to bind to.

Mispriming can be reduced by using nested primers, and by adding the DNA polymerase once the DNA has been denatured by heating ("hot-start PCR"). Saunders et al (1993) therefore used these techniques.

Nested primers.

Nested primers are designed to amplify the specific product produced by the first round PCR, and not any other product caused by mispriming. As with all PCR it is critically dependant on the primers used. The nested primers failed to amplify product in the current study, although the outer primers did produce product in the second round. The nested primers matched the known sequence of *por A*, but sequencing of the product found a two base pair difference with primer 3. This mismatch may partly explain the poor results with nested primers. However nested primer 4 matched the product exactly but did not amplify much product when combined with the outer primer 1. Repeat sequencing did not confirm the mismatch at the binding site of primer 3. The poor amplification by the inner primers remains unexplained.
Using the outer primers in both rounds initially gave sensitive results, then the sensitivity decreased. This may be due to some of the other reagents.

Polymerase enzyme.

*Taq* polymerase was used throughout the experiments, although different batches were used. Sensitivity of the method may vary with new batches of reagent (Saunders et al 1993). The decrease in sensitivity may have been due to a new batch of *Taq* polymerase, although it did not improve when another batch was used. Saunders et al (1993) found that *Tub* polymerase consistently showed superior amplification than *Taq* polymerase. They were unable to explain this, but using *Tub* polymerase may have improved the sensitivity in the present study.

Stringency of the reaction.

To prevent mispriming a stringent method was used. The lowest magnesium concentration possible was used so that any annealing of primer to non-target DNA was destabilised. Further mispriming was avoided by having primers with a high melting temperature. The lower the annealing temperature, the greater the likelihood of primers binding to non-target DNA. However if the annealing temperature is too high any binding of the primers will be prevented. An annealing temperature just below the primers melting temperature (the temperature at which the double bonds binding the primer to the target DNA break) will decrease mispriming. The annealing temperature in Saunder's method of 50-70°C are high, and just less than the melting temperature of the primers. These stringent conditions were effective in that no false positive results occurred, even when large amounts of non-meningococcal DNA were tested, as in the specificity experiments.

However it is possible that in making the method so stringent the sensitivity was decreased.

9.3.1.c Lack of "gold standard" for diagnosis.

Although there may be a strong clinical suspicion of MCD, cultures may be negative or yield an organism not thought to be pathogenic which may or may not be the causative factor (Brazilian Purpuric Fever Study Group 1987). This highlights the lack of a "gold standard" for the early diagnosis of MCD. The diagnosis may eventually be confirmed by detecting a rising antibody titre against the meningococcus (Jones & Kaczmarski 1993). However this method requires the patient to survive long enough to mount a measurable antibody response, and is of limited value in patients who die. It is thus very difficult to calculate the true sensitivity of PCR in MCD. Ni et al (1992a) claimed a 91% sensitivity. They found positive PCR results in 10 of 11 CSF samples that were also positive by either microscopy or culture, and one positive PCR result in culture negative CSF. Saunders et al (1993) attempted to detect meningococci in CSF that was negative by all other techniques, but in which there was a clinical suspicion of MCD. Four out of seven samples were positive by PCR, but the false positive and false negative rates are unknown. PCR has thus been used to detect meningococci in only a small number of CSF samples, and the predictive value is unknown. The current study was unable to produce a consistently sensitive method capable of detecting the low numbers of meningococci not detected by other methods.

The nested PCR method took approximately 12 hours work to give a result. In practice this meant two working days. It was thus much slower than microscopy or antigen detection, and similar to culture. PCR may have a role in detecting meningococci when other techniques have failed, particularly in those involved in vaccine trials (Zollinger et al 1991), but is unlikely to be a useful routine diagnostic method at present.

### 9.3.2 Other uses for PCR.

PCR may not provide a rapid diagnostic test for MCD, but it still is a helpful technique for studying MCD. The other principle molecular technique for detecting microorganisms is hybridisation; the binding of a specific DNA or RNA probe to it's target (Naber 1994). PCR is much more sensitive and rapid than hybridisation and does not require highly purified samples. One major use of PCR in microbiology is to make template for determining bacterial gene sequences as in this study.

#### 9.3.2.a Confirmation of sequence.

Sequence of PCR product from *N meningitidis* Bnt P1.4.

The sequence of the product was very similar to the published sequence of the *por A* gene (Barlow et al 1989). Differences in sequence could be due to misincorporation of bases during PCR, misreading during sequencing or genuine variation. The *por A* gene does show quite marked variability due to genetic exchange and subsequent mutation (Feavers et al 1992; McGuiness et al 1993).

The structure of protein 1, the protein coded for by *por A* has been derived (van der Ley et al 1991; Maiden et al 1991) and has eight hydrophilic loops that project out from the surface of the bacterium (See Figure 9.6). The two longest loops, loops 1 and 4, are the areas with the most variation between subtypes and are referred to as variable regions 1 and 2 (McGuinness et al 1990). Most variation is seen in variable region 2 and this is where the P1.4 epitope is located (Maiden et al 1992). The current study also found most variation in variable region 2, with less variation in region 1.

The sequence for variable region 2 was very similar to that published for other P1.4 subtypes (Maiden et al 1992). The differences did not occur in the epitope to which the subtyping monoclonal antibody binds (McGuinness et al 1993).

The sequence for variable region 1 was identical to that for P1.7, except for a two amino acid deletion (Maiden et al 1991). The P1.7 subtyping antibody reacts with an epitope at the apex of loop one (McGuinness et al 1990). Amino acid deletions may "mask" the P1.7 epitope, by moving it's position down the side of the loop so that it is not exposed to the subtyping antibodies (McGuinness et al 1993). The sequence encoding this epitope was present in the *por A* sequenced, but failed to react with P1.7 antibody during subtyping, suggesting a "masked" P1.7 subtype.

Two semivariable regions also occur on Class 1 protein on loops 5 and 6 (McGuinness et al 1993)(See Figure 9.6). The current study also found variation here with a two amino acid deletion in loop 6 in both PCR products sequenced.

Sequence of PCR product from *N flava*

The sequence of the product from the *N flava* variant, showed great homology to the published *por A* sequence, and changes in the semivariable regions similar to those found in the other product sequenced. The sequence in variable region 1 was also similar to that found in non-subtypable meningococci (McGuinness et al 1993). This organism thus possesses the *por A* gene. The organism may be a strain of *N flava* which has acquired the gene by interspecies variation, although this is rare. Alternatively it may be a non-capsulated meningococcus, which has acquired the ability to utilise sucrose. The organism was isolated from a contact of a child who had septic arthritis due to a group B meningococcus. It is most likely that it is a meningococcus.

9.3.2.b The importance of subtyping meningococci.

Class 1 outer membrane protein is the antigen used for subtyping meningococci (Frasch et al 1985) and is also a valuable epidemiological marker (McGuinness et al 1991), as well as a potential vaccine component (Zollinger et al 1991). Deletion or substitution of just one amino acid in variable region 2 is associated with an increased resistance to bactericidal antibodies (McGuiness et al 1991; Rosenqvist et al 1993). Class 1 protein can thus influence virulence of the bacterium. If further vaccines based on Class 1 proteins are developed (van der Ley & Poolman 1992), it will become even more important to subtype meningococci from clinical samples.

A major limitation of the standard typing methods is the inability to subtype all isolates with the currently available monoclonal antibodies. PCR of *por A* gene can give comprehensive typing of subtypes, is more reliable and gives more information than monoclonal antibody typing and is able to type isolates untypable with monoclonals (McGuinness et al 1993; Kertesz et al 1993). "Masked" epitopes of P1.7, as found in the current study, can also be identified (McGuiness et al 1993). It may also be possible to subtype directly from clinical samples, even if they are culture negative (Maiden et al 1992; Saunders et al 1993). This rapid method of subtyping may be particularly useful during outbreaks of MCD, allowing rational and timely public health measures to be taken. Identification of the different epitopes could also enable these to be incorporated into a vaccine (van der Ley & Poolman 1992).

9.3.2.c Classification of meningococci.

PCR may also be useful for typing disease and carrier strains (Woods et al 1994), or identifying pathogenic organisms by typing ribosomal RNA (Greisen et al 1994; Muralidhar & Steinman 1994). In the current study PCR enabled reclassification of an organism thought to be *N flava*, on standard typing. The presence of the *por A* gene suggests this organism is a meningococcus. No other *Neisseria* species other than meningococci were detected by PCR, demonstrating the specificity of the method.

### 9.3.3 Conclusions

In this study PCR was not able to detect meningococci in clinical samples, however it did help classify *Nesseria* species, reveal a "masked" P1.7 subtype and give information on the sequence of a possible vaccine component. As Class 1 protein vaccines are developed, comprehensive serosubtyping using PCR will provide vital bacteriological surveillance and information on the epitopes to include in the vaccine.

# CHAPTER TEN. DISCUSSION

# CHAPTER TEN. DISCUSSION

Two studies of MCD in Merseyside children have been performed. A retrospective study of children admitted to RLCHs between 1977 and 1993, and a prospective study in four Merseyside hospitals from September 1992 until April 1994. The aims were to; 1) study changes in the incidence of meningococcal septicaemia and mortality from meningococcal disease; 2) determine the features of early MCD that parents and doctors notice; 3) relate interleukin-10 to disease severity, outcome and other cytokines; 4) determine the presence of true or relative adrenal insufficiency in MCD; 5) relate plasma fibronectin, vitamin A and nutritional status to disease severity and outcome and evaluate fibronectin's role as a marker for MCD; 6) explore the possibility of laboratory diagnosis by polymerase chain reaction techniques and 7) study the possible impact of a conjugate group C meningococcal vaccine.

## 10.1 SUMMARY OF CONCLUSIONS FROM THE STUDIES.

1) Life threatening MCD does not present as meningitis, but as septicaemia. The mortality from MS is significantly greater than that from other presentations. The proportion of cases presenting as MS is increasing. This increase cannot be explained by changes in referral pattern or serogroup. Despite this increase, mortality remains about 11% and showed a trend towards a decrease in MS.

2) The early features of MCD that parents notice are non-specific; fever, vomiting and lethargy. These are followed by rash, cyanosis and deterioration. Parents are the first to notice the rash of MCD, and this is the commonest reason for them seeking medical advice. Parents do not recognise or seek medical advice about the features of meningitis.

3) Delays in diagnosis occur when doctors do not recognise the rash of MCD, especially if the rash is maculopapular. Pre-admission penicillin is often given if a diagnosis of MCD is made, but rarely if meningitis is diagnosed.

4) High levels of the anti-inflammatory cytokine IL-10, are associated with disease severity and death in MCD. IL-10 levels correlate strongly with levels of the pro-inflammatory cytokines and have a pattern similar to TNF-$\alpha$. Survivors have higher IL-10 levels for a given level of TNF-$\alpha$ and an imbalance between these two cytokines may lead to the development of shock and death.

5) No cases of true adrenal insufficiency were found, but children who died had significantly lower cortisol levels than survivors of MCD. A subgroup with severe MCD and initial cortisol levels below 800 nmol/l had a high mortality. This subgroup could be identified by an initial creatinine above 65 $\mu$mol/l. In survivors, cortisol levels fell rapidly over the first 48 hours of treatment. Cortisol levels did not correlate with TNF-$\alpha$ or IL-6 levels.

6) Decreased levels of plasma fibronectin were associated with disease severity and death. Fibronectin levels correlated with IL-6, and increased after 1 week. Low plasma fibronectin was a poor predictor of MCD.

7) Nutritional status and plasma retinol levels were not associated with disease severity or death. However lower weight-for-age and deficient vitamin A levels were more common in those with MM+MS.

8) The PCR techniques used were not sensitive enough to detect meningococci in clinical samples likely to be negative by other methods. PCR was highly specific for meningococci and was useful for classifying organisms and providing information on the structure of a potential vaccine target, Class 1 outer membrane protein.

9) Group C meningococci were responsible for 20% of MCD seen at RLCHs. There was a significant increase in group C disease since 1986. Most children with group C infection were under 2 years old, and could not be protected by the current polysaccharide vaccine. Immunisation with a conjugate group C vaccine, completed at 4 months, could have prevented 86% of cases, including all deaths. Mortality from group C infection was 14%. Eleven of the 50 deaths (22%) were due to group C meningococci. Since 1986 group C meningococci caused 30% of deaths from MCD. A conjugate group C meningococcal vaccine might decrease the mortality from MCD by up to 30%.

The increasing proportion of cases of MCD presenting as septicaemia alone presents a number of challenges. Much publicity about MCD refers to it as "meningitis", and parents are told the features of meningitis that they should look out for. As the proportion of cases with septicaemia alone increases, this information is becoming increasingly inaccurate and the features of the more serious septicaemia may be ignored (Thomson & Hayhurst 1993). This study shows that parents rarely notice the signs of meningitis in children even when they are present, and hardly ever seek medical advice because of them.

The hallmark of MS is the vasculitic rash. Parents were often the first to notice the rash and rash was the commonest reason for seeking medical advice in this study. When a rash was present in severe MCD, over three quarters of parents sought advice because of it. Parents sought advice significantly more often for the rash of MCD, differentiating it from other petechial rashes. Publicity about MCD should thus focus on the life threatening presentation; septicaemia characterised by a vasculitic rash.

Doctors also often refer to MCD as "meningitis" (Tarlow & Geddes 1992). In this study when MCD was diagnosed as meningitis, children were significantly less likely to receive pre-admission penicillin. To increase the numbers of cases given pre-admission penicillin attention should focus on septicaemia, diagnosed by the vasculitic rash, rather than meningitis. Such "on the spot" treatment may help decrease mortality (Cartwright et al 1992c).

Avoidable delays in diagnosis occurred when the rash of MCD was not recognised. This occurred particularly when a maculopapular rash was present and may have contributed to two deaths in this study. This early presentation of septicaemia needs highlighting for "doctors of first contact with MCD" (Welsby & Golledge 1990). Neither low fibronectin levels nor PCR techniques provided a rapid diagnostic test for MCD, although CRP above 10 mg/l had a positive predictive value of 78%. CRP levels are raised in children with MCD presenting with a maculopapular rash (Marzouk et al 1993). Markers of MCD in children presenting with maculopapular rashes are being examined in this cohort and a previous cohort of Merseyside children (Marzouk et al, manuscript in preparation).

To reduce "the interval between the onset of symptoms, diagnosis and treatment", parents, GPs, and junior hospital doctors all need to be "familiarised with the clinical features of MCD and the disastrous consequences of therapeutic delay" (Slack 1982). Information for all these groups should focus on septicaemia and its characteristic rash.

Once admitted to hospital aggressive management of severe MCD may help decrease mortality (Sinclair et al 1989). Searching for signs of meningitis in cases of septicaemia may delay diagnosis and treatment (Farmer 1993). Treating septicaemia as meningitis (with fluid restriction rather than fluid loading) may increase mortality (Nadel et al 1995).

The retrospective study showed a trend towards lower mortality in both MS and the most severe cases, following the start of the research program into MCD. This may be due to increasing familiarity with the disease and vigorous intensive care co-ordinated by the research fellows. This decrease in mortality however was not statistically significant.

The GMSPS identifies children at high risk of dying, who might benefit from early intensive care. All children who died in this study had a GMSPS of 8 or more. The clinical score of GMSPS also correlated well with laboratory variables; cytokines and fibronectin, confirming its ability to predict both prognosis and cytokinaemia (Marzouk 1995). A modified GMSPS, developed from a previous cohort, may be applied to this cohort to examine it's characteristics (Marzouk, unpublished data).

## 10.2 THE NEED FOR FURTHER STUDIES.

### 10.2.1 Conventional treatments.

Conventional treatments such as corticosteroids and blood products may be beneficial in MCD. Cortisol levels were significantly lower in children dying from MCD, although none had absolute adrenal insufficiency. A subgroup of children with severe disease may have had relative insufficiency with levels below 800 nmol/l. This group could mostly be identified by raised creatinine levels on admission.

A trial of replacement doses of hydrocortisone in this group is now needed to assess whether steroid replacement is beneficial. This would require a large multicentre study, but could run in tandem with other trials of new agents in MCD. Surrogate markers of outcome, such as blood pressure, could be used in a pilot study.

Fibronectin levels were also significantly lower in children who died from MCD. Again a trial of fibronectin replacement in MCD is required to demonstrate benefit. Animal studies suggest that fibronectin enhances the action of immunotherapy (Hill et al 1984). Future trials of immunotherapy in MCD should therefore study fibronectin levels in those enrolled in the study, or consider the co-administration of fibronectin.

Lower nutritional status and vitamin A levels were associated with meningitis rather than septicaemia and were not associated with mortality. Further studies in areas with endemic vitamin A deficiency and a high mortality from meningitis are needed to assess the potential benefit of vitamin A supplementation in MCD. Such supplementation cannot be justified from the current study.

### 10.2.2 Novel treatments.

The mortality from meningococcal septic shock has remained high despite conventional treatments. This has stimulated research into novel methods of treatment, most aimed at decreasing the levels of endotoxin or the circulating inflammatory mediators (Nadel et al 1995).

Only two randomised controlled trials of anti-endotoxin therapy in children with severe MCD have been performed. Neither anti-J5 plasma (J5 Study Group 1992) nor Pentaglobin/Polymixin E affected mortality (Marzouk 1995). Other novel treatments are thus being studied.

10.2.2.a Plasmapheresis and whole blood exchange.

The removal of pathogenic substances (ie endotoxin and cytokines) from the blood of patients with MCD might decrease mortality. This has been found to be so in a few small studies of plasmapheresis and whole blood exchange in MCD. However the studies have used historical controls and none is large enough to be conclusive (Pollack 1992). Interestingly plasmapheresis did not lower endotoxin levels (van Deuren et al 1992), although levels of TNF-$\alpha$, IL-1$\beta$ and IL-6 did decrease (Drapkin et al 1989, Westendorp et al 1992). A definitive controlled trial of plasmapheresis and whole blood exchange in MCD has not yet been published. A trial in Glasgow was abandoned due to poor results and technical difficulties.

Methods of blocking the cytokine cascade involve either prevention of cytokine production or inhibition of cytokines once produced. Most experience and success is in blocking cytokine translation with corticosteroids.

10.2.2.b Corticosteroids

Steroids in Bacterial Meningitis.

Despite the low mortality from meningococcal meningitis (MM), long term complications such as epilepsy (1.4%), psychomotor retardation (2.1%), and hearing loss (5%-10%) may occur (Fortnum 1992; Baraff et al 1993).

Animal models show that pharmacological doses of corticosteroids, especially dexamethasone, down regulate many components of the inflammatory response, by blocking cytokine production (Sáez-Lorens et al 1990). In particular steroids reduce brain water, decrease intracranial pressure and lower cerebrospinal fluid lactate levels (Tauber et al 1985). However the timing of administration of steroids in relation to antibiotics is critical. Corticosteroids must be given with, or shortly before antibiotics if maximum benefit is to be achieved (Tauber & Sande 1989; Mustafa et al 1990).

Controlled trials of dexamethasone given with or before antibiotics have shown a decrease in sensorineural deafness and neurological sequelae in children with bacterial meningitis (Lebel et al 1988; McCracken & Lebel 1989). These improvements occurred only in children treated with cefuroxime, a second generation cephalosporin now shown to be inferior to the third generation cephalosporins (Schaad et al 1990). Critics commented that steroids may not be beneficial if meningitis was treated with other antibiotics (Kaplan 1989). This was refuted by two further studies where children given dexamethasone 20 minutes before the first dose of cefotaxime or ceftriaxone (Odio et al 1991; Schaad et al 1993).

Both showed decreased neurologic sequelae in the steroid treated group. A meta-analysis of all children treated with ceftriaxone found a significant decrease in sequelae in those also given steroids (Schaad et al 1993).

Only 16% of children in these studies had MM (Schaad et al 1995). Most had meningitis due to *H influenzae*, and a subsequent study only found beneficial effects in *Haemophilus* meningitis (Wald et al 1995). These trials may thus not be applicable to MM.

One large prospective placebo controlled study had a large proportion of patients with MM (Girgis et al 1989). Steroid therapy had no significant effect on mortality or neurological morbidity in the 267 patients with MM, although it did significantly reduced mortality in those with pneumococcal meningitis. The patients in this study had very severe MCD, 57% being comatose on arrival, and may not be comparable to MCD on Merseyside. A more recent trial found no neurological or audiological sequelae in 59 children with MM given dexamethasone (Syrogiannopoulos et al 1994). Unfortunately there was no placebo group with which to compare this result.

There are no specific studies on the role of corticosteroids in meningococcal meningitis. Because of the relatively low rate of sequelae after MM (~6.6%, Baraff et al 1993) this would require a large multicentre trial (Tarlow et al 1992). At present there is thus little evidence that corticosteroids are beneficial in MM. Might steroids be beneficial in MS?

Corticosteroids protective effects against endotoxin.

Steroids block the transcription of the inflammatory cytokines, TNF-α and IL-1β, and thus could be protective against the harmful effects of endotoxin. Numerous investigators have documented an increased survival after high dose corticosteroid therapy in various animal models of gram negative sepsis (Hinshaw et al 1982). However trials of methylprednisolone given within 2-4 hours of the onset of shock did not show a significant reduction in mortality in adults (Bone et al 1987; Veterans Administration Study Group 1987). One trial also showed an increased mortality amongst patients with a raised creatinine at enrolment in the treatment group (Bone et al 1987). Both studies showed a decreased rate of resolution of secondary infection in those receiving glucocorticoids. These trials included patients with a wide variety of infections, and with the post surgery/trauma type of septic shock. They did not include children, nor patients with meningococcal septic shock. They may thus not be applicable to children with MCD.

High dose dexamethasone did produce a significant decrease in mortality in children and adults with severe typhoid, although patients did not receive inotropic or ventilatory support (Hoffman et al 1984). This study may be more applicable to MCD than trials in adult septic shock. The patients were young, without other underlying diseases, and infected with the same organism which was sensitive to the antibiotics given.

However, no trials of high-dose steroids in meningococcal septic shock have been performed. It is suggested that a trial of dexamethasone given with the first dose of antibiotic should be considered in MCD (Booy & Krull 1994). Such a trial of high dose dexamethasone would have to run separately from any trials of replacement doses of hydrocortisone.

Cytokine production may also be blocked by preventing endotoxin stimulating macrophages. The use of anti-endotoxin antibodies in septic shock (in particular HA-1A) illustrates the difficulties of using these new treatments.

10.2.2.c New Anti-endotoxins

HA-1A, an IgM monoclonal antibody against the toxic lipid A portion of endotoxin, decreased mortality in adults with gram-negative bacteraemia and shock (Ziegler et al 1991). However HA-1A did not consistently bind endotoxin or alter cytokine levels in vitro or in animal models (Quezado et al 1993) and its mode of action was questioned (Baumgartner et al 1991). HA-1A had no overall effect, but did decrease mortality in a subgroup with gram negative bacteraemia. This led to a suggestion of toxicity to those with non-gram negative bacteraemia (Tanio & Feldman 1991). Patients in the placebo group were older, had worse prognostic scores and a higher incidence of organ failure and inadequate antibiotic treatment (Carlet et el 1991). These worse prognostic factors may have led to a higher mortality in the control group.

A re-interpretation of the data found a marginally significant decrease in mortality in those with gram negative bacteraemia; suggestive, but not conclusive evidence of HA-1A's effectiveness (Warren et al 1992). A second trial of HA-1A in adult septic shock was thus undertaken. This trial was stopped following an interim analysis which showed a trend for increased mortality with HA-1A in those with non-Gram negative bacteraemia. HA-1A was thus voluntarily withdrawn from sale (Luce 1993). Further analysis found that HA-1A was not effective in reducing mortality in those with gram negative bacteraemia (32% vs 33%) (McCloskey et al 1994). Any further trials will be restricted to groups with gram negative infections who might benefit, as in the current trial in MCD.

A murine anti-endotoxin monoclonal antibody, E5, did not improve survival from sepsis in two trials. A third trial in adults with gram negative bacteraemia is underway (Lynn & Cohen 1995).

Other endotoxin binding compounds are being studied. Bactericidal/permeability-increasing protein (BPI) is a protein which naturally occurs in neutrophils granules. BPI binds to the lipid A portion of endotoxin significantly better than monoclonals like HA-1A (Marra et al 1994). BPI blocks the presentation of endotoxin to macrophages by lipopolysaccharide-binding protein and thus downregulates cytokine release and inflammation. The ratio of BPI to lipopolysaccharide-binding protein varies with infection (Opal et al 1994). BPI decreases mortality in animals given endotoxin, even when administered 1-2 hours after endotoxin (Fisher et al 1994a). Clinical trials of BPI in septic shock and MCD are awaited.

Endotoxin Neutralising Protein (derived from the Horseshoe crab, *Limulus polyphemus*) protects rabbits from meningococcal septic shock and will be studied in humans (Alpert et al 1992).

Endotoxin stimulates macrophages via CD14 receptors. Monoclonal antibodies against CD14 prevents endotoxin stimulating macrophages (Wright et al 1990). Studies of these antibodies in sepsis are awaited.

10.2.2.d Interrupting the Cytokine cascade.

Other new forms of treatment involve blocking particular cytokines, TNF or IL-1.

Anti-Tumour Necrosis Factor-α agents.

Anti-Tumour Necrosis Factor-α antibodies decreased mortality from MCD in an animal model (Nassif et al 1992). Trials in adults with severe sepsis found a more rapid reversal of shock, but no decrease in mortality (Lynn & Cohen 1995).

The outer membrane portion of TNF-α receptors can be shed and then bind to TNF-α, preventing it from stimulating an intact receptor. These soluble TNF-α receptors (sTNFr) thus regulate the effects of TNF-α. Raised levels of sTNFr were found in MCD (Villard et al 1993; van Deuren et al 1994), with high sTNFr/TNF-α ratios being associated with a better prognosis (Girardin et al 1992). Unfortunately sTNFr therapy increased mortality when given to adults with sepsis (Suffrendini 1994).

Anti Interleukin-1 agents.

A specific inhibitor of IL-1 activity, IL-1 receptor antagonist (IL-1ra), is produced by macrophages. These inhibitors prevent IL-1 from binding to its receptor (Dinarrello & Wolff 1993). Trials of IL-1ra in adults with sepsis found no overall benefit (Fisher et al 1994b). However a retrospective analysis showed a 20% decrease in mortality in those with a greater than 24% risk of dying. A second trial was halted when an interim analysis showed no evidence of benefit (Lynn & Cohen 1995).

Interleukin-10

Interleukin-10 can inhibit the production of TNF-α, IL-1β and IL-6 (de Waal Malefyt et al 1991b; Fiorentino et al 1991)and increase production of their inhibitors (de Waal Malefyt et al 1993; Joyce et al 1994). Interleukin-10 has improved survival in septic shock in an animal model (Howard et al 1993). In the current study IL-10 levels correlated strongly with levels of the pro-inflammatory cytokines. The ratio of IL-10 to TNF-α seemed to influence mortality, a ratio above 1.7 being associated with survival. However levels of IL-10 did not increase once TNF-α levels were over 800 pg/ml, the level at which all deaths occurred. Whether giving exogenous IL-10 to children with MCD and high levels of TNF-α is beneficial will require another large multicentre trial.

Anti-TNF antibodies may decrease IL-10 production (Wanidworanum & Strober 1993) and thus decrease sTNFr and IL-1ra release. Inhibition of anti-inflammatory cytokines when blocking pro-inflammatory cytokines needs monitoring.

Summary of novel treatments.

Trials of anti-endotoxins and anti-cytokines have been disappointing thus far. Significant effects are often only found after posthoc subset analyses. These may be misleading when the overall treatment effect is not significant (Counsell et al 1994). Too much attention has focused on cytokines' role in disease, at the expense of understanding their potential role in host resistance and the ability to eradicate infection (Hinds 1992; Giroir 1993). An effective immune system is needed to fight infection. Anti-cytokine therapy must find the balance between inhibiting excessive host responses and abolishing essential defences and natural protective mechanisms.
The vast array of interactions of the pro- and anti-inflammatory cytokines are only just starting to be unravelled (Mercier 1993). We are therefore unlikely to find one "magic bullet" to improve mortality in sepsis and may need a combination of agents (Hinds 1992; Giroir 1993; Mercier 1993), possibly including an opsonin like fibronectin.

10.2.2.e Blocking the end results of the inflammatory cascade.

One further method of decreasing mortality is not by interrupting the cytokine cascade, but by blocking its final effects. The inflammatory cascade results in the induction of nitric oxide synthase. This synthesises nitric oxide which produces the marked vasodilation seen in sepsis.

Inducible nitric oxide synthase can be inhibited, and this may restore the blood pressure in septic shock (Petros et al 1991). Trials of nitric oxide synthase inhibitors are awaited.

Prostacyclin.

In view of the intense peripheral vasoconstriction found in meningococcal septic shock, the use of vasodilators could seem justified. Prostacyclin, a vasodilating prostaglandin, is a logical choice since epithelium treated with meningococcal endotoxin is deficient in prostacyclin (Heyderman et al 1991). Prostacyclin also inhibits platelet adhesion and could prevent the micro-thrombi found in MCD. Although prostacyclin has been shown to be beneficial in adult septic shock (Bihari et al 1987), no trials of its use in MCD have been performed.

10.2.3 Preventative measures.

Conventional or novel therapies have not produced a significant decrease in MCD since the introduction of antibiotic treatment. Prevention of MCD would be the most effective way to decrease mortality. Chemoprophylaxis of MCD prevents few cases (Cooke et al 1989) and prevention will thus require immunisation. However until recently there were no effective vaccines for the majority of cases of MCD in the UK; young children infected with serogroups B or C meningococci. The group B polysaccharide is similar to a fetal brain antigen and is poorly immunogenic (Finne et al 1987) and group C polysaccharide vaccines are ineffective in children under two (Taunay et al 1974).

10.2.3.a Conjugate vaccines.

Protein conjugation of polysaccharide vaccines makes them immunogenic even for young infants. A group C conjugate vaccine has been developed (Costantino et al 1992). The current study èstimates that such a vaccine could have prevented 86% of cases of group C infection, and reduced the mortality since 1986 by 30%. Trials of conjugate group C vaccines are currently underway in Gloucestershire and Oxfordshire. Once the safety and immunogenicity of these vaccines is known, a large phase III study involving the whole UK would be needed to demonstrate efficacy and their ability to reduce mortality from MCD (Fairley et al 1994).

A conjugate group B vaccine has also been developed (Jennings et al 1986). However it is not know whether it will be safe to break the tolerance to the "self antigen" expressed on foetal brain cells. This is especially relevant in pregnant women and young children, the ultimate target group of such a vaccine.
Other conjugate vaccines include an *E coli* K92 vaccine. This induced cross-reacting antibodies to group B meningococci in mice (Devi et al 1991). The safety of such a vaccine in man is not known.

10.2.3.b Group B vaccines.

Since group B meningococci are responsible for most MCD in the UK, a vaccine against this serogroup would have the greatest impact on mortality. Such a vaccine however is unlikely to be available for some time.

Group B polysaccharide is poorly immunogenic, and attempts have been made to make vaccines from other components of the outer membrane. Most have used endotoxin depleted outer-membrane vesicles containing a variety of proteins. A vaccine based on Class 1 Outer Membrane Protein 7 was used in Chile, but was only 51% effective and immunity was short lived (Zollinger et al 1991). A group B outer membrane vesicle combined with group C polysaccharide vaccine was developed in Cuba, and was 83% effective in school children (Sierra et al 1991). However this same vaccine was ineffective in Brazilian children under 4 years of age (de Moraes 1992). A Norwegian outer-membrane vaccine based on serotype 15 had only a 57% efficacy in school children (Bjune et al 1991).

These outer membrane vaccines show some efficacy, but like the polysaccharide vaccines are less immunogenic in young children. Purified outer membrane component vaccines may improve efficacy. The class 1 proteins appear to be the most immunogenic outer membrane protein. Seven sub-types cause 80% of group B infection worldwide. A multivalent class 1 outer membrane protein vaccine containing these 7 subtypes may thus prove effective (van der Ley & Poolman 1992).

Other potential vaccine targets are other outer membrane proteins (class 2 or 3), pili, transferrin binding protein and detoxified endotoxin (Reviewed by Frasch 1995). The safety and immunogenicity of these vaccines is not known.

10.2.4 Conclusions

The greatest reduction in mortality from MCD will come when an effective group B vaccine is available. Until that time mortality may be reduced by helping parents and doctors to recognise the early features of the disease and by encouraging prompt, appropriate treatment. Such advice has been given for 30 years.

> "Success will require a team with a predetermined plan of therapy and with the necessary drugs and equipment immediately available."
> (Steihm & Damrosch 1966)

New techniques may now help to decrease mortality. Rapid methods for confirming the diagnosis and assessing severity need to be refined. Novel therapies targeting the complex inflammatory process of MCD need to be assessed in large randomised trials. However these treatments are only likely to be beneficial if early appropriate therapy has been given. Further studies seeking to improve parents and doctors awareness of MCD, as well as assessing novel treatments and developing new vaccines are required.

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ORIGINAL PAPER

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# Mortality from group C meningococcal disease: a case for a conjugate vaccine?

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**Abstract** This 17-year retrospective review of children with meningococcal disease (MCD) has determined the mortality due to serogroup C, in order to assess the potential impact of a group C conjugate vaccine. Four hundred and forty-nine cases of MCD were admitted to our hospitals during 1977–1993; 78 due to group C, 11 of whom died. There was a significant increase in the proportion of cases due to group C from 1986 onwards (10% vs 21%), and an increase in the total number of cases of MCD (151 vs 298). The currently available group C polysaccharide vaccine has low efficacy below 2 years of age and could not have prevented 54 cases of group C disease. A conjugate group C vaccine administered between 2 and 4 months of age could have prevented 68 cases, including all fatal cases. The recent increase in MCD is partly due to an increase in group C disease. A meningococcal group C conjugate vaccine could prevent most cases of infection due to group C, and decrease the mortality from MCD by up to 30%.

**Key words** Meningococcal disease
Conjugate vaccines

**Abbreviation** *MCD* meningococcal disease

## Introduction

*Neisseria meningitidis* is an important cause of mortality and morbidity in children [8]. Although meningococcal disease (MCD) in the United Kingdom has mainly been caused by serogroup B meningococci [4] (for which an effective vaccine is not available), serogroup C organisms have recently increased in prevalence [6].

Serogroup C meningococci are currently responsible for 25% of MCD in England and Wales [7] and infection could be prevented by the use of a polysaccharide vaccine. However this vaccine is not effective in children under 2 years of age [10]. In the United States of America children with group C disease are significantly older than those with group B disease, with 73% aged over 2 years [2]. Children over 2 years of age with group C disease could have their disease prevented by the currently available vaccine. If children with group C disease are below this age then protein conjugation of the vaccine [3], similar to the new *Haemophilus influenzae* type b vaccines, could offer protection to children as young as 4 months.

The mortality associated with group C disease is greater than that with group A disease [5], and may be similar to or greater than that due to group B disease [8]. Data are needed to estimate the benefit of vaccinating children against group C meningococci.

In order to evaluate the possible benefits of vaccination, we aim to answer four questions. Firstly, what proportion of meningococcal disease, seen in our hospitals over a 17-year period, was due to group C? Secondly, what was the mortality in children with group C disease? Thirdly, how many of these children were over 2 years of age and could have been protected by the current vaccine? And finally, how many were 4 months or older and could be protected by a conjugate vaccine?

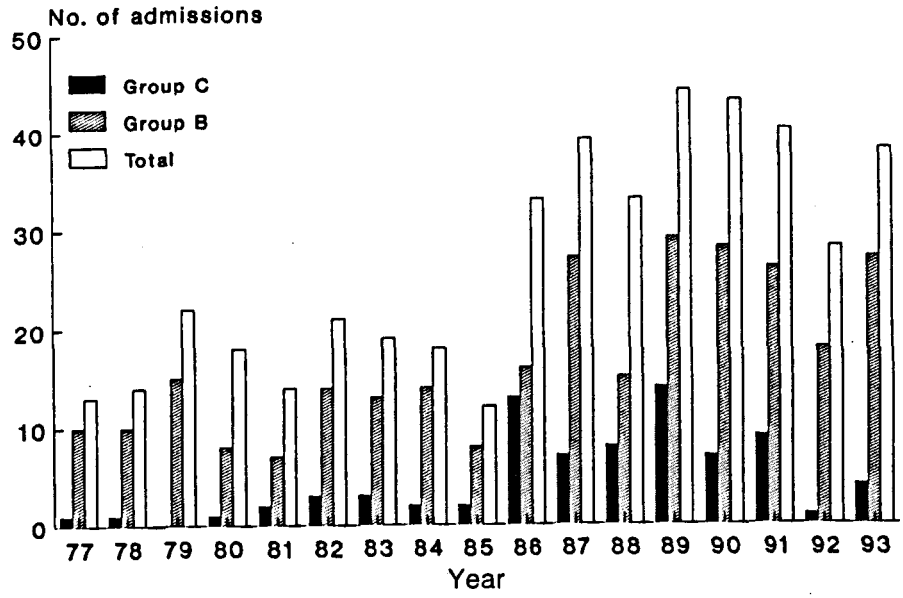


Fig. 1 Annual number of admissions of children with MCD to the Royal Liverpool Children's Hospitals by serogroup, 1977–1993

## Methods

The Royal Liverpool Children's Hospitals (Alder Hey and Myrtle Street) admit all children from the Liverpool and South Sefton health districts. Since January 1990 all inpatient facilities have been at Alder Hey. The Regional Paediatric Intensive care unit at Alder Hey receives referrals from other local district hospitals. Cases of MCD seen in both hospitals between January 1977 and December 1993, were identified from microbiology records, the Intensive Care register, and from the records of two prospective studies of MCD. Case notes or the Research Fellows data (from OM and AR) for these children were examined (by AT and AR).

Children were included in the study if they had positive cultures for *N. meningitidis* in blood, CSF or synovial fluid; or a clinical presentation compatible with MCD disease together with either; detection of meningococcal antigen in serum or CSF, Gram-negative diplococci seen in the CSF or a positive throat swab for *N. meningitidis*.

Isolates of *N. meningitidis* were sent to the Meningococcal Reference Laboratory, Manchester for serogrouping.

Significance testing was done using the Mann-Whitney U-test or Chi squared with Yates correction where appropriate.

## Results

Four hundred and forty-nine children were admitted with 451 episodes of MCD; 52 were transferred from other hospitals for intensive care. Information was available for 449 episodes. Fifty children (11%) died. Serogrouping was available for 384 (86%) cases; 285 (74%) of which were group B. Group C disease occurred in 78 (20%) cases.

The total number of cases of MCD rose from 1986 onwards; data were therefore compared for the years 1977–1985 and 1986–1993 (see Fig. 1).

The proportion of cases due to group C meningococci increased significantly from 1986 (10% vs 21% $P$=0.003), and there was a significant decrease in the number of cases due to serogroups A and W–Z (10% vs 2% $P$=0.0004) (See Table 1). There was no change in the overall proportion of cases due to group B meningococci over this time, although the total number of cases increased (99 vs 186).

Table 1 Mortality by serogroup for 449 admissions with MCD. Number of cases (%)

| Serogroup | Year of admission | | | | | |
|---|---|---|---|---|---|---|
| | 1977–1985 | Deaths | 1986–1993 | Deaths | Total | Deaths |
| Group B | 99 (66) | 14 | 186 (62) | 19 | 285 | 33 |
| Group C | 15 (10) | 1 | 63 (21) | 10 | 78 | 11 |
| Other[a] | 15 (10) | 0 | 6 (2) | 1 | 21 | 1 |
| Unknown | 22 (14) | 2 | 43 (14) | 3 | 65 | 5 |
| Total | 151 | 17 | 298 | 33 | 449 | 50 |

[a] Other serogroups found; A ($n$ = 4), W135 ($n$ = 2), X ($n$ = 1), Y ($n$ = 4), Z ($n$ = 2), A–D ($n$ = 2), A/Y ($n$ = 1), X–Z ($n$ = 1) and non groupable ($n$ = 4, 1 died)

Mortality due to group C increased from 1 of 15 cases (6%) during 1977–1985 to 10 of 63 cases(16%) in 1986–1993. During the latter period group C meningococci were responsible for 10 (30%) of the 33 deaths due to MCD. All cases of fatal group C disease occurred in children 4 months of age or older. Table 2 compares the ages of children with group B and group C infection. The median ages (range) were 14 months (0.3–168) for group B and 14.5 months (1–162) for group C. These ages were not significantly different and the proportion of children aged less than 2 years or over 4 months was also similar.

Clinical presentations were also similar. Septicaemia without meningitis occurred in 68 cases with group B infection (24%) and 19 cases of group C infection (24%).

Table 2 Comparison of ages and outcome in 363 cases of MCD due to group B or group C meningococci

| | Group B | | Group C | |
|---|---|---|---|---|
| | No.cases (%) | Deaths | No.cases (%) | Deaths |
| Ages | | | | |
| In those >2 years | 98 (34) | 12 | 24 (31) | 6 |
| In those≥4 months | 256 (90) | 28 | 68 (87) | 11 |
| Totals | 285 | 33 | 78 | 11 |

Mortality was slightly higher in children with group C disease (14%) compared to children with group B (11.6%), but this difference did not achieve statistical significance.

## Discussion

The proportion of cases of MCD due to group C meningococci in our population was 78 (20%) of the 384 tested, however this proportion has increased significantly since 1986. This is not just a local phenomenon as the incidence of Group C disease has been reported to have increased in the rest of the north of England during the study period [1]. Since 1985 there has been a rise in the incidence of cases of both group B and group C MCD in England and Wales [6], although fewer cases were seen during 1991–1992 [7]. This trend is reflected in our data. Eleven of the 50 deaths (22%) from MCD were due to group C disease. However since 1986 group C meningococci were responsible for 10 of the 33 deaths (30%) from MCD.

A significant difference in ages between those with groups B and C disease was not demonstrated in our study. This is contrary to the results from a hospital based study from the United States of America [2]. In the study of Baker et al. [2] only 27% of children with group C disease were less than 2 years of age, whereas in our study 69% were aged 2 or less. Baker et al. found group C disease was more common in adolescents and young adults than group B disease [2]. This does not appear to be the case in our study.

Adolescents over 16 years of age are unlikely to be admitted to our paediatric hospitals and so an increase in group C disease in these young adults could have been missed in the present study. However data from England and Wales shows that group B sulphonamide-resistant strains cause as much infection in adolescents and young adults as group C strains [6]. A genuine difference in age between those with groups B and C disease is therefore unlikely.

Current strategies for preventing MCD involve giving chemoprophylaxis to close contacts of the disease, and offering vaccination to contacts of cases with group C disease. In our study only five cases occurred in contacts, the vast majority of cases were unconnected. To prevent MCD thus requires vaccination of the whole population, not just those in contact with cases. No vaccine is currently available for group B meningococci. The currently available polysaccharide vaccine against group C meningococci, effective in children over 2 years of age, would only have prevented 24 of the 449 cases of MCD seen in our hospitals.

Techniques to improve the immunogenicity of polysaccharide vaccines have focused on the conjugation of polysaccharides to proteins such as diphtheria or tetanus toxoid [3].

This strategy has proved very successful in producing the new vaccines against *Haemophilus influenzae* type b. Widespread usage of this vaccine has led to a dramatic decrease in *H. influenzae* meningitis [9].

Conjugate vaccines against group C meningococci have now been developed and are in clinical trial. If a protein-conjugated group C meningococcal vaccine could protect children 4 months of age and older, then 68 cases of MCD (15%), including 11 fatal cases, could have been prevented during the period of our study. Use of such a conjugate vaccine since 1986 could have prevented 30% of the deaths in our series due to MCD. This assumes a 100% vaccine uptake, in reality uptake may be 85%–90%. However even at this level the carriage rate and epidemiology may be sufficiently altered to prevent deaths from group C meningococci.

The proportion of cases of MCD in England and Wales due to group C meningococci decreased in 1992 [7]. It is possible that group C disease only increased during the recent upsurge in cases of MCD, and may now decrease to previous levels. Further prospective, population based studies of MCD will be needed to fully assess the impact of group C vaccines.

In conclusion our study shows that an increasing proportion of cases of MCD in children over the past 17 years has been due to serogroup C. Since 1986, group C meningococci were responsible for 30% of the deaths from MCD in our hospitals. The currently available vaccine would not protect the majority of children who contract group C disease, but a conjugate vaccine might decrease the mortality from MCD by up 30%. The development and usage of such a vaccine may have a significant impact on the mortality from MCD.

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ORIGINAL PAPER

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# The changing presentations of meningococcal disease

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**Abstract** Meningococcal disease (MCD) can present as meningitis, meningitis plus septicaemia or septicaemia alone. This 17-year retrospective study sought to determine if the proportion of cases presenting as septicaemia alone was increasing. Four hundred and forty-nine children with MCD were admitted between 1977 and 1993, 50 children died (11%). The proportion of cases with septicaemia alone increased from 7% in 1977–1985 to 36% in 1990–1993 ($P < 0.0005$). Mortality was highest in children with septicaemia alone (19%). Despite the increase in septicaemia, overall mortality did not alter over the 17 years.

**Conclusion** MCD should not be thought of as "meningitis", since 33% of cases now present as septicaemia alone. Nearly one in five children with septicaemia alone die. Information and publicity about MCD should focus on septicaemia, characterised by a petechial rash, as the life-threatening presentation.

**Key words** Meningococcal disease · Septicaemia · Meningitis · Mortality

**Abbreviations** *MCD* meningococcal disease · *MM* meningitis alone · *MM+MS* meningitis plus septicaemia · *MS* septicaemia alone

## Introduction

Infection with *Neisseria meningitidis* commonly presents as meningitis. The term "meningococcal meningitis" is thus widely used to describe all meningococcal infections (i.e. both meningitis and septicaemia) [6]. Recent reports however suggest that meningococcal disease (MCD) is presenting less often as meningitis [3]. An increase in meningococcal septicaemia is important since this presentation carries the highest mortality [1].

We have previously described the three clinical presentations of MCD to our hospitals; meningitis alone (MM), meningitis plus septicaemia (MM+MS) and septicaemia alone (MS) [8]. We now update our previous study to determine whether the proportion of cases of meningococcal septicaemia has increased.

## Methods

Data were collected on children with MCD admitted between January 1977 and December 1993. Children were either directly admitted to our hospitals or were tertiary referrals to the Regional Paediatric Intensive Care Unit from other hospitals.

Inclusion criteria and data collection for the study have been presented elsewhere [5], and excluded children without positive cultures or antigen for *N. meningitidis* in blood, CSF or throat swab. Children were divided into three groups; MM, MS and MM+MS [8].

The severity of illness was assessed retrospectively using the score devised by Kahn and Blum [4], in which the presence of three or more risk factors is associated with a 79% risk of mortality.

Significance testing was by the Mann-Whitney U test or chi-square, with Yates correction where appropriate.

**Table 1** Comparison of clinical presentation in cases of MCD over time. Data shown as number of episodes (%), except ages (*DGH* Referral from other district general hospital)

| | 1977–1985 ($n$ = 151) | 1986–1989 ($n$ = 149) | 1990–1993 ($n$ = 149) |
|---|---|---|---|
| Age in months median (range) | 13 (0.3–163) | 16 (1–168) | 16 (0.8–178)*[3] |
| Deaths | 17 (11.3) | 13 (8.7) | 20 (13.4) |
| MS | 11 (7)*[1] | 41 (28) | 54 (36)*[4] |
| MM+MS | 102 (68)*[1] | 81 (54) | 74 (50)*[3] |
| MM | 38 (25) | 26 (17) | 21 (14)*[3] |
| Septic arthritis | 0 | 1 (0.6) | 0 |
| DGH* | 4 (3)*[1] ($n$ = 146)[a] | 13 (9)*[2] ($n$ = 146)[a] | 35 (24)*[4] ($n$ = 145)[a] |
| Kahn ≥3 | 16 (11) | 18 (12) | 28 (19)*[3] |

[a] Kahn score not calculated on 12 children due to lack of data
*[1] Difference between 1977–1985 and 1986–1989, $P < 0.05$
*[2] Difference between 1986–1989 and 1990–1993, $P < 0.0005$
*[3] Difference between 1977–1985 and 1990–1993, $P < 0.05$
*[4] Difference between 1977–1985 and 1990–1993, $P < 0.0005$

**Table 2** Comparison of age, referral pattern, disease severity, serogroup and mortality for three presentations of meningococcal disease. Number of episodes (%), except ages

| | MM ($n$ = 85) | MM+MS ($n$ = 257) | MS ($n$ = 106) |
|---|---|---|---|
| Age in months median (range) | 11 (0.8–178) | 14 (0.3–168) | 19.5 (2.5–163)*[4] |
| DGH ($n$ = 52) | 6 (7) | 31 (12) | 15 (14) |
| Kahn ≥ 3 ($n$ = 62) | 0*[1] | 35 (14)*[1] | 27 (25)*[3] |
| Group B ($n$ = 284) | 53 (62) | 163 (63) | 68 (64) |
| Group C ($n$ = 78) | 11 (13) | 48 (19) | 19 (18) |
| Deaths ($n$ = 50) | 1 (1.2)*[1] | 29 (11.3)*[2] | 20 (18.8)*[5] |

*[1] Difference between MM and MM+MS, $P < 0.01$
*[2] Difference between MM+MS and MS, $P < 0.05$
*[3] Difference between MM+MS and MS, $P < 0.01$
*[4] Difference between MM and MS, $P < 0.01$
*[5] Difference between MM and MS, $P < 0.0005$

## Results

Four hundred and fifty-one episodes of MCD occurred in 449 children admitted during the 17-year study period. Data were available for 449 episodes in 447 children. Fifty (11%) children died, 10 of whom were brought in dead. It was impossible to calculate the prognostic score for the 10 children brought in dead and for 2 other children who died, because of insufficient data.

The survey was divided into three time periods which contained approximately equal numbers of cases (Table 1). The proportion of cases with MS increased from 7% in 1977–1985 to 36% in 1990–1993 ($P < 0.0005$). The proportion of cases with MM+MS and MM both showed significant decreases. Mortality was not significantly different in the three time periods, despite increases in the more severe forms of disease.

A potential confounder is the increasing number of tertiary referrals. The data were therefore re-analysed to exclude tertiary referrals (data not shown). The increase in cases of MS remained highly significant ($P < 0.0005$). However the increase in disease severity and age at presentation disappeared, suggesting that this increase was due to a rising number of older children referred for intensive care from other hospitals.

Mortality was greatest in the group with MS (18.8%) compared with other presentations (Table 2). Disease severity (i.e. the proportion with a Kahn score of 3 or more) was also greatest in the group with MS.

## Discussion

Our study shows a significant increase in the proportion of cases of MCD presenting as septicaemia without meningitis. Mortality was significantly greater in those with MS compared to those with a meningitic component to their disease.

Despite increases in factors that might increase mortality, the mortality did not change significantly over the 17 years. This may represent an improvement in mortality, since an increase in disease severity has led to increased mortality in other centres [1]. There can be no room for complacency however, as one in ten children admitted to our hospitals with MCD died.

An increasing reluctance to perform a lumbar puncture in a child with an obvious meningococcal rash may explain the fall in cases of meningitis [2]. To remove this possible bias, children with a rash and meningitic signs but who did not have a lumbar puncture, were included in the meningitis plus septicaemia group. A true rise in septicaemia is supported by the fact that the proportion of cases of septicaemia has risen in other recent reports from the United Kingdom [3].

An increase in meningococcal septicaemia is important since it carries the highest mortality [1]. The press [7], the public and the medical profession [6] often refer to all meningococcal infections as "meningitis". Previously this was mostly correct. However over a third of cases now do not have meningitis at all, but the more lethal septicaemia. Information for the public about MCD stressing the signs of meningitis is thus becoming increasingly inaccurate. Focusing attention on "meningitis" can mean that the features of septicaemia are ignored [7]. Information should therefore stress the features of septicaemia, in particular the vasculitic rash [7]. Septicaemia has a different pathophysiology to meningitis. Doctors treating MCD need to recognise the difference between septicaemia and meningitis because the treatments may differ [6].

Life-threatening MCD does not present as meningitis but as septicaemia, often with a rash. This potentially fatal presentation has increased dramatically in the last few years. The emphasis in MCD needs to be shifted away from meningitis and towards septicaemia. Publicity about the features of septicaemia, especially the vasculitic rash, should replace information that refers to MCD as "meningitis".

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Short paper

# Children who are seen but not referred: hearing assessment after bacterial meningitis

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**Abstract**

Bacterial meningitis is an important cause of hearing loss in children. Previous studies have shown that a proportion of survivors of childhood bacterial meningitis do not have a formal hearing assessment. To confirm this finding amongst children treated for bacterial meningitis in our hospital, a retrospective audit was performed. The hospital case notes and community audiological records were examined to see how many children were referred for hearing assessment after their illness, and how many actually attended. Between 1984 and 1991, 194 children were directly admitted to our hospital with bacterial meningitis. Thirteen children died, and hearing assessment was carried out in 135 of the 181 survivors (75%), 15 of whom had evidence of sensorineural hearing loss. The major reason for hearing not being assessed was non-referral (31 out of 46 cases), 12 children did not attend for assessment despite referral, and three moved shortly after discharge. Thirty of the children remaining in the area who had no assessment (9.7%) were however seen in hospital out-patients.

Routine referral for hearing testing at discharge, with re-referral at out-patient attendance, could help increase the number of children assessed after bacterial meningitis.

**Key words:** meningitis; hearing impairment; screening; children; audit

## Introduction

Bacterial meningitis is an important cause of death and handicap in childhood. Sensorineural hearing loss is the commonest serious complication of meningitis occurring in about 10% of survivors (Baraff *et al.*, 1993; Fortnum and Davis, 1993; Dodge *et al.*, 1984) and is the single most important cause of acquired hearing loss in children (Davis and Wood, 1992). The majority of cases of meningitis occur in young children, in whom hearing loss may critically affect the development of speech, but in whom, paradoxically, hearing loss may go undetected unless a formal assessment is done. Nearly 90% of British paediatricians are aware of the need for formal hearing assessment after meningitis claiming to refer all children for audiology (Fortnum and Hull, 1992). A recent study by Fortnum and Davis (1993) however, found that only 77% of survivors of childhood bacterial meningitis had such an assessment. The only other similar study found that 70% of survivors of meningococcal disease had a hearing assessment (Dawson and Wardle, 1990). In order to increase this proportion it is necessary to know why children are not assessed, and develop appropriate strategies to combat this.

A preliminary retrospective audit in our hospital found that only 69% of survivors of bacterial meningitis had a hearing test result in their case notes (Riordan and Thomson, 1993). The current study is a further presentation of these data with larger numbers and including audiology data.

This study sought to confirm the study by Fortnum and Davis (1993) by determining how

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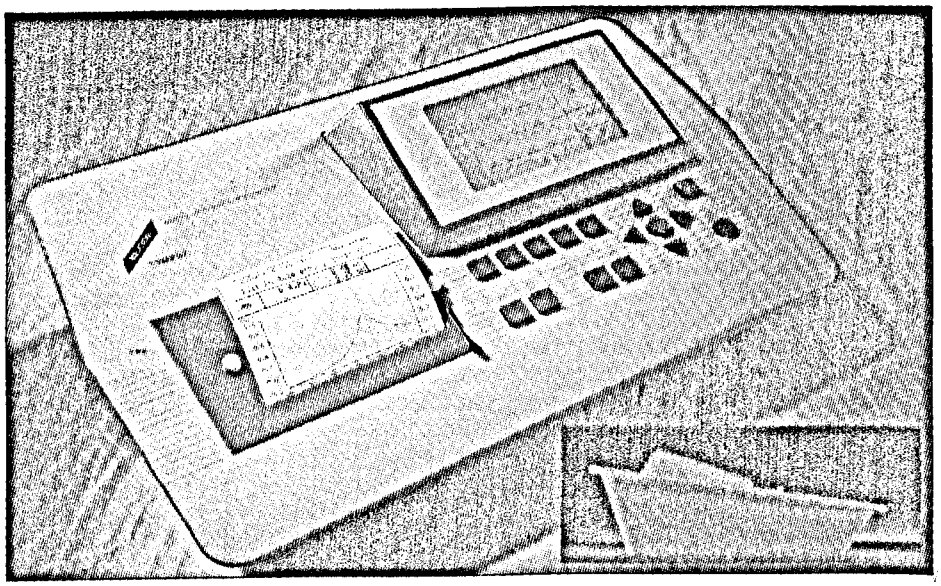

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# Hearing assessment after meningitis and meningococcal disease

Andrew Riordan, Alistair Thomson, Judith Hodgson

**Abstract**

**A method to increase audiology referral after meningitis or meningococcal disease was audited in 89 children. A standardised proforma increased referrals from 78% to 96% over a two year period. However, only 73% of children had a hearing test. The major reason for hearing not being tested changed from non-referral to non-attendance.**

(*Arch Dis Child* 1995; 72: 441–442)

Keywords: meningitis, sensorineural deafness, audit.

Sensorineural hearing loss occurs in 10% of children surviving bacterial meningitis or meningococcal disease.[1 2] Partial or unilateral hearing loss can cause linguistic disabilities, but may be missed by informal testing at the bedside or in outpatients. All children should therefore have a formal audiological assessment after meningitis.[2] Hearing loss can also occur after meningococcal septicaemia without meningitis.[3] All children with meningococcal disease, not just those with meningitis, should therefore have audiological follow up.

A recent audit in our hospital found that only 75% of survivors of meningitis had a hearing assessment.[4] The major reason for hearing not being assessed was non-referral. As a result of our audit a simpler method of referral was adopted.

We now present an audit of this new referral method. The standard set was for all children with meningococcal disease or bacterial meningitis to be referred for and have a formal audiological assessment.

## Methods

From 1 April 1993 a standardised proforma for audiology referral was available on all medical wards. Before this referral had been by letter. Medical staff were asked to complete the form when discharging children with meningitis or meningococcal disease. Forms were sent to one of us (JH) at community services, and forwarded to the appropriate local audiology service.

*Audiology referrals and hearing test results after meningitis and meningococcal disease. Data shown as number (%)*

| | April 92–March 93 (n=41) | April 93–March 94 (n=48) |
|---|---|---|
| Referred by hospital | 30 (73) | 42 (88) |
| Other referral | 2 (5) | 4 (8) |
| Known to audiology services | 32 (78) | 46 (96) |
| Did not attend hearing test | 5 (12) | 11 (23) |
| Hearing tested | 27 (66) | 35 (73) |
| Sensorineural loss | 2 (5) | 3 (6) |

A list of children with a discharge diagnosis of bacterial meningitis or meningococcal disease was compiled for the year before, and the year after the introduction of the new referral method as part of a prospective study (A Riordan, unpublished data). After one year of the new referral method, audiology records were studied to see how many children had been referred and how many had had a hearing test. The referral rates were compared with those for the previous year.

## Results

During the two years audited 96 children were directly admitted to our hospital with bacterial meningitis or meningococcal disease. Eighty nine children survived and were included in our audit. Referrals for audiology were only made for these children, except for one other child. This child had a possible viral meningitis, and was excluded from the audit.

The number of children admitted was similar for the two 12 month periods (table). The causative organisms were also similar (data not shown). However, the proportion of children known to the audiology services was much greater during the latter year (78% *v* 96%) (table). This was in part due to an increase in referrals on the new referral form, but also due to increased referrals from other sources. Despite the increased referrals for audiology, the proportion of children who had their hearing tested increased to only 73%. This was due to an increase in non-attendance for hearing test after referral. Five of the 62 (8%) children assessed were found to have sensorineural hearing loss.

## Discussion

Having identified non-referral as the major reason for hearing not being assessed after meningitis,[4] we implemented a new referral method. This audit shows the effectiveness of this simpler method of referral, with 96% of cases now being known to the audiology services. This improvement was due to an increasing referral rate from medical staff (73% to 88%) as well as from other professionals. The proportion of children who actually had a hearing assessment changed very little, however (66% to 73%), and is similar to that previously reported by ourselves[4] and others.[1 5] The main reason for non-assessment changed during the audit from failure to refer to failure to attend for audiology.

The increasing rate for those who did not attend is worrying. A non-attended appointment at a specialist centre wastes resources,

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and is more difficult to rectify than non-referral. Hospital staff can be made aware of the need for hearing assessment after meningitis in large groups. Parents, however, will need to be seen individually and may not appreciate the need to return for hearing assessment.

Eleven of the 16 children who defaulted audiology appointments did attend paediatric outpatients. Encouragement to attend hearing assessment then, or appointments on the same day, could help increase the number of children tested. Such a system will require close cooperation between audiology and paediatric services and continuous audit.

Increasing referral to the audiology services after meningitis and meningococcal disease up to 96% did not substantially increase the number of children assessed. Strategies to decrease non-attendance now need to be devised and the audit repeated.

We would like to thank Frank McIntyre for help in data collection and the Johanne Holly Trust for financial support.

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# "NORMAL" CEREBROSPINAL FLUID IN BACTERIAL MENINGITIS PRESENTING WITH SEIZURES

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*(Received 2 August 1993; in final form 28 September 1993)*

Objective — To determine the proportion of cases of childhood bacterial meningitis presenting with seizures, which have normal cerebrospinal fluid (CSF) values.
Design — Retrospective review of hospital case notes of children admitted with bacterial meningitis over an 8 year period.
Results — Of 163 children with meningitis, 27 presented with seizures. Four of these 27 (14.8 per cent) had completely normal CSF on arrival. Treatment was commenced because of physical signs despite these normal results. One further child with abnormal CSF protein and glucose levels was not treated for 19 hours because the CSF cell count was normal.
Conclusion — Normal CSF values occur in 14.8 per cent of cases of bacterial meningitis presenting with seizures. All values must be examined before CSF is regarded as normal. Treatment should be based on physical signs despite normal results.

KEY WORDS: Bacterial meningitis, seizures with fever.

## INTRODUCTION

Bacterial meningitis in young children may present with fever and seizures, when other signs of early meningitis may be absent. For this reason many paediatricians recommend that young children who have had a seizure with fever should have a lumbar puncture[1–3]. The finding of normal cerebrospinal fluid may reassure the physician that the child does not have meningitis[4]. However lumbar puncture may be performed before inflammation is apparent in the cerebrospinal fluid and so delay the diagnosis and treatment of bacterial meningitis[5].

We have therefore reveiwed case notes of those children with bacterial meningitis who presented with seizures, to determine whether normal cerebrospinal fluid values led to delay in treatment.

## METHODS

We reviewed case notes of all children, between the ages of 3 months and 6 years, admitted with positive cerebrospinal fluid cultures between January 1984 and December 1991. There were 163 such children directly admitted to our hospital and the case notes of all 163 children were studied retrospectively.

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Presented in part to the Paediatric Research Society, Bath, April 2nd 1993.

Children who had their first seizure after admission were excluded from the seizure group.

Signs of meningitis were defined as: neck stiffness, a raised anterior fontanelle or coma. A petechial rash was regarded as a marker for septicaemia.

Normal cerebrospinal fluid values were defined as less than $6 \times 10^6$ leucocytes per litre, protein of less than 0.45 g/l and a cerebrospinal fluid to blood glucose ratio of greater than 0.4.

There was no hospital policy on the need for lumbar puncture after febrile convulsion over the period of the study.

## RESULTS

The clinical details of the 163 children with meningitis are shown in Table I.

Normal cerebrospinal fluid cell counts were more common in the children presenting with seizures, and occurred in 5 of the 27 cases. Further details of these cases are shown in Table II. Two children in this group (Cases 1 and 2) had clinical

**Table I** Laboratory and clinical features of 163 children with bacterial meningitis

| | No. presenting with seizures [%] | No. presenting without seizures [%] | p value[a] |
|---|---|---|---|
| Total number of children | 27 | 136 | |
| Age in months [Median (Range)] | 15 (4–58) | 11.5 (3.68) | NS |
| Physical signs on admission: | | | |
| Neck stiffness | 8 [30] | 76 [56] | 0.022 |
| Petechial rash | 7 [26] | 63 [46] | NS |
| Raised anterior fontanelle | 9 [33] | 30 [22] | NS |
| Coma | 6 [22] | 7 [5] | 0.009 |
| None | 7 [26] | 18 [13] | NS |
| Prior Antibiotics | 11 [41] | 41 [30] | NS |
| Causative organism: | | | |
| *N. meningitidis* | 8 [30] | 87 [64] | 0.002 |
| *H. influenzae* | 11 [40] | 40 [29] | NS |
| *S. pneumoniae* | 8 [30] | 9 [7] | 0.001 |
| Cerebrospinal Fluid on admission: | | | |
| White Cell count < 6 | 5 [18.5] | 8 [7] | NS |
| Protein < 0.45 g/l | 6 [22] | 21 [17] | NS |
| Glucose > 3 mMol/l | 13 [48] | 49 [36] | NS |
| Glucose/blood glucose > 0.4 | 12 [44] | 53 [39] | NS |
| All values normal | 4 [14.8] | 9 [6.6] | NS |
| Outcome: | | | |
| Deaths | 2 [7.4] | 10 [7.4] | NS |

[a]p value using $Chi^2$ (with Yates correction where applicable), except ages where the Mann-Whitney U test was used.

**Table 2** Clinical and laboratory data for children with "normal" cerebrospinal fluid.

| Pt. | Age [months] | Temp °C | Symptom duration [days] | Signs on exam | Blood culture | Cerebrospinal fluid results | | | | | | Treatment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | White Blood Cells | Red Blood Cells | Gluc [mMol/l] | Prot [mg/l] | Orgs seen | Culture | |
| 1 | 21 | 37.7 | 1 | Rash | Nil | 3 | 3 | 4.2 | 0.2 | nil | N men | Immediate on ITU |
| 2 | 25 | 38.2 | 1 | Rash | N men | 4 | 3 | 3.9 | 0.2 | nil | N men | Immediate |
| 3 | 40 | 39.0 | 0.5 | Rash later | N men | 5 | 3 | 1.8* | 0.24 | nil | N men | Delayed 12 hours until rash developed |
| 4 | 7 | 39.7 | 1 | None | Pneumo | 3 | 1 | 0.2 | 1.86 | nil | Pneumo | Delayed 19 hours until cultures positive |
| 5[a] | 28 | 38.4 | 2 | None | Pneumo | 1 | 34 | 3.9 | 0.38 | nil | Nil | Delayed 52 hours until LP repeated |
| 5[b] | 28 | | | Neck stiff | Nil | 50 | 78 | 0.6 | 2.35 | + + + | Pneumo | Immediate |

Key

Pt. = patient
Temp = Temperature on arrival
Signs on exam = Signs of meningitis on arrival
Gluc = Glucose concentration
Prot = Protein concentration
Orgs seen = Organisms seen on microscopy
N men = *N meningitidis*
Pneumo = *S pneumoniae*
ITU = Intensive care unit
LP = Lumbar puncture

*Simultaneous blood glucose = 1.4 mMol/l

[a]Lumbar puncture on admission
[b]Repeat lumbar puncture

findings suggesting meningococcal septicaemia on arrival and both received immediate antibiotic treatment.

The third child (Case 3) developed a petechial rash 12 hours after admission and was then treated for meningococcal disease.

Case 4 illustrates the problems of concentrating on the cell count when interpreting cerebrospinal fluid results. She presented with a simple seizure and had a lumbar puncture on admission. Cerebrospinal fluid showed only 3 white cells and this was taken as evidence that she did not have meningitis. This was despite the fact that both glucose and protein levels were abnormal. Treatment was delayed until cultures became positive. She developed septicaemia and renal failure but recovered with ventilatory and inotropic support and dialysis. She now has some neurological, hearing and renal impairment.

Case 5 had normal cerebrospinal fluid initially, which was sterile on culture. However, 52 hours later he developed neck stiffness which necessitated a repeat lumbar puncture to diagnose pneumococcal meningitis.

## DISCUSSION

There have been many case reports of bacterial meningitis with "normal" cerebrospinal fluid (summarised in a literature review[6]). Normal cerebrospinal fluid cell counts are found in 2 per cent of all cases of bacterial meningitis in children over 4 weeks of age.[7] However we have found normal cerebrospinal fluid cell counts in 18.5 per cent of children with meningitis presenting with seizures. This finding confirms a study by McIntyre et al[8] where bacterial meningitis was not initially diagnosed in four out of nine cases presenting with seizures, because the cerebrospinal fluid appeared normal at hospital admission.

Cerebrospinal fluid protein and glucose concentrations may also be normal in 7 per cent and 22 per cent of cases of bacterial meningitis respectively[7]. However abnormal results may be ignored if there is a normal cell count, as illustrated by case 4.

Children with meningitis but normal cerebrospinal fluid fall into two groups.

The first group comprises children whose cerebrospinal fluid appears completely normal, but which later grows a pathogen. These children may have clinical signs of meningitis or septicaemia[6] and if so treatment should not be delayed because of a "normal" lumbar puncture. (Cases 1, 2 and 3.)

The second and more controversial group, include those children in whom meningitis develops following a lumbar puncture. The suggestion that a lumbar puncture performed during bacteraemia might actually cause meningitis was made over 70 years ago but remains unproven[9,10]. (Case 5 is a possible example.)

In summary, the diagnosis of meningitis presenting with seizures may be difficult, despite obtaining cerebrospinal fluid, since completely normal values can occur in 14.8 per cent of cases. All values must be examined, not just the cell count, before the cerebrospinal fluid is regarded as normal. Even completely normal cerebrospinal fluid does not exclude meningitis or preclude its development, even within 24 hours, since repeat lumbar puncture may be positive in bacteraemic children[6].

Management should be determined by the child's clinical condition. Where there are signs of meningitis or septicaemia, antibiotic treatment should not be

delayed. Alternatively, if a child remains unwell following a seizure, or develops signs of meningitis despite a previously normal lumbar puncture, a repeat lumbar puncture should be done. These risks, and the need for repeat lumbar puncture, should be borne in mind when recommendations are made for the use of lumbar puncture in children presenting with fever and seizures.

*Acknowledgements*

We would like to thank the Johanne Holly Trust for financial support.

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# Bacterial meningitis in the first three months of life

FAI Riordan, APJ Thomson, JA Sills, CA Hart

**Summary**

**A retrospective study of infants with bacterial meningitis admitted to our hospital during 1949–52, highlighted the lack of 'classical' signs of meningitis in these infants.[1] We carried out a similar review of 44 infants aged less than three months, admitted during 1982–91. We also determined the causative organisms and their antiobiotic sensitivities.**

**Symptoms and signs were similar in the two series. Forty infants in the later series were either febrile, irritable or had seizures on the day of admission. Overall mortality fell from 30% to 11%.**

**Between 1982 and 1991 Group B *Streptococcus* and *Neisseria meningitidis* were the commonest causes of meningitis. All organisms, except one, were sensitive to ampicillin and/or cefotaxime.**

**Bacterial meningitis should be suspected in young infants who are febrile, irritable or having seizures. Initial treatment with ampicillin and cefotaxime is appropriate.**

Keywords: bacterial meningitis, infants

## Introduction

Bacterial meningitis in the first few months of life presents a number of difficult clinical problems in diagnosis and treatment. The symptoms and signs of meningitis may be non-specific, and diagnosis relies heavily on a high index of suspicion.

Forty years ago Haworth[1] noted that the 'classical' signs of meningitis (neck stiffness and/or a raised anterior fontanelle) occurred less often in infants less than three months of age. He reported on 13 infants under three months of age with bacterial meningitis admitted to our hospital between July 1949 and April 1952. The diagnosis of meningitis was delayed in four infants, all of whom died. Since this study, group B *Streptococcus* has become a major pathogen for young infants.[2] We aim to see if the clinical presentation of bacterial meningitis in young infants has changed since Haworth's study. We also aim to determine whether delay in diagnosis still occurs.

Controversy also exists over the most appropriate initial antibiotic regimen for young infants with meningitis. The organisms that cause bacterial meningitis in infants vary with the age of the child. In the neonatal period Gram-negative bacteria (particularly *Escherichia coli*), and more recently Group B *Streptococcus* (GBS), and *Listeria monocytogenes*, are common causes.

In children over three months of age *Neisseria meningitidis*, *Haemophilus influenzae* type b (Hib) and *Streptococcus pneumoniae* cause almost all community-acquired cases.[3] Infants between one and three months of age can develop meningitis with either the neonatal or the childhood group of pathogens. Antibiotic guidelines based on knowledge of the common pathogens recommend a combination of cefotaxime and ampillicin.[4] Before adoption, these guidelines need validating in a clinical setting.

Neonatal meningitis has a high mortality, especially in premature and low birth weight infants.[3] Initial treatment with penicillin, cefotaxime and gentamicin is recommended.[5]

Almost half of all cases of neonatal meningitis are admitted to hospital directly from home[3] and will therefore not be treated on a neonatal unit but on a paediatric ward. An antibiotic regimen to cover meningitis in all children under three months of age admitted to paediatric wards may help to simplify treatment.

We have reviewed infants under three months of age with bacterial meningitis admitted to our hospital over a 10-year period. Our aim was to examine the initial clinical presentation and to determine the causative organisms and their antibiotic sensitivities. An appropriate initial antibiotic regimen could then be suggested.

## Patients and methods

The case-notes of all children less than three months of age with positive cerebrospinal fluid (CSF), cultures, admitted to our hospital between January 1982 and December 1991, were reviewed. Cases were identified from microbiology records and ward admission books. The case-notes for all but three children were eventually traced, and information from previous research was available on two of these children. Infections complicating myelomeningocoeles, ventricular shunts, or occurring after surgery were excluded.

Forty-five episodes of meningitis occured in 44 children over the 10-year period. (One child was re-admitted following partially treated *E coli* meningitis.) Six infants had been born

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before 37 weeks of gestation, but all been discharged from a neonatal unit before their admission with meningitis.

Twenty-nine infants were directly admitted to our hospital, one of whom was re-admitted, and 15 were tertiary referrals.

Five children died, three of whom had neonatal meningitis (see table 1).

## Results

SYMPTOMS AND SIGNS

Presenting symptoms and signs were noted from case-notes, referral letters and casualty cards and compared with those found by Haworth[1] (table 2).

Symptoms and signs did not differ between the two groups except for the incidence of poor feeding. Forty children in our series presented with either fever, irritability or seizures on the day of admission. Two further infants had poor peripheral perfusion and cyanosis. The 'classical' signs of meningitis (neck stiffness and/or a raised anterior fontanelle) were absent in over 50% of infants in both series. A clinical diagnosis of meningitis was made in only 43% of these infants in Haworth's series, but in 81% of these infants in our series. Delay in diagnosis occurred in four children in Haworth's series. These infants were initially thought to have pneumonia (two), jaundice, or failure to thrive. All these children died. In our series delay in diagnosis occurred in seven cases. Five children were initially referred to other specialities because of presumed surgical (three) or cardiac (two) symptoms, one child presented with afebrile seizures, and one with stomatitis. None of these children died. There was an overall decrease in mortality from 30% to 11%, but this failed to achieve statistical significance.

**Table 1** Causative organism of meningitis in infants under three months, Alder Hey Children's Hospital 1982–91. Figures are given as the number of admissions with the number of deaths in parentheses

| | *Age in completed week* | | | |
|---|---|---|---|---|
| *Organism* | *0–3* | *4–8* | *9–13* | *Total* |
| *N meningitidis* | | 8 | 5 | 13 |
| *H influenzae* | | 1 | 1(1) | 2 |
| *S pneumoniae* | 1 | 2(1) | 1 | 4 |
| Group B Strep | 8(2) | 4 | 3 | 15 |
| Group A Strep | 3(1) | | | 3 |
| *Listeria* | 2 | | | 2 |
| *Escherichia coli* | 2 | 2* | | 4* |
| Other** | 1 | | 1 | 2 |

*One child with relapsed *E coli* meningitis re-admitted aged five weeks.
**_Streptococcus milleri_ (one week old) and _Enterobacter agglomerans_ (10 weeks old).

**Table 2** Presenting symptoms and signs and outcome of infants less than three months with bacterial meningitis

| | *1949–52* | *1982–91* |
|---|---|---|
| *Symptoms* | (n = 13) | (n = 42) |
| Poor feeding | 5 (38%) | 32 (76%)* |
| Fever | NA | 29 (69%) |
| Irritable | 7 (54%) | 25 (60%) |
| Lethargic | 1 (8%) | 14 (33%) |
| Vomiting | 5 (38%) | 13 (31%) |
| *Signs* | (n = 13) | (n = 40) |
| Temperature ≥ 38°C | NA | 28 (70%) |
| Irritable | NA | 28 (70%) |
| Seizures day 1 | NA | 14 (35%) |
| Full fontanelle | 5 (38%) | 18 (45%) |
| Neck stiffness | 3 (23%) | 5 (13%) |
| No 'classical' signs | 7 (56%) | 22 (55%) |
| *Outcome* | (n = 13) | (n = 45) |
| Delay in diagnosis | 4 (30%) | 7 (15%) |
| Deaths | 4 (30%) | 5 (11%) |

NA = information not available
*Significant at $p < 0.05$ by $chi^2$.

ORGANISMS

The organisms grown from the CSF of the children admitted between 1982 and 1991 are listed in table 1. Ten infants aged between one and three months (36%) had infections with 'neonatal' organisms. Both cases of coliform meningitis in this age group were due to a relapse of infection. (One child had previously been treated at our hospital and one at a referring hospital.)

Antimicrobial sensitivities were available for 41 organisms, 28 of which were seen on microscopy of the CSF. Of the 17 organisms isolated from neonates, 11 were sensitive to either penicillin or cefotaxime and four organisms (*E coli* and *L monocytogenes*, two each) were sensitive to gentamicin. (Sensitivities were unavailable for two cases of GBS meningitis.) However all 15 organisms with known sensitivities were sensitive to either ampicillin or cefotaxime, making the addition of gentamicin unnecessary.

Amongst the 26 organisms with known sensitivies in the one to three month age group, all except one were sensitive to either ampicillin or cefotaxime or both. The exception was a case of relapsed meningitis due to *E coli* resistant to ampicillin. This organism was sensitive to cefuroxime, but sensitivity to cefotaxime was not tested. (This case occurred in 1982.) (Sensitivities were again unavailable for two cases of GBS meningitis.)

## Discussion

Symptoms and signs of bacterial meningitis in young infants appear not to have changed over the past 40 years despite changes in the causative organisms.[2] (There were no cases of GBS meningitis in Haworth's series.) A high index of suspicion is still necessary as over half the cases do not show the 'classical' signs of bacterial meningitis. Most cases in our series, however, were either febrile, irritable or had seizures on the day of admission. Other studies have found that fever and irritability are the commonest signs of bacterial meningitis in young infants.[6,7] Awareness of these non-specific symptoms of meningitis appears to be increasing, as a delay in diagnosis occurred in a much smaller proportion of cases in our series (31% in Haworth's series, 16% in ours). There

is no room for complacency, however, as the five infants initially referred to our surgical and cardiac colleagues demonstrate.

Our study demonstrates the wide number of pathogens causing meningitis in the first three months of life, with GBS and meningococci predominating. All except one of these organisms were sensitive to either ampicillin or cefotaxime, or both. (The exception being a relapsed case of menigitis due to *E coli* sensitive to cefuroxime, and thus likely to be sensitive to cefotaxime, although not tested.) The four cases where sensitivities were unavailable were all caused by GBS. These would almost certainly be sensitive to ampicillin and cefotaxime.

Studies from the US found that Hib[8] or GBS[6] were the commonest causes of meningitis in infants aged between one and three months, and recommended treatment with ampicillin combined with either chloramphenicol[8] or cefotaxime.[6] No similar study from the UK, focusing on this age group, has been published although de Louvois *et al* have studied meningitis in children under one year of age.[3] In this study, 10% of neonatal meningitis was caused by the common childhood organisms, and 5% of meningitis between the second and sixth month of life was caused by neonatal organisms.

Penicillin and chloramphenicol were the most commonly used antibiotics for both neonates and older infants. Chloramphenicol and gentamicin both have serious side-effects and require monitoring of blood levels.[9] The efficacy and safety of ampicillin and cefotaxime make them an attractive choice for young infants with bacterial meningitis.[4] This combination is increasingly popular amongst directors of programmes in paediatric infectious disease in the US.[10] Our study shows that this combination could be used in the UK as the initial treatment of meningitis in infants aged between one and three months. Neonates admitted to a children's ward with meningitis could also be treated with this regimen, instead of one using gentamicin combined with cefotaxime and penicillin.[5]

**Bacterial meningitis in infants**

- classical signs often lacking
- group B *Streptococcus* and *N meningitidis* are the usual causes
- high index of suspicion for diagnosis
- treat initially with ampicillin and cefotaxime

Our study cannot make any recommendations about treating meningitis on the neonatal unit, although a combination of ampicillin and cefotaxime has been recommended by others.[11,12]

In conclusion, the diagnosis of bacterial meningitis in young infants continues to require a high index of suspicion. Meningitis should be suspected in any infant less than three months of age who is febrile, irritable, has seizures or is in a poor condition. Initial treatment on a paediatric ward with ampicillin and cefotaxime is appropriate until the causative organism is identified.

We would like to thank Dr Omnia Marzouk and Dr Huw Thomas for providing data, Paula Thomas for help in tracing case-notes, and the Johanne Holly Trust for financial support.

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# LETTERS TO THE EDITOR

## The dangers of soft bedding for infants

EDITOR,—The paper by Bolton *et al* on the possible relationship of rebreathing in bedding to cot deaths raises only one aspect of the way in which bedding probably contributes to many of these deaths.[1] The work of Bolton *et al* largely replicates the experiments of Kemp and Thach who used the faces of rabbits to study the effects of breathing into baby nest bean bags.[2] After 35 deaths of babies face down in these bags, the US Consumer Safety Commission banned their production and the planned introduction of these bags into this country was abandoned.

We have believed for many years that a proportion of babies found face down as cot deaths die as a result of asphyxiation in their mattresses.

There are two factors in addition to rebreathing, one is the form of the surface in which the face is placed and the other, the softness and compressibility of the nose in young babies. When we were working upon means of obstructing breathing in babies we found that in some children a weight of only 10 g on the end of the nose would completely compress the nose in some children of 2 months of age.

In 1978 we showed, when we used machinery much like that used by Bolton *et al*, but used cadaver heads not a model, that considerable obstruction can be produced to breathing and the effects of regurgitated milk can in some situations produce almost complete obstruction.[3] At that time we concluded that the best sleeping surface for a baby would be a bale of hay!

Many babies who are found dead face down in their cots have much regurgitated material in their nostrils.

For the last 15 years we have been attempting to develop a sleeping surface on which a child who becomes face down in its cot will not asphyxiate if it regurgitates and also that the effects of rebreathing are minimised. Such a sleeping system has been produced and has been tried out in the wards of the Sheffield Children's Hospital. This was described in the *Health Visitor* in 1990[4] and is currently being produced for general sale.

The New Zealand infant mortality situation is an intriguing one. In the early years of this century New Zealand led the world in its low infant mortality and it has only been during the last 20 or so years that the cot death rate there soared and seemed to rise more in the whites than in the Maoris but did not rise in the Pacific Islanders.

In 1986 when the New Zealand cot death rate was high, I was invited by the Plunket Society and their Minister of Health, to report on the situation and I visited New Zealand and looked into the actual circumstances and pathology of deaths in many centres throughout the country. There were a number of factors that could have accounted for the increased infant mortality rate but what appalled me most were the cots and bedding. It had become the fashion for babies to sleep directly on sheepskins and the softer and more fluffy the sheepskins the better. Infants were placed prone on these, often in cots with no hard base so that the babies were deep in woolly nests. I voiced my horror to everyone possible. At that time their sheep industry was in recession and there was a drive to sell sheepskins for baby cots to the world!

Returning from New Zealand I stopped off in Hong Kong, which had a very low cot death rate. There I was taken around the villages by a social worker to see babies in their homes. There I do not recall seeing any cots or mattresses at all. The babies were simply lying on a piece of sheeting directly on the floor with somebody in the room with them the whole time.

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## Early presentation of meningococcal disease after media publicity

EDITOR,—A petechial rash is often an early sign of meningococcal disease, but parents rarely seek medical advice about it.[1] Press publicity about a recent outbreak of meningococcal disease mentioned the vasculitic rash in only 27% of articles, although this was present in 93% of cases.[2] Increased public awareness of the significance of a petechial rash may lead to earlier presentation of meningococcal disease. Such earlier presentation may save lives.[3] We report two cases where parents sought early medical advice about a vasculitic rash after being alerted to its significance by two television programmes.

A 2 year old boy became febrile and irritable over the course of an afternoon. At 7 pm his mother recognised the development of a petechial rash as the herald of meningococcal disease, having seen a similar rash on the television programme *The Time, The Place* that morning.

His mother immediately sought medical advice and insisted on her child's admission to hospital. Meningococcal septicaemia was diagnosed on arrival and he required immediate resuscitation and antibiotics, and he was transferred to the regional paediatric intensive care unit for two days of inotropic and ventilatory support. He has subsequently made a full recovery.

The second child, aged 1 year, was admitted three months later. Her parents awoke to find her covered in a petechial rash. On the evening before admission they had watched a feature on meningococcal disease on the programme *That's Life*. They brought her immediately to hospital where she was admitted to the paediatric intensive care unit. She was given antibiotics, ventilated, and required the use of three inotropes for three days. She has also now made a full recovery.

The parents of both children sought medical advice because they recognised a petechial rash after seeing it on television. These two cases show that appropriate publicity about the presenting features of meningococcal disease can lead to early presentation and successful treatment if accurate information is given. A television campaign similar to one screened in Norway, highlighting the features of meningococcal disease, especially the vasculitic rash, could lead to further cases being treated earlier.

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## National follow up of *Haemophilus influenzae* meningitis

EDITOR,—In view of the recent introduction of a vaccine to protect children against *Haemophilus influenzae* type b infection, it is timely to report preliminary findings from a five year follow up of 440 children surviving *H influenzae* meningitis in infancy. This forms part of a national follow up of 1794 children who had meningitis from a variety of causes in their first year of life between 1985 and 1987. These cases were reported by hospital paediatricians at the time of diagnosis through an active reporting system. Methods of case identification, details of initial illness, and immediate outcome (case fatality rate 4·1% for children with *H influenzae* meningitis) have been previously reported.[1] Age at the time of diagnosis is known for 433/440 of these children, 87% (381) were aged between 4 and 12 months.

Information on health and development of these children at 5 years of age is being sought from general practitioners (GPs) and parents by postal questionnaire, together with similar details for a control population matched for age and sex. Data obtained from GPs are currently available for 373/440 (85%) children surviving *H influenzae* meningitis in their first year.

A total of 255 (68%) children were reported by GPs to have no health or developmental problem, a further 88 (24%) had minor problems such as squint, conductive hearing loss, speech or language delay, and 30 children (8%) had significant neurodevelopmental problems. These include 14 (4%) with a sensorineural hearing loss, nine (2%) with multiple developmental problems with mental impairment, and five (1%) with epilepsy or suspected epilepsy. The age at diagnosis is known for 29 of the 30 children with significant problems: 27 were aged between 4 and 12 months, one was less than 1 month, and one was 3·8 months. With a suggested potential efficacy of at least 90%,[2] immunisation with *H influenzae* type b vaccine could have protected 24 of these children from the disease.

Rates of significant neurodevelopmental problems after *H influenzae* meningitis reported from other studies range from 8% to 37%.[3,4] Our findings are preliminary and should be treated with caution as data collection is not yet complete. Further detailed analysis of these data from GPs as well as information from parents and from both sources for the control population is in progress.

A vaccine providing protection against *H influenzae* type b infection was introduced

ch clinical studies and animal models

ng meningococcal meningitis in the ...y may be extremely difficult. General ...hers should not give dexamethasone in the ...s many patients would be treated inappro... ...ly. There have been no placebo controlled ... of dexamethasone to support its use in ...ningococcal septic shock, but trials of steroids ... adult patients with other forms of Gram negative ...psis have shown no benefit.[1]

We stand by our recent recommendation that ...neral practitioners should give parenteral anti... ...iotics before admission to all patients suspected of ...aving meningococcal disease.[4] In all five health ...istricts that participated in a study there was a ...end towards increased survival when benzyl... ...enicillin was given before admission, with ...reatest benefit in the patients who were most ill— ...ose with a haemorrhagic rash.[5]

We therefore endorse the chief medical officer's ...vice. During the winter general practitioners ...ould ensure that they carry benzylpenicillin ...hong their emergency drugs. If they see a patient ... whom they suspect meningococcal disease, ...pecially if a rash is present, they should give ... dose, ideally intravenously, before urgently ...ranging admission to hospital. Admitting ...ediatricians can remind general practitioner ...lleagues over the telephone of the value of this ...atment. Whether or not benzylpenicillin is ...ven, immediate transfer to hospital is the next ...gent priority.

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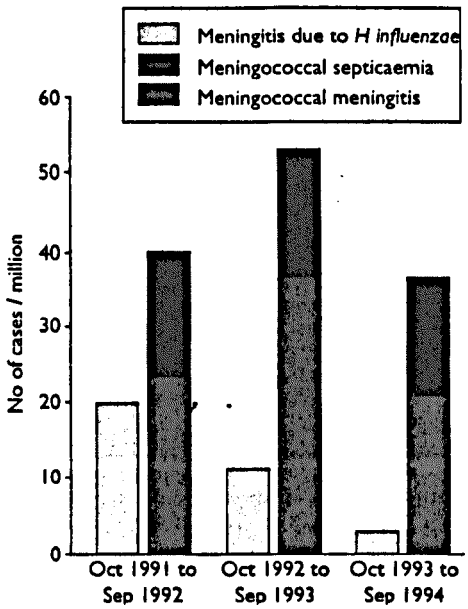
## ...ief medical officer's guidelines are right

...TTOR,—We regard as unconvincing the evidence ... support C S Nanayakkara and R Cox's con... ...ding comments in their letter on the initial ...nagement of suspected meningococcal infec...n.[1] The incidence of *Haemophilus influenzae* ...e b infection has decreased since the intro... ...ction of the immunisation programme. In ...mingham it decreased from 19·8 per million ...pulation for the year October 1991 to September ...92 to 2·0 per million for the year October 1993 to ...ptember 1994. The figure compares this with the ...idence of meningococcal disease over the same ...iod.

Meningococcal infection will therefore increase ... proportion of the total number of cases of ...ningitis, particularly in childhood. Meningo... ...cal disease may also present as septicaemia, ...ich has a much worse prognosis. Thus the most ...propriate management of infection with *Neisseria ...ingitidis* will become even more important. The ... of dexamethasone in the early treatment of meningococcal infection is unclear and remains under review. Most studies agree, however, that in suspected meningococcal disease antibiotics should be given as soon as is appropriate.[2-4] Even Peltola—cited by Nanayakkara and Cox—urged prompt management.[5]




*Number of cases of meningitis due to H influenzae, meningococcal meningitis, and meningococcal septicaemia per million population, October 1991 to September 1994 (source: Birmingham Communicable Disease Unit)*

In the future, when a general practitioner is presented with a case of meningitis the cause is more likely to be *N meningitidis* than *H influenzae* type b. Nanayakkara and Cox present insufficient evidence to support the statement that early use of benzylpenicillin by general practitioners may be inappropriate or even harmful. Indeed, general practitioners would need to consider whether withholding benzylpenicillin in order to adminster dexamethasone would do more harm than good to their patients.

The chief medical officer's current guidelines on meningococcal disease are based on the scientific data available. Their use should be encouraged by all doctors involved in the early management of the disease.

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## On the spot treatment needed

EDITOR,—C S Nanayakkara and R Cox's letter highlights the confusion that exists between meningococcal meningitis and meningococcal septicaemia.[1] Life threatening meningococcal disease presents not as meningitis but as septicaemia, often with a rash.[2] Giving penicillin before admission to hospital may decrease the mortality from meningococcal disease[3]; penicillin should therefore be given to those most at risk of dying—that is, those with septicaemia characterised by a purpuric rash. Information and publicity about meningococcal disease should focus on septicaemia, characterised by a petechial or purpuric rash, rather than on meningitis.[2]

In a recent prospective study in Merseyside the most important factor affecting whether children with meningococcal disease received penicillin before admission was the admitting doctor's diagnosis. When meningococcal disease was diagnosed 26 (84%) of 31 children were given penicillin. Of the 19 children diagnosed as having meningitis, however, only three (16%) were given penicillin ($P<0.0001$, Fisher's exact test). To increase the numbers of children given penicillin before admission, attention should focus on meningococcal disease and not meningitis.

There is little evidence that children with bacterial meningitis benefit from antibiotics before admission,[4] although they may benefit from dexamethasone given with, or before, the first dose of antibiotic.[5] There is no evidence supporting the use of dexamethasone in meningococcal septicaemia, and antibiotics should not therefore be withheld before admission from those with a purpuric rash because steroids are unavailable.

The rapid progression of meningococcal septicaemia requires immediate antibiotic treatment to be given by the first doctor to see the patient. Penicillin should thus be recommended before admission for patients with meningococcal disease presenting with a petechial or purpuric rash. Such on the spot treatment might help reduce the mortality from this devastating infection.

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## Authors' reply

EDITOR,—F A I Riordan and colleagues highlight the clinical confusion that exists between meningococcal meningitis and meningococcal septicaemia. We would be interested to learn the outcome of the prospective study on Merseyside, particularly in those children in both groups who did not receive penicillin before admission.

The authors emphasise that numbers of children given penicillin before admission could be increased if attention was focused on meningococcal disease, not meningitis. We agree that meningococcal disease presenting clinically with a petechial or purpuric rash requires penicillin before admission. But this distinction is not made or emphasised in the advice given by the chief medical officer[1] or the Communicable Disease Surveillance Centre.[2] Confusion may still exist in the indications for penicillin before admission in children without a rash, who may still have serious meningococcal disease.