Prevalence and Prediction of Serious Bacterial Infection in Febrile Children: A Role for Cytokines?

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2007

Dedication

To my parents, for giving me the meaning of living for others

Declaration

I hereby declare that this thesis has been wholly composed by me (Dr. OG Osman) and I own all work it contains. The thesis is written solely for fulfulment of MD from the University of Edinburgh and has not been submitted for any other degree or professional qualification.

Abstract

Prevalence and Prediction of Serious Bacterial Infection in Febrile Children: A Role for Cytokines?

Background: To date, no consensus has been reached regarding the evaluation and management of young febrile children, and no single laboratory test has been shown to reliably identify young children at high or low risk of having serious bacterial illness. Knowing the local prevalence of SBI is essential in formulating management strategies because extrapolation from studies done elsewhere is difficult.

Aims: i) To determine the prevalence of SBI in young febrile children served by a paediatric hospital. Ii) To evaluate the role of cytokines in predicting SBI in these children.

Hypothesis: IL-6, IL-8 and sICAM-1 can accurately predict SBI in young febrile children.

Methodology: The study population was children seen at the emergency department of the Edinburgh Royal Hospital for Sick Children. All children ≤5 years old with a temperature of ≥38.5°C were studied over a 12-month period. Demographic, clinical and laboratory data were collected prospectively. Serum IL-6, IL-8 and sICAM-1 were measured by ELISA. The performance characteristics of the cytokines in predicting SBI were compared to those of the traditional tests (WBC, ANC, CRP, ESR).

Results: 618 patients fulfilled the study criteria of age and temperature. 26.7% of the patients had a SBI. The commonest SBI was pneumonia. 2.7% of blood cultures were positive, over half were streptococcus pneumoniae. 40% of positive blood cultures were from patients discharged with an apparently benign illness. The modest gain in the post-test probability of SBI was comparable between the cytokines and the traditional tests. Serum IL-6 and IL-8 was elevated in all patients with bacteraemia or meningitis. A model based on the respiratory rate, CRP, and sICAM-1 correctly identified 70% of SBI.

Conclusion: The prevalence of SBI, and bacteraemia, in young febrile children is still high in spite of the decline in immunizable diseases. Highly febrile young children should continue to be evaluated with a blood culture and close follow up, and UTI should be excluded in all infants. IL-6 and IL-8 appear to be sensitive markers for bacteraemia and meningitis and their role requires further evaluation.

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Abbreviations

ABC	Absolute band count
ACTH	Adrenocorticotrophic hormone
AFUO	Acute fever of unknown origin
AIDS	Acquired immunodeficiency syndrome
ANC	Absolute neutrophil count
AOME	Acute otitis media with effusion
ARDS	Acute respiratory distress syndrome
AVP	Arginine vasopressin
BCG	Bacille Calmet Guarine
BMT	Bone marrow transplant
BI	Bacterial infection
CBC	Complete blood count
CD	Cluster differential
CF	Cystic fibrosis
CI	Confidence interval
CSF	Cerebrospinal fluid
DNA	Deoxyribonucleic acid
EPO	Erythropiotein
G+ve	Gram positive
GCSF	Granulocyte colony stimulating factor
GM CSF	Granulocyte monocyte colony stimulating factor
GN	Glomerulo-nephritis
gp	Glycopreotein
GTP	Guanidine triphosphate
GvHD	Graft versus host disease
G-ve	Gram negative
HIV	Human immune deficiency virus
hpf	High power field

Abbreviations cont.

IBD	Inflammatory bowl disease
IFN	Interferon
IgG	Immune globulin G
ITP	Inositine triphosphate
ITU	Intensive therapy unit
IU	Intrauterine
JRA	Juvenile rheumatoid arthritis
kDa	Kilo-dalton
L.mono	Listeria monocytogenes
LE	Leucocyte esterase
LFA	Leucocyte function antigen
LP	Lipo-polysacharide
LR	Likelihood ratio
MCP	Monocyte chemo-attractant protein
MHC	major histocompatibility complex
MPG	Mesangio-proliferative glomerulo-nephritis
MW	Molecular weight
N.menin	Nieserria meningiditis
NAP	Neutrophil attractant/activator protein
NF	Nuclear factor
NPV	Negative predictive value
OB	Occult bacteraemia
OBI	Occult bacterial infection
OM	Otitis media
OR	Odds ratio
PCV7	Heptavalent pneumococcal conjugate vaccine
PPV	Positive predictive value
ROC	Receiver operator curve
SBI	Serious bacterial infection

Abbreviations cont.

SCID	Severe combined immune deficiency
sICAM-1	Soluble inter-cellular adhesion molecule
SIRS	Systemic inflammatory response syndrome
SLE	Systemic lupus erythromatosis
SPA	Supra-pubic aspirate
SRE	Serum responsive element
TB	Tuberculosis
TGF	Thymocyte growth factor
TGF	Transforming growth factor
TNF	Tumour necrosis factor
UTI	Urinary tract infection
WBC	White blood cell count
YOS	Yale observation scale

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The thesis

A study of the prevalence of serious bacterial infection in young febrile children and the role of cytokines in its prediction.

Issues pertaining the management of febrile young children have been reviewed in this thesis, which has been written in three parts. The first part discusses the prevalence of serious bacterial infection (SBI) and the controversy surrounding the management of febrile children. The clinical evaluation and laboratory tools used to identify serious illness in these patients are outlined. The inflammatory cytokines and their potential role as markers of illness in febrile children are discussed in the second part. The third part is a study of the prevalence of serious infection in febrile children seen in the emergency department of a paediatric teaching hospital. The performance of three cytokines in predicting serious illness is tested and compared with the conventional markers of infection.

Hypothesis:

Plasma interleukin-6, interleukin-8 and sICAM-1 can accurately predict SBI in febrile young children.

Aims:

1. To determine the prevalence of SBI in children ≤5 years of age attending A&E with a temperature ≥38.5 °C.
2. To determine the utility of cytokines (IL-6, IL-8 and sICAM-1) in predicting SBI in these children.

Part One

Background

Chapter I

Prevalence of Serious Bacterial Infection in Febrile Children

I. 1. Febrile illness in children

Clinicians differ in the magnitude of temperature by which they define the presence of fever in children (*Zerr et al. 1999*). The upper limit of normal body temperature has been agreed to be 37.7°C (99.9°F) in adults and 37.9°C (100.2°F) in children. The lowest and highest (99th percentile) temperature varies with the time of the day, with the lowest occurring at 6 am and the highest at 6 pm. A temperature of 38.0°C (100.4°F) or greater in the infant or child represents abnormal temperature elevation or fever (*McCarthy, 1995*). Paediatric patients experience approximately 4-6 episodes of infectious disease per year during the first 5 years of life (*Procop et al. 1997*). Of these, fever is one of the most frequently encountered complaints and accounts for as many as 20% of paediatric emergency department visits (*Nelson et al. 1992, Henretig et al. 1996*). A similar proportion of total sick child visits in general practice is also for a febrile illness (*Pantell et al. 1980, Wright et al. 1981*). In England and Wales, between 2 and 3 million children attend accident and emergency (A&E) annually, representing 25% of all A&E attendance. A third of these children present with an infectious illness (*Simons et al. 1999*). The highest rate (98%) of GP consultation, the percentage of registered individuals consulting their GP, in England and Wales is for the under fives and the commonest reason for these consultations is for infectious diseases (*Polnay, 1993*).

Episodes of fever in the first 3 months of life are less common than in the age range of 3 to 36 months. At times, a young infant may not mount a fever response to an

acute infection and may even be hypothermic. In one study of 575 patients in the first 2 months of life who were being followed for primary care, only six (1%) developed fever. In the same clinic, 55 of 390 children (14%) between 9 and 12 months of age developed a fever (*Baker et al. 1993*). Furthermore, it appears that young infants react with lesser degrees of fever than older patients. In another report, only 2 of 149 (1.3%) consecutive febrile young infants (0-2 months old) had fevers of 40°C (104°F) or more compared with 26 of 332 (8%) consecutively seen febrile children aged 3 to 36 months from the same institution (*Baker et al. 1989*). An increase in the occurrence of fever is seen between November and March in children aged 3 to 36 months of age, coinciding with the circulation within the community of acute infections caused by respiratory and gastrointestinal viral pathogens such as respiratory syncytial virus and rotavirus. There is also an increase in the frequency of febrile illness in infants younger than 90 days of age during this period. However, 40% of febrile episodes in these younger patients occur in July through September, due to the circulation of enteroviruses at that time of the year. This could also be due to immature thermoregulation predisposing infants to elevated body temperatures at high ambient temperatures during summer months *(McCarthy et al. 1995*).

Acute fever of unknown origin (AFUO) is used to describe a child who develops high fever of relatively sudden onset, but who displays no localizing signs of infection or other cause discernible by history. This type of illness accounts for 2-4% of infections in outpatients of all ages and is an important diagnostic problem especially in young children (*Murray et al. 1981*). The majority of children presenting with a febrile illness, however, have symptoms and/or signs of respiratory tract disease and a virus can be identified in a third to a half of these patients (*Martin et al. 1978*). Acute otitis media, URTI, and viral syndrome are the most common diagnoses made, accounting for more than 70% of febrile episodes seen in a paediatric accident and emergency department. In this group of patients a viral aetiology can be implicated in up to 86% of cases, when investigated thoroughly, with aseptic meningitis being the commonest diagnosis (*Krober et al. 1985, Nelson, 1992*). Nevertheless, up to 25% of febrile illnesses show no localizing signs or symptoms (*Wright. et al. 1981*) and a significant proportion of these

patients will have a serious bacterial infection (*McCarthy et al. 1982, Dagan et al. 1985, Bonadio et al, 1994, Berger et al. 1996, Kuppermann et al. 1998*) (*Table 1*). It has been demonstrated that nearly half of serious bacteraemic illnesses are diagnosed as viral illness when first seen (*Forman, 1991*).

I. 2. **Definition of serious bacterial infection**

Studies varied widely in what constituted a serious bacterial infection. The definitions used depend to some extent on the objectives and methodology of the study. Some authors define SBI as a positive culture of urine, blood or CSF (*Pantel et al. 1980, Bachur et al. 2001*). Other studies put more emphasis on the pathophysiology involved and potential for serious complications (*Dagan et al 1985*). Bonadio et al, studying infants aged 8-12 weeks, define SBI as bacterial meningitis, Salmonella enteritis, and UTI, (positive tests of urinalysis were considered indicative of UTI) excluding pneumonia (*Bonadio et al. 1994*). In an earlier study of older children, the same authors also exclude pneumonia from the definition of SBI (*Bonadio et al. 1990*). Other authors also omit pneumonia from their definition of SBI *(Anbar et al. 1986*). Dagan et al consider culture-positive purulent otitis media to be SBI, while excluding radiologically diagnosed pneumonia (*Dagan et al. 1988*). In an appraisal of the Rochester criteria for the detection of SBI in febrile infants, Jaskiewicz et al consider pneumonia to be serious bacterial infection. They define bacterial pneumonia as a focal infiltrate on chest radiogram in association with a bacterial pathogen isolated from the blood or the presence of capsular polysaccharide in the urine detected by countercurrent immuno-electrophoresis (*Jaskiewicz et al. 1994*). Baker et al consider pneumonia to be SBI only if cultures of blood or respiratory secretions grow a known respiratory bacterial pathogen (*Baker et al. 1999*). The authors of the practice guidelines for the "Management of Fever without Source in Infants and Children" define SBI as meningitis, sepsis, bone and joint infections, UTI, pneumonia, and enteritis. No bacteriological proof of pneumonia is required (*Baraff. 1993*).

In the era of wide vaccination coverage of previously dreaded infections, such as those caused by Haemophilus influenzae type B, some meningococcal strains, and more

recently pneumococci, less emphasis has been placed on bacteraemia while the concept of systemic inflammatory response syndrome (SIRS) has attracted more attention (*Table 2*). It is important, nevertheless, to have a specific bacteriological diagnosis in order to effect prompt and targeted treatment and thus reduce cost and discomfort and also implement evidence-based preventive measures. Definitive identification of SBI requires a positive culture of CSF, blood or urine or an identifiable bacterial focus by physical examination or radiographic testing. However, the reality of clinical practice dictates that establishing a causative agent for many apparently infectious illnesses is difficult to achieve and is not always accomplished. Illnesses managed as bacterial infections include meningitis with clinical and cytological indicators of a bacterial pathogen, urinary tract infection, cellulitis, epiglottitis, sinusitis, and osteomyelitis. Other illnesses are clearly viral, for example measles, varicella, roseola, erythema infectiosum, herpangina, hand/foot/mouth syndrome, croup, bronchiolitis, and hepatitis. There are, however, syndromes in which a number of agents may be causative: bacteria, viruses, mycoplasmas, chlamydiae, or fungi. These include pharyngitis, pneumonia, infectious diarrhea, and fever in the young child with no focus of infection. In evaluating these syndromes, epidemiologic and clinical features may be used more frequently than laboratory testing.

In daily clinical practice, a specific microbiologic diagnosis may not be pursued, whether an antibiotic is prescribed or not, except perhaps in the highly or persistently febrile child. Instances when a viral diagnostic evaluation may be worthwhile include; i) infants at risk for serious disease from RSV or influenza A, where antiviral therapy is a consideration; ii) infants taking antibiotics where bacterial cultures and antigen detection tests are negative and demonstration of a viral aetiology might allow earlier discontinuation of antibiotics; and iii) where knowing the etiology is of prognostic importance. In an emergency room setting, when it comes to ordering investigations and deciding upon disposal of the patient, clinical judgment is frequently subjective and following a standard of management based on evidence-based guideline, if one is available, is not always straightforward. It is impractical to obtain a precise bacteriological diagnosis in each and every febrile patient presenting to a busy

emergency department. To study the incidence of serious bacterial infections, it will not suffice to obtain blood cultures only, as these will reveal only the minority (2-5%) of patients who are bacteraemic. Even for hospitalized patients the diagnosis recorded at discharge is often non-specific and does not necessarily reflect the true status of the patient. Many such diagnoses are presumptive and the causative organism may not be mentioned. Diagnoses such as otitis media, tonsillitis and chest or lower respiratory tract infection, are usually made to reflect the site of symptoms or signs without any indication of a possible causative organism. These infections can be potentially serious but are not considered so unless a local or remote septic or reactive complication has occurred. The middle ear is part of the secretory immune system, where antigens react with immune cells in its lamina propria to produce local, and systemic, immune response. Immunoglobulins and chemical mediators of inflammation can be found in middle ear effusion. Otitis media is associated with elevated levels of circulating cytokines, and this response can vary among different causative micro-organisms. It has been demonstrated that the immunologic response and cytokine profile differ between, for instance, viral and bacterial otitis media (*Heinkkinen et al, 1998*). Unless microbiologic diagnosis has been made by myringotomy, which is not routinely practiced, the inclusion of patients with otitis media can adversely affect the performance characteristics of any marker of infection that is sought to differentiate between viral and bacterial causes of infection. Moreover, the clinical diagnosis of otitis media can on occasion be questioned, as redness of the tympanic membrane can be part of a simple upper respiratory catarrh or due to excessive crying.

Otitis media is one of the most frequent causes for medical consultations in children. Up to 40% of clinic visits by children below 5 years are due to acute otitis media with effusion (AOME) and 5% to 10% of all well child visits result in a diagnosis of either acute otitis media or otitis media with effusion (*Klein et al. 1994*). AOME is a clinically identifiable suppurative disease of the middle ear and is characterized by an opaque and/or bulging tympanic membrane. Bacterial pathogens can be isolated from middle ear fluid in approximately two thirds of patients who have AOME. Streptococcus pneumoniae is found in approximately 30% to 50% of infected middle ears.

Haemophilus influenzae causes 20% to 27% of all cases of AOME. The majority of these cases are caused by non-typeable strains, but up to 36% have been caused by type b, and a quarter of these could be associated with bactaeremia or meningitis. Moraxella catarrhalis is isolated in approximately 7% to 23% of cases of AOME (*Del Beccaro et al. 1992*). Other bacteria reported to cause AOME include: group A Streptococcus, Staphylococcus aureus, alpha-haemolytic Streptococcus and Pseudomonas aeruginosa. The rate of bacteraemia in patients with isolated clinical otitis media is comparable to those in children with no clinically identifiable focus of infection (*Shutzman et al. 1991, Lee et al. 1998*). Therefore, it has been advised that otitis media should not be considered a cause of fever and febrile infants and children with otitis media should be evaluated for an occult infection (*Luszczak et al. 2001*). Viruses have also been associated with AOME and have been cultured or detected by antigens in up to 42% of children with AOME. The virus isolated most commonly is rhinovirus, followed by respiratory syncytial virus. Other causative viruses include adenovirus, parainfluenza, coronavirus, influenza, and enteroviruses (*Arola et al. 1990*). The microbioligical diagnosis of AOME rests on obtaining purulent middle ear fluid for culture by needle tympanocentesis. The most specific symptom of otitis media is otalagia, and clinical diagnosis is based on the presence of a retracted, dull or bulging tympanic membrane. Pneumatic otoscopy, tympanometry, and acoustic reflectometry can add objective evidence to the status of the middle ear but are not available to a physician working in a busy paediatric emergency room where diagnosis of AOME largely depends on the appearance of a red tympanic membrane.

The clinical distinction between viral and bacterial pharyngitis is also difficult as all can cause fever, sore throat, pharyngitis with or without exudate, and enlarged cervical nodes. Group A beta-hemolytic streptococcus (GABHS) is the major cause of bacterial pharyngitis, while EBV, adenovirus, the coxsackie A viruses, and HSV are the major causes of viral pharyngitis. Infections which can be detected by more specific and easily obtained tests, such as urinalysis and chest radiograph, can also impose considerable difficulty and confusion in interpretation. Because bacterial aetiology can

be difficult to establish, the serious nature of the infection is sometimes determined by the need for interventions such as hospitalization or specific treatment.

I. 3. Prevalence of serious bacterial infection

The incidence of all infectious diseases peaks in children in the first four years of life, and serious infections have the highest rates in the same age group (*Van den Bruell, 2006*). The prevalence of serious bacterial infections varies between different populations and universal extrapolation of findings from local studies is therefore difficult. Comparison between different studies is further complicated by the varying definitions of SBI between studies, as detailed above. The disease patterns vary between and within populations with changing demographic characteristics and the continuing implementation of varied preventive measures, such as newly introduced vaccines. With elimination of H. influenzae type b and vaccine-serotype pneumococcal invasive diseases, the incidence of serious bacterial infections in febrile children is likely to decrease, to the extent that some experts predict current strategies of testing and empiric antibiotic therapy will become obsolete (*Baraff, 2000*). However, the current vaccine is only 90% effective in preventing invasive pneumococcal disease (*Black et al. 2000*), and the number of children with fever seen in emergency rooms or in general practice is unlikely to change (*Klein et al. 2002*). Infections caused by pneumococcal serotypes not in PCV7, Neisseria meningitides, Salmonella spp., group A Streptococcus, Staphylococcus aureus and gram-negative enteric bacteria will continue to occur, together with those occurring due to vaccine failure. It follows that infectious disease presenting as acute febrile illness will continue to be a common cause for hospitalising children. It is widely realised that a high proportion of such children are unnecessarily admitted and subjected to stressful and expensive treatment and tests. Therefore, the challenge of distinguishing the febrile child with invasive bacterial disease, who requires aggressive therapy, from the febrile child who has a viral infection and requires only symptomatic therapy, will persist.

I. 3. a. Occult bacteraemia

Occult bacteraemia, defined as a febrile state in which a major pathogen circulates in the blood for hours to days in temporary balance with the body's immune defenses (*Baron, 1980*), occurs in 3-12% of febrile illnesses in young children showing no focal signs (*Schwartz et al. 1982, Anbar et al.1986*). Bacteraemia also frequently occurs in association with the common focal bacterial infections of young children, such as otitis media (*Schutzman et al. 1991*). It has been estimated that 10-15% of children with bacteraemia, if not promptly treated, will develop more serious illness such as septicaemia, meningitis, pneumonia, osteomyelitis, and septic arthritis (*Dagan et al. 1985*). The potential for bacteraemia evolving into a serious illness largely depends on the causative organism (*Table 3*).Before the introduction of Haemophilus infulenzae type b (Hib) vaccine, Hib accounted for 25% of occult bacteraemia in young children (3-36 months) and it caused 42% of the complications identified at follow up (*McGowan et al. 1973, Teele et al. 1975, McCarthy et al. 1976, Hoekelman et al. 1979, Wood et al. 1990, Forman et al. 1991*). Hib also caused most cases of bacterial meningitis in this age group (*Barraf 1993, Shapiro et al. 1993*). Streptococus pneumoniae caused around 65% of bacteraemic infections (*Forman et al. 1991*). In areas where Hib immunisation rate is high, this once dreaded organism is now almost completely eliminated (*Lee et al. 1998*). In Finland (*Peltola et al. 1992*) and the USA (*Lee & Harper, 1998*), where Hib vaccination was introduced in 1986 and 1990 respectively, a large reduction of invasive Hib disease has been documented. A similar decline has also been witnessed in the UK after Hib vaccination was introduced in 1992 (*Booy et al. 1995*). However, meningitis and other serious illnesses due to Haemophilus influenzae continue to occur in areas and individuals not completely covered by the vaccine (*Luca et al. 2004*). Furthermore, concerns are raised of possible resurgence of invasive disease in the same age group targeted by vaccination programmes. This has been blamed partly on incomplete vaccination coverage and partly on the considerable lot-to-lot variation in both the composition and the immunogenecity of conjugate vaccines. There are also concerns about the ability of the available conjugate vaccines to provide long-term protection and the possibility of an increase in the proportions of older children and adults who are

susceptible to Hib infections due to the reduction in the prevalence of colonization with Hib in the population. In the United Kingdom, invasive Haemophilus influenzae type b disease has been increasing since 1998 despite high levels of immunization. Low levels of protective Hib IgG were found in children, especially those born prematurely, who had a full primary course of immunization with combined conjugate Hib vaccine (*Steinhoff et al. 2003*).

In the post Hib vaccine era, Streptcoccus pneumoniae accounts for more than 90% of all cases of occult bacteraemia, while Salmonella, Neisseria meningiditis, group A streptococcus, and group B streptococcus are responsible for the rest (*Lee et al. 1998*) (*Table 4*). The incidence of invasive pneumococcal infections has been estimated as 10 per 100,000 children under 16 years of age and 45.3 per 100,000 children less than age 2, and an increase has recently been observed in some parts of the western world after the wide use of Hib vaccine (*Baer et al. 1995*). It has been speculated that this surge could be related to the disappearance of Haemophilus influenzae type b disease and changes in the pneumococcal carriage rates among recipients of Hib vaccine. It is biologically more plausible, however, to suppose that non-serotype b H. influenzae would be likely to replace Hib in the nasopharynx and cause more frequent invasive disease. No such change has been recorded in the rate of invasive pneumococcal disease, or that of non-type-able and type-able Haemophilus influenzae infections (other than type b) in areas of the United Kingdom where Hib has largely been eradicated (*Booy et al. 1995*). The prevalence of invasive pneumococcal disease continues to change with the recent introduction of conjugated heptavalent pneumococcal vaccine (PCV7). O'Brien et al reported on the epidemiology of invasive pneumococcal disease before the introduction of a conjugate heptavalent vaccine. The estimated incidence in children below 5 years of age was 272 infection episodes per 100,000 child years, with the highest rate in the 6-11 month old children. Only 60% of isolated pneumococci were serotypes contained in the pentavalent vaccine (*O'Brien et al. 2004*). Following the introduction of heptavalent pneumococcal vaccine, a study of highly febrile young children (2-36 months of age) who were well enough to be discharged from the emergency department, reported an incidence of occult pneumococcal bacteraemia of

0.91% (*Stoll et al. 2004*). Nonetheless, another study has shown a shift in pneumococcal colonization towards non-vaccine serotypes and an increase in infections with other virulent organisms after vaccination (*Bogaert, et al, 2004*). More worrying is the demonstrated increased prevalence of very-high-level resistant pneumococci (*Schrag et al. 2001*).

The incidence of bacteraemia varies among different age groups (*Table 5*). No racial, geographic, or socio-economic predictors of bacteraemia have been demonstrated (*Dershewitz et al. 1983*). Nevertheless, there are many important confounding factors that affect the reported incidences of occult bacteraemia. Changes in the size of population, paediatricians' criteria (temperature and age) for ordering blood cultures in febrile children, culture techniques and the coverage rates of Hib and other vaccines are some examples. Hospitalized patients may have higher rates of bacteraemia than outpatients, because hospitalized children may look more ill and often have serious diseases that are more likely to be associated with bacteraemia. Bacteraemia is rarely seen in a primary care setting because of the relative infrequency of the condition, the treatment with antibiotics at the onset of the illness, the policy of obtaining blood cultures judiciously, and the referral of sick children to emergency rooms hours after being seen. Thus, findings from studies based in primary care might underestimate the prevalence of bacteraemia in children seen at hospital emergency departments where a selected population is represented. The importance of bacteraemia as a cause of acute fever of unknown origin will diminish as effective vaccines are developed and effectively used.

I. 3. b. **Bacterial meningitis**

Bacterial meningitis, the most dreaded serious bacterial infection, has the highest incidence in children younger than 5 years, with over 70% of cases occurring in those below 2 years of age. The incidence of neonatal meningitis, 0.25-1 case/1000 live births, remains relatively stable. 30% of newborns with clinical sepsis will have associated bacterial meningitis (*Kumar et al. 2003*). S. agalactae, L. monocytogenes and gram negative organisms cause most of these infections. In children older than 2 months, H.

influenzae, S.pneumoniae, and N. meningiditis, account for 90% of bacterial meningitis (*Baraff, 1993*). Meningitis caused by organisms other than these in a child over two months should prompt a search for an immunological or anatomical abnormality. In Western Europe, half cases of meningitis are due to S. pneumoniae, a third are caused by N. meningiditis , while Hib accounts for less than 3% of the cases (*Perrocheau et al. 2004*). In Eastern Europe, where vaccination with conjugate Hib and meningococcal C vaccines is not widespread, meningococcal meningitis accounts for two thirds of cases and Hib causes about a quarter of meningitis in children below 5 years of age (*Luca et al. 2004*). In developing countries, where the high cost of the vaccine is a major obstacle to its mass application, Hib meningitis is still an important public health problem (*Gomez et al. 1998*). Before the advent of Hib vaccine, the average annual incidence of meningitis in the Western countries was 30 to 70 per 100000 (*Wenger et al. 1990*). H. influenzae was the most common organism. Currently, the likelihood of Hib meningitis occurring in a child who has received at least two doses of Hib vaccine approaches zero (*Kaplan et al. 1997*). Since the introduction of Hib vaccine, S. pneumoniae has become the most common cause of bacterial meningitis in this age group. The annual incidence of meningitis in children under 5 years of age in the United States has been relatively stable for N. meningiditis (4-5/100,000) and S. pneumoniae (2.5/100,000) since 1980. Seven of the 84-pneumococcal serotypes (4, 6B, 9, 14,18F, 19F, and 23F) account for 80% of invasive disease in children. The universal use of the heptavalent conjugated pneumococcal vaccine (PCV7) will certainly have an impact on the prevalence of meningitis in this age group. Group B and C are the most common serogroups of N. meningiditis causing meningitis in the Western World (*Kaplan et al. 1997*). Meningococcal bacteraemia has the highest propensity to cause meningitis (*Table 3*).

The mortality and morbidity of bacterial meningitis vary with the etiologic organism. For instance, the estimated mean probability of mortality for H.influenzae is 3.8%, for N.meningditis 7.5% and for S.pneumoniae 15.3% (*Baraff and Schringer 1993*). Pneumococcal meningitis has a particularly high rate of neurological complication. In a multi-centre surveillance involving eight children's hospitals in the United State, Arditi et al reported 25% and 32% incidence of motor deficits and

moderate to severe hearing loss, respectively, among the survivors of pneumococcal meningitis (*Arditti et al. 1998*). The most common serotypes of S.pneumoniae recovered from children with meningitis were the same as those isolated from children with other systemic infection, many of which are not included in the heptavalent pneumococcal vaccine. There was also a high rate of resistance to penicillin (19.3%) and ceftriaxone (7.2%). Because of this organism-specific variation in sequelae, the substantial reduction in H.influenzae infections may not lead to as striking a reduction in the incidence of neurologic sequelae of this disease.

I. 3. c. **Urinary tract infection**

UTI is the most common occult SBI in young febrile children seen in a paediatric emergency unit occurring in between 7% and 11% of the patients (*Roberts et al. 1983, Krober et al. 1985, Bauchner et al. 1987, Pulliam et al. 2001)*, and is frequently seen with associated bacteraemia; especially in young infants (*Crain et al. 1990, Jaskiewicz et al. 1994, Craig et al. 1998*). In spite of its frequency and potential severity, UTI is often felt to be a simple problem. It is important to identify children with UTI among those who present with fever to institute early treatment and evaluate their renal tract and watch for recurrence of infection, because the risk of renal damage increases as the number of recurrences increase. Up to 50% of young children with their first episode of UTI can be expected to have an underlying urinary tract abnormality (*Rickwood et al. 1992, Zamir et al. 2004*). Proper evaluation of UTI will provide the opportunity to prevent kidney damage and consequent complications. On the other hand, it is critically important to use proper criteria to diagnose UTI to avoid the risks of over-diagnosis; repeated patient visits, use of antimicrobials, exposure to radiation, and excessive costs.

The prevalence of UTI varies by age and sex. In the newborn period, approximately 1-2% of both girls and boys can be expected to develop UTI. In young febrile infants ≤2 months old, the prevalence of UTI is similar in infants evaluated for sepsis, those suspected of UTI and infants with other illnesses and no suspected UTI (*Hoberman et al. 1993*). The prevalence of UTI in children younger than 2 years of age with fever without source is high, around 5%. The prevalence in girls younger than one

year of age (6.5%) is twice that in boys (3.3%). Between one and two years, the prevalence in girls is 8.1%, and in boys it is 1.9%. The rate in circumcised boys is low, 0.2%-0.4%, but it is 5 to 20 times higher in uncircumcised boys (*Bergman et al. 1999*). UTI in children younger than 2 years warrants special attention because they are at higher risk for UTI, they show no symptoms referable to the urinary tract, are more likely to have factors predisposing them to renal damage, and they are at higher risk of developing renal scarring from an episode of UTI. The incidence and severity of VUR are greater in this age group, with the most severe form (intrarenal reflux or pyelotubular backflow) virtually limited to infants (*Woodard et al. 1976*).

Although the management of UTI is seemingly straightforward, there exists a considerable variation in the methods of diagnosis, treatment and evaluation of children with UTI. UTI has been defined, variably, as urethritis, cystitis, or pyelonephritis. Urethritis is dysuria, frequency or enuresis with pyuria, but colony count of $<10^4$ /ml of urine. Cystitis is as above, with a colony count of $> 10^4$ /ml of urine. Haematuria may be present, but no casts, flank pain, fever, or systemic toxicity. Pyelonephritis is febrile UTI, often with flank pain and symptoms of cystitis. The colony count may be $<10^5$ /ml of urine. No diagnostic test except the radionuclide renal scan has been shown to distinguish cystitis conclusively from pyelonephritis. Indirect tests for localisation of the site of UTI, such as a reversible defect in the renal concentrating ability and high levels of antibody titres to the infecting E. coli, and non-specific tests of inflammation, such as elevated WBC or CRP, do not provide confirmatory evidence that the febrile young child with UTI has pyelonephritis. Fever as a marker of pyelonephritis (defined by a positive scan) provides a wide range of sensitivity (53% to 84%) and specificity (44% to 92%) (*Bergman et al. 1999*). Nevertheless, the presence of fever in a child with bacteruria and pyuria has been accepted as a marker of renal parenchymal involvement (pyelonephritis) (*Johnson et al. 1999*) and indicates an increased frequency of underlying urinary tract abnormalities (*Roberts et al. 1983, Woodard et al. 1976*).

The symptoms and signs of UTI are usually non-specific in young children and vary with age. In the newborn, late-onset jaundice, hypothermia, signs of sepsis, failure to thrive, vomiting, and fever are possible signs of UTI. In infants and preschool

children, additional findings include diarrhoea and strong-smelling urine. The school-age child is more like the adult in terms of signs and symptoms of UTI, but enuresis, strong-smelling urine, and vomiting may be holdover features from the younger age group. Other historic findings that should be sought include voiding problems and family history of UTI or proven vesico-ureteric reflux.

Laboratory tests useful in diagnosing UTI include urinalysis and urine culture but it is important to focus on the technique of urine collection (The *Royal College of Physicians, 1991*). Commonly used methods of urine collection in young children include clean catch specimen (*Ramage et al. 1999)*, sterile adhesive bags (*Waddington et al. 1997*), urine collection pads (*Feasey et al. 1999*, *Macfarlane et al. 1999*), and urine collection from disposable nappies (*Ahmed et al.1991*) and sanitary towels (*Vernon et al. 1992*). These methods have been reported with variable parental acceptance, success and contamination rates (*Lewis et al. 1998*, *Vernon et al. 1995, Roderck et al. 1997, Liaw et al. 2000*, *Giddens et al. 1998*). In children, clean-catch midstream specimens are often contaminated, especially in girls by some vaginal reflux of urine. Midstream specimens in circumcised males are more reliable. Bag urine specimens are frequently contaminated in both males and females and are not recommended for use in the diagnosis of symptomatic patients who may have UTI. The combination of a 5% prevalence of UTI and a high rate of false positive results from bag urine (specificity of 70%) results in a positive culture of urine collected in a bag to be a false-positive result in 85% of the time (*Bergman et al. 1999*). The false-positive rate would be 93% in febrile boys and 99% in circumcised boys, who have 2% and 0.2% respective prevalence of UTI. If taken, bag urine specimens are useful only when negative, provided no prior antimicrobial treatment has been received. Transurethral catheterization does not eliminate completely the possibility of contamination in girls and uncircumcised boys. SPA has been considered the gold standard for obtaining urine. However, technical expertise and experience are required, with a variable success rate (23% to 90%), and many parents and physicians perceive the procedure as unnecessarily invasive. Decision analyses and cost-effectiveness studies based on different age and gender prevalence of UTI have shown that it is cost-effective to pursue the diagnosis of UTI by invasive

means and to perform imaging studies in girls and uncircumcised boys younger than 2 years of age. The evidence is equivocal for circumcised boys younger than one year, but it is generally held that they deserve the same diagnostic evaluation. Invasive diagnostic procedures are not justified in circumcised boys older than 1 year with unexplained fever. Analysis of bag urine is a reasonable screening test in these boys, as long as they do not appear so ill as to warrant the initiation of antimicrobial treatment. Those who will be given antimicrobials on clinical grounds should have a specimen obtained for culture that is unlikely to be contaminated (*Bergman et al. 1999*).

Commercial test strips are used for rapid urine testing for infection. These utilize colour indicators that detect nitrite, leucocyte esterase, and blood. The nitrate test is the most specific for UTI and is based on the principle of nitrate being converted to nitrite in the bladder by bacteria. Therefore, it is best done on first-morning or concentrated urine specimens where the bacteria have had sufficient opportunity (4 to 6 h) to metabolize the nitrate to nitrite in the bladder. Urine analysis is the best tool to identify patients who might need treatment and further investigation and, in practice, most clinicians would treat any febrile child who has positive findings on urinalysis, before culture results are available. However, the sensitivity of the test is so low that the risk of missing UTI is unacceptably high (*Table 6*). Microscopic examination of unspun urine is a simple, rapid and practical diagnostic method and, when combined with a Gram stain, is highly predictive of UTI and allows immediate initiation of treatment (*Hoberman et al. 1993*). Microscopic analysis of a centrifuged urine specimen looking for white blood cells or bacteria is also an indirect evidence of UTI. A bacterial count under high power objective of >100 bacteria per field corresponds to $>10^5$ cfu/mL. However, neither bacteruria nor pyuria are sensitive or specific enough (*Table 6*).

It has been suggested that, in febrile children, significant sterile pyuria should not be dismissed as a nonspecific response to fever but should be considered as UTI in the absence of an alternative explanation, and should be investigated accordingly, as up to a third of such patients can have an underlying urological abnormality (*Buys et al. 1994, Giddens et al. 1998*). The diagnostic utility of pyuria alone for identifying true UTI has been quoted as 96% sensitive, and 95% specific, the 4% false negatives being attributed

to early UTI (*Hoberman et al. 1996*). Furthermore, it has been shown that urine culture guided by urinalysis was the most cost-effective strategy in the management of febrile children *(Kramer et al. 1994)*. However, pyuria can occur in the absence of UTI and can occasionally be absent in patients with confirmed urinary tract infection (*Kumar et al. 1996, Crain et al. 1990*). Turner and Coulthard found moderate pyuria ($10\text{-}100\times10^6$/l) in 43% and obvious pyuria ($100\text{-}700\times10^6$/l) in 9% of febrile children without UTI. They demonstrated that pyuria was the result of an increase of urinary leucocyte excretion and not a direct reflection of a raised blood neutrophil count (*Turner & Coulthard, 1995*). Therefore, urinalysis can only suggest the diagnosis of UTI. No element of the urinalysis or combination of elements is as sensitive and specific as urine culture, which is the standard test for the diagnosis of UTI. A properly obtained urine specimen that has not been left at room temperature for more than 30 minutes should be submitted for culture. Urine usually is cultured by inoculating a small volume into culture media, or using the dipslide technique that permits inoculation of urine on agar media in the general practice setting. The latter technique has a reported sensitivity in the range of 87% to 100%, and specificity 92% to 98% (*Bergman et al. 1999*). Criteria used for the diagnosis of UTI in adults include symptoms plus pyuria on urinalysis plus a single clean-catch urine specimen that grows greater than 10^5cfu/mL of a single organism. This definition cannot be applied in children who often do not have the classic symptoms of UTI and pyuria may not be present in up to 50% of children who have proven UTI and often is present in those who do not have UTI.

I. 3. d. **Pneunomia**

Pneumonia accounts for 13% of all infectious illnesses in the first 2 years of life (*Denny & Clyde, 1986*). In the US, the annual attack rates of pneumonia are 4 cases/100 preschool children, 2/100 children in the 5-9 age group, and 1 case/100 in older children (*Sherman J, 2004*). WHO estimates that 3 million children die worldwide from pneumonia each year. Although most of these fatalities occur in developing countries, pneumonia remains a significant cause of morbidity in industrialized countries. Occult pneumonia, i.e. without clinical signs indicative of chest infection, is a common

occurrence in febrile young children (*Leventhal et al.1982, Bachur et al. 1999*). The conjugate pneumococcal vaccine is estimated to reduce clinical pneumonia by 10%, radiographic pneumonia by 32% and pneumonia with definite consolidation by 73% and may reduce the need for chest radiography in febrile children with no clinical signs of pneumonia (*Black et al. 2000*).

The clinical diagnosis of pneumonia is suggested by a constellation of symptoms such as fever, cough, and tachypnoea. More specific pointers are sought in the physical findings of respiratory distress (grunting, flaring and retractions) and consolidation (dullness on percussion, rales, bronchial breathing, aegophony and whispering pectoriloquy). As these clinical signs are often derived from examining hospitalized or very ill patients, they may not be as useful in ambulatory patients who are less likely to show all, or any, of the signs. These signs, besides being difficult to illicit in young children, lack sensitivity and specificity in detecting pneumonia. Signs suggestive of pneumonic consolidation, such as crepitations, are frequently detected in patients with bronchiolitis. Tachypnoea is considered the best clinical sign distinguishing pneumonia from uncomplicated upper respiratory tract infection (*Harari et al. 1991*). Signs of decreased breath sounds and rales are detectable in 46% and 55%, respectively, of children with pneumonia (*Tan et al, 1998*). However, many children who have pneumonia, including those with bacteraemic pneumococcal pneumonia, can have no respiratory symptoms or signs and present with non-specific symptoms.

Pneumonia appears as an opacity (or opacities) on the chest x-ray film, meaning that a large number of pathologic processes may mimic pneumonia radiographically. Furthermore, the radiographic definition of pneumonia and interpretation of chest radiographs vary widely among radiologists. This variation might not be important in routine clinical practice but is a cause of great confusion and inaccuracy in clinical research. In a study of the effect of chest radiograph on the pre-x-ray diagnosis and management, Alario et al classified a CXR as normal or abnormal (hyperaeration, peribronchial thickening, patchy or peribronchial infiltrates, subsegmental or segmental infiltrates, atelectasis, lobar consolidation, pleural effusion, or other specific abnormalities) (*Alario et al. 1987*). Studying the utility of chest radiograph in the

evaluation of the febrile infant, Bramson et al grouped radiographs into four categories: Normal CXR; type 0 (normal), typel (hyperinflation). Abnormal CXR; type 2 (hyperinflation plus peribronchial thickening), Type 3 (hyperinflation, peribronchial thickening and pulmonary infiltrates) (*Bramson et al. 1993*). Patterson et al considered focal parenchymal opacification, but not peribronchial thickening, as evidence of lower respiratory tract infection (*Patterson et al. 1990*). Zukin et al, classified chest radiographs as normal, pneumonia, major abnormalities (atelectasis, effusion), or any abnormality (*Zukin et al. 1986*). Leventhal et al considered the presence of an infiltrate positive evidence of pneumonia (*Leventhal et al. 1982*), while Kramer et al, defined pneumonia on the bases of the presence of one or more focal areas of consolidation or infiltrates (*Kramer et al. 1992*). On the other hand, Bachur et al defined pneumonia as consolidation reported in definite terms (*Bachur et al. 1999*). This wide variation in radiographic characterization and definition of pneumonia makes comparison between studies a difficult task.

Even within defined categories, there can be considerable inter- and intra-observer variability in the radiographic diagnosis of pneumonia, a disagreement that does not improve with increasing experience (*Young et al. 1994, Cochrane et al.1952*). Part of this variation could be due to unblinded radiologists being biased by the treating physician's clinical suggestions *(Kramer et al. 1992)*. Generally, chest radiographs with dense lobar or segmental opacities are interpreted as pneumonia whereas patchy opacities are not considered pneumonia (*Young et al. 1994*). Although radiographic appearance is not an aetiologic diagnosis, it has been generally agreed that a lobular or segmental consolidation suggested a bacterial process, while diffuse or ill-defined densities and infiltrates suggested a viral process (*Tew et al. 1977, McCarthy et al.1981*). Therefore, decisions about therapy are often based on the radiographic appearance, and in routine clinical practice a focal opacification will usually prompt clinicians to start antibiotic therapy in the appropriate clinical setting (*Patterson et al. 1990*). In research studies, a clinical diagnosis of pneumonia has to be supported by roentgengraphic findings compatible with pneumonia- the chest roentgenogram is the gold standard.

The chest radiograph is one of the diagnostic tests most frequently obtained in the evaluation of young febrile children. Views differ as to the necessity of obtaining a chest x-ray in this process. Alario et al demonstrated that the chest radiograph resulted in a change of the pre-x-ray diagnosis in 21% and pre-x-ray management plans in 16% of the patients (*Alario et al. 1987*). Bachur et al found 38 (26%) cases of pneumonia in 146 patients without clinical evidence of chest infection and called for routine chest radiography in highly febrile children with leukocytosis and no alternative major source of infection (*Bachur et al. 1999*). In a series of 330 consecutive children younger than 2 years with high fever, McCarthy et al reported 52 cases of pneumonia, 27 of which were not clinically suspected (*McCarthy et al. 1990*). On the other hand, of 41 patients without pulmonary findings studied by Leventhal, none had an abnormal CXR. The authors claim that if the criterion of at least one pulmonary sign had been used to order a CXR, no case of pneumonia would have been missed, and the number of roentgenograms would have been reduced by 30% (*Leventhal et al. 1982*). Other workers who found much lower rates of occult pneumonia, also called for the abandoning of the use of chest radiograph as part of routine septic work up in febrile infants and young children, to be done only in those who show clinical signs of pneumonia (*Bramson et al. 1993, Zukin et al. 1986, Patterson et al. 1990*). Occult pneumonias do occur, but which children need chest radiography remains unclear. Chest radiography should probably be performed in the evaluation of febrile young children with no identifiable source, even in the absence of respiratory symptoms. Such children could be early in the course of infection, and other children probably offer suboptimal clinical examinations. The true prevalence of bacterial pneumonia is unknown because only a subset of children receive a chest radiograph, it is difficult to differentiate viral from bacterial pneumonia and blood cultures are only positive in 3-5% of febrile children with pneumonia.

Defining the aetiology of pediatric pneumonia is complex for several reasons. First, a wide variety of microorganisms can cause pneumonia. Second, epidemiologic, clinical, and routine laboratory features associated with different organisms can be similar. Third, specimen for specific bacterial etiology is not easy to obtain. Pneumonia

is an infection of the terminal air exchange units of the lungs. Procedures such as bronchoscopy or broncho-alveolar lavage are rarely carried out in routine clinical practice. It follows that the anatomic differentiation between lower and upper respiratory tract infection is arbitrary and any organism implication is even more speculative. The frequently used terms of lower respiratory tract or chest infection are imprecise and do not help in anatomic or microbiologic characterization of the infection. The microbiology of pneumonia is protean, encompassing viruses, mycoplasma, chlamydia, bacteria, fungi, protozoa and metazoa. Extensive testing with cultures, serology and PCR identify a possible microbiologic cause of pneumonia in only a small proportion of patients (*Claesson et al. 1989' Wubbel L et al. 1999*). The commonly encountered types of pneumonia in routine clinical practice are those caused by viruses, mycoplasma and bacteria. Most pneumonias in young children, including those with radiographic evidence of segmental consolidation, are caused by viruses (*Stashwick et al. 1981, Bettenay et al. 1988*). Epidemics of viral respiratory infections occur throughout the year, RSV and influenza predominate in winter while parainflunezae occurs in summer and autumn (*Martin et al. 1978*). Whereas serologic methods are used to confirm a diagnosis of pneumonia caused by viruses, mycoplasma pneumoniae and chlamydia psittaci, the diagnosis of bacterial pneumonia is usually based on cultures from the blood or the respiratory tract, or on detection of capsular antigens in respiratory secretions or urine. Respiratory pathogens may colonize the upper respiratory tract, which creates problems in interpretation of respiratory secretion cultures. Bacterial antigen detection tests on urine in children who have pnuemonia may have both false-positive (eg, otitis media, recent Hib vaccination) and false-negative results. Juven et al studied community acquired pneumonia in hospitalized children using multiple diagnostic techniques. A causative agent was identifiable in 85% of the patients; 62% viral, 53% bacterial and 30% with concomitant viral and bacterial infections. Streptococcus pneumoniae was implicated in 37% of the cases with a single (0.8%) positive blood culture (*Juven et al. 2000*). Although most pneumonias in infants and children are nonbacterial and do not need antimicrobial therapy, physicians treat more than 80% with antibiotics because of the difficulty in defining etiology, parental and community pressures to use antibiotics,

and the desire not to withhold antibiotics when a potentially serious bacterial infection could be present *(Young et al. 1994)*. Bacteraemia is rare in community-acquired pneumonia in developed countries, occurring in only 1-3% of paediatric outpatient pneumonia cases. However, patients with risk factors, such as immunodeficiency, neonates or hospitalized children or those from the developing countries have higher rates of bacteraemic pneumonia (*Banya et al. 1996*). Bactaeremic pneumococcal pneumonia is characterized by high fever, leucocytosis, and ill appearance, and the majority of patients will have lobar or segmental consolidation (*Toikka et al. 1999*).

Table 1: Diagnoses in 305 febrile infants <60 days of age*

	Number	% of total
Viral illness	256	83.8
Bacterial illness		
Meningitis/Bacteraemia†	11	3.6
Urinary tract infection	7	2.3
Enteric pathogen	8	2.6
Soft-tissue infection	6	2.0
Total bacterial	32	10.5
Other	17§	5.5

*Adapted from *Caspe et al. 1983.*
†Of these 11 patients, two had meningitis and bacteraemia and nine had bacteraemia only.
§Four (1.3%) had pneumonia.

Table 2: Serious illnesses during 996 episodes of acute infectious illness in febrile children <36 months of age*

Diagnosis	Number	%
Bacterial meningitis	9	0.9
Aseptic meningitis	12	1.2
Pneumonia	30	3.0
Bactaeremia	10	1.0†
Focal soft-tissue infection	10	1.0
Urinary tract infection	8	0.8§
Bacterial diarrhea	1	0.1
Abnormal electrolytes, abnormal blood gas	9	0.9
Total	89	8.9

* *McCarthy, 1988.*
†Some studies have noted a mean occurrence of bactaeremia of approximately 4% if the fever is >39°C (102.2°F).
§In some studies, the occurrence of urinary tract infection has been as high as 5% in febrile children.

Table 3: Incidence of invasive infection following bacteraemia*:

Organism	Severe invasive disease (%)	Meningitis (%)
Streptococcus pneumoniae	5.7	3.4
Haemophilus influenzae	22.1	13.9
Neisseria meningiditis	43.5	34.8

*: Steele RW, 2007

Table 4: Organisms isolated from blood in outpatients who have occult bacteraemia*

Organism	Number[1]	Number[2]	Number[3]
S pneumoniae	130	23	164
H influenzae type b	29	2	9
Neisseria meningitidis	5	0	2
Salmonella sp	0	2	7
Others	0	0	10

*: *McCarthy 1995.*
1: Alario AJ et al, 1998

2: Jaffe DM et al, 1991

3: Fleisher GR et al, 1994

Table 5: Incidence of bacteraemia in febrile children by age.*

AGE	Incidence of bacteraemia (%)
0-6 monoths	1.2
7-12 months	7.7
1-2 years	5.9
2-4 years	5.3
>4 years	3

*: Steele RW, 2007

Table 6: Sensitivity and specificity of components of the urinalysis, alone and in combination*.

Test	Sensitivity % (Range)	Specificity % (Range)
Leucocyte esterase	83 (67-94)	78 (64-92)
Nitrite	53 (15-82)	98 (90-100)
Leucocyte esterase or Nitrite positive	93 (90-100)	72 (58-91)
Microscopy: WBCs	73 (32-100)	81 (45-98)
Microscopy: bacteria	81 (16-99)	83 (11-100)
Leucocyte esterase, or nitrite, or microscopy positive	99.8 (99-100)	70 (60-92)

* *Bergman et al. 1999.*

Chapter II

Management of Febrile Children

II. 1. A Continuing Controversy

Despite the myriad of reports and attempts to define criteria that predict serious illness in febrile children, the management of these patients remains a major practice inconsistency within emergency medicine (*Green et al. 1999*). In the early seventies, Mcgowan et al described unsuspected bacteraemia in 4% of apparently well children brought to an urban walk-in clinic who had a temperature of 38.5°C or higher and no focus of infection (*McGowan et al. 1973*). It was then shown that, untreated, approximately 10% of patients with bacteraemia could be expected to acquire serious infectious morbidity, including meningitis (*Long et al. 1994*). Three decades on, paediatricians still differ widely in the evaluation of young non-toxic-appearing febrile children and the controversy continues (*Baraff, 2000*).

When confronted by a febrile young child, who does not look seriously ill and in whom no focus of infection is evident, the clinician must make a series of decisions:

- Whether to hospitalize the child or to observe as an outpatient and what follow-up to arrange if the child is not admitted.
- Whether to perform diagnostic tests to identify an occult bacterial infection.
- Whether to prescribe empiric antibiotic treatment if no focus of infection is identified, and what route, oral or parenteral, to use.

As consensus on these issues remains elusive (*Kramer et al. 1997*), the increasing complexity of medical care and the desire to control costs and improve quality have led to a growing use of clinical practice guidelines. One widely recommended approach, based on meta-analysis of original articles and the opinions of an expert panel, uses risk-stratification algorithms. The recommendations of this approach are summarized as follows:

- All toxic-appearing infants and children and all infants under 28 days of age should be hospitalized for full septic screen and parenteral antibiotic therapy.
- Febrile infants 28 to 90 days of age defined as low risk by specific clinical and laboratory criteria may be managed as outpatients if close follow-up is assured.
- Older children with fever less than 39.0°C without source need no laboratory tests or antibiotics.
- Children age 3 to 36 months with fever of 39.0°C or more and whose white cell count is 15,000/mm^3 or more should have a blood culture and be treated with antibiotics pending culture results.
- Urine cultures should be obtained from all boys 6 months of age or less and all girls 2 years of age or less who are treated with antibiotics.
- Low risk criteria in febrile non-toxic looking infants less than 12 weeks old were defined as:
  1. Previously healthy.
  2. Having no focal bacterial infection identifiable on physical examination.
  3. Having negative laboratory screening:
     i. White blood cell count of 5,000 to 15,000/mm^3.
     ii. Less than 1,500 bands/ mm^3.

iii. Normal urinalysis.

iv. When diarrhoea present, less than 10 WBCs/hpf in stool.

- A sepsis evaluation, when indicated, included:
  1. A culture of CSF, blood and urine.
  2. A complete blood cell and differential count.
  3. Examination of CSF for cells, glucose and protein.
  4. A urinalysis obtained by catheter or suprapubic aspiration.

This guideline (*Baraff, 1993*) was published in the official journal of the American Academy of Pediatrics in 1993, but it was not officially endorsed by the academy or any other professional organization. However, because it was developed by authors who were widely recognized in the field, it was expected to have an important impact on both clinical practice and health care policy. The guideline was meant to help physicians navigate the complexity of medical issues and reduce their uncertainty and perception of liability.

However, years after its publication, there was poor compliance among clinicians across the specialties concerned with the management of febrile children (*Zerr et al. 1999*) and many paediatricians have not found it very helpful (*Christakis et al. 1998*) and it did not seem to have changed the behaviour of many physicians (*Young et al. 1995, Wittler et al. 1998*). Reasons for non-compliance included lack of awareness of the guideline, particularly among family physicians, a perceived negative relationship with the patient's family when obtaining laboratory tests, feeling that their served population was different from those in an academic setting, and distrust for clinical applicability of scientific reports (*Zerr et al. 1999*). Many physicians rejected such a resource-intensive approach in favour of the simplicity of clinical judgment alone and argued that it was based on studies fraught with design and analysis flaws and outdated data (*Manger et al. 1993, Kramer et al. 1997*). Many studies, especially those favouring outpatient antibiotic treatment, were observational which are inherently biased:

- Because they were not randomly assigned, the patients in each group were not necessarily similar.

- Some of the untreated children may have an unrecognized focus at the initial visit e.g. missed pneumonia when no chest x-ray is performed, or red tympanic membranes dismissed as due to crying or viral catarrh.
- Most of the treated children already had identified foci of infection, so the "subsequently" identified infections were fewer in this group.

The experimental studies also had drawbacks:

- Many studies analyzed only bacteraemic patients, which on average constituted only 3% of the patients, and ignored the outcomes in the overwhelming majority of febrile children, with other serious infections.
- Blood cultures are not 100% sensitive and bacteraemia can be intermittent or occur late in the course of a febrile illness, so many serious infections may not have been included in the analysis.
- Some studies included no placebo controls, therefore non-blinding could have influenced the diagnoses and outcome at follow-up.
- Categorization into definite or probable bacterial infections produced intrinsic bias.

The evidence from randomized trials does not demonstrate a benefit of either orally or intramuscularly administered antibiotics in reducing the risk of meningitis or of other serious bacterial infection (*Kramer et al. 1997, Bauchner et al. 1997*). Moreover, these studies have been performed at single institutions and, therefore, their usefulness may differ among different medical centres. There were also major differences among studies that determined risk criteria (see above). These differences, along with the small number of children with serious illness, resulting in wide confidence intervals, added to the difficulty in universal applicability of the low risk criteria (*McCarthy et al. 1994*). As there is no valid mechanism of adjusting for these biases in the absence of compelling evidence, the guideline was considered to reflect the authors' own beliefs and biases (*Schriger et al. 1997*). More importantly, the changing epidemiology of infectious disease, such as the decline in Hib disease and the anticipated reduction in invasive pneumococcal disease, has the largest impact on the validity of the guideline. Nevertheless, the guideline is regarded as an evidence-based structured methodical

strategy that could minimize adverse sequelae from occult infections, save lives and perhaps lower liability risk. It could also partially compensate for the insensitivity of clinical examination (*Green et al. 1999*). The controversy surrounding the guideline may simply reflect different beliefs that rise from different models of the problem, such as hospital vs primary care practice (*Schriger et al. 1997*). The main areas of controversy will now be discussed in some detail.

II. 1. a. Hospitalisation vs. outpatient treatment

Because febrile young children are at risk for potentially life-threatening infections and because findings from the outpatient evaluation may be misleading (*Procop et al. 1997*), a large proportion of these children are admitted to hospital. Infectious diseases are the most common reasons for hospitalization among young children (*Soulen et al. 1994, McConnochie et al. 1995*). This high admission rate represents a major use of resources which might be allocated more appropriately to effective out-patient management of these would-be inpatients. Inappropriate hospitalization is defined as time during which an inpatient fails to receive clinically appropriate services. Several studies have shown that a substantial proportion of child hospitalization is medically unnecessary (*Kemper 1988, Soulen et al. 1994*). A large proportion of children admitted would unnecessarily run the potential risks of hospitalization (*De Angelis et al. 1983*). Obviously, there are factors other than the severity of the medical illness that may affect the decision to hospitalize a child. These factors include the degree of parental anxiety, parental education, distance from a health care facility, whether the family has a telephone or a car, and the physician's intuition about a child or the situation. Lowered thresholds for admission may reflect concerns about the adequacy of home care, or the level of experience of the attending physician.

Demographic factors have their effect on hospitalization rates. For example, the increasing numbers of single parent families and the continued high rates of teenage pregnancy leave little support for children in the community (*Goodman et al. 1994*). These factors can be equally as important as medical pathology (*McConnochoie et al. 1997*). Nevertheless, these factors do not by themselves explain all of inappropriate

hospital use, nor do they solely account for the vast variation in admission rates among institutions serving populations of similar social and ethnic make up (*Perrin et al. 1989*). As an example, the study by Soulen et al showed that only 6% of admissions were identified as social by the admitting physicians (*Soulen et al. 1994*). There is less variation in hospitalization rates among hospitals for conditions in which the diagnosis is certain and admission obligatory, for example bacterial meningitis and appendicitis, reflecting similar prevalence (*Goodman et al. 1994*). Meanwhile, hospitalization practices differ markedly in the face of similar disease severity for illnesses like gastro-enteritis, viral illnesses and fever of unknown aetiology, aseptic meningitis and abdominal pain (*Dershewitz et al. 1983, Perrin et al. 1989*). Furthermore, there is ample evidence that much hospitalization is discretionary, and these admissions account for the majority of the difference in admission rates among hospitals within and between health districts (*McConnochoie et al. 1995*). Factors other than funding mechanisms contribute to inappropriate hospitalization, although the organization of health services and the availability of hospital beds may also influence rates of hospitalization (*Wennberg et al. 1984, Gloor et al. 1993*). The growth of ambulatory care has contributed to the lowering of hospitalization rates (*Gloor et al. 1993*). Good quality follow-up assures both parents and physicians of the safe management of children with avoidable hospitalization conditions (*Flores et al. 2003*).

Hospitalization is the most expensive component of medical care and accounts for a large proportion of expenditure for child health care (*Kemper et al. 1988*). It has been shown that admission rate, not length of stay, is the primary determinant of this cost and of decisions determining the use of hospital beds (*Wennebrg et al. 1984*). Cost-containment efforts directed at limiting the length of hospitalization may therefore not reduce inappropriate hospital use. So, in this respect, admission policies are more important than length-of-stay decisions. Reducing delays in care due to lack of available inpatient beds is an important goal for health authorities. The financial burden on the family is also often overlooked when hospitalization is considered. Costs incurred by the family may include missed workdays, travel expenses, baby-sitting for siblings of the patient, hotel accommodation, and meals. Hospitalization exposes children to the risk of

nosocomial infection (*Dashefsky et al. 1983*) and of iatrogenic events, such as adverse drug reactions and unnecessary invasive procedures (*Startwell et al. 1974, Couch et al.1981, Steel et al. 1981*). As many as 20% of young febrile infants were found to have suffered a complication as a result of hospitalization. An appreciable proportion of these complications occurred in patients who probably did not require hospitalization. In addition to the therapeutic complications, these infants were also subjected to a large number of diagnostic misadventures (*DeAngelis et al. 1983*). Hospital days are threatening to young patients and disruptive of families. Hospital admission in early childhood can lead to acute emotional distress, which may persist for some time after the child returns home (*Douglas et al. 1975*). There is also evidence that prolonged or repeated hospital admission in early childhood can increase the risk of behaviour disturbance or delinquency in adolescence (*Quinton et al. 1976*). Obviously, recovering at home in a familiar environment is a preferable approach, if possible. Thus, every admission that is avoided reduces potential morbidity, mortality, and cost created by nosocomial infections and iatrogenic complications.

One major reason for calling for outpatient management of febrile young children is the relatively low incidence of serious bacterial infection in these patients (*Roberts et al. 1983, De Angelis et al. 1983, Krober et al. 1985, Long et al. 1994, Edwards et al. 1996*). Hence, given the iatrogenic complications and cost of unnecessary hospitalization, most of these children can be safely discharged to home observation provided close-follow up is ensured. De Angelis reported that a third of febrile infants younger than two months of age were managed as outpatients and none of these infants suffered morbidity as a result of not being admitted. The majority of these patients did not even have a complete septic work up (*DeAngelis et al. 1983*). Other studies have shown, however, that unacceptably high rates of infectious morbidity, and mortality, occurred in febrile children not hospitalized on the basis of the outpatient assessment (*Bratton, 1977*). Dashefsky reported that half of episodes of meningococcaemia occurred in febrile patients who were not suspected of having serious invasive disease. A large proportion of these patients suffered significant morbidity, and a few of them died (*Dashefsky et al. 1983*). Similar outcomes were also reported in patients discharged

with an obvious clinical diagnosis such as isolated otitis media (*Schutzman et al. 1991*). This demonstrates an urgent need in improving the accuracy of our assessment of young febrile children, to reduce the cost and complications of unnecessary hospitalizations on one hand, while avoiding the consequences of missing serious disease on the other. Given the inadequacy of clinical assessment alone, there is certainly a need to develop some objective tools to aid in this delicate process.

II. 1. b. Laboratory testing

Screening laboratory tests are used to assist in determining the extent of the diagnostic evaluation and to help decide whether empiric antibiotic therapy should be initiated in patients in whom there is no evident focus of bacterial infection. In a hospital-based outpatient setting, where physicians caring for children have varied levels of experience and where follow up may not be consistent, these tests can be useful adjuncts in the evaluation and management of febrile children (*Bennish et al. 1984*). However, studies examining indicators, such as WBC, ESR and CRP, have shown that these parameters are not helpful in the acute care setting of the emergency department (*Browne et al. 1997*). It follows that the greatest area of controversy is the necessity of performing laboratory studies on the febrile child who appears well and has no abnormalities on history and physical examination to suggest serious illness (*McCarthy et al. 1997*). In this regard, many argue that the guideline, discussed above, reflects a climate of unnecessarily increased and invasive diagnostic testing:

- The diagnostic tests called for are difficult to obtain for many primary care practitioners. In addition, the volume of blood required for these tests and the delay in obtaining results make them impractical.
- The costs incurred in performing these tests are formidable. For instance, routinely performed, individually inexpensive tests are estimated to account for 25% of costs in hospital and ambulatory settings. Of routine laboratory procedures, WBC and electrolytes ranked as high as 2nd and 9th in overall costs (*Gombos et al. 1998*).
- The risk of serious outcomes is too low as to justify the time, expense, and invasiveness of the routine risk-stratification approach. Close follow-up or return

emergency department visits will identify those children who progress to serious bacterial illness.

- Routine performance of these tests may not be justifiable in terms of risk *vs* benefit and the parents` values for these risks and benefits (*Kramer et al. 1997*). It can be argued that parents might not necessarily be willing to tolerate the risk of serious, however rare, sequelae (*Bauchner et al. 1997*). However, it has been demonstrated that parents may prefer less testing and treatment and may be more willing to take a greater risk of rare adverse outcomes (*Kramer et al. 1994, Green et al. 1999*). The risks incurred in performing these tests include the pain and discomfort of the procedure, the waiting time before the procedure is performed and results are available, the need for repeat culture due to contamination or transient bacteraemia, and unnecessary hospitalization and invasive treatment due to false positive urine analysis or to contaminated blood cultures (*Thuler et al. 1997*). Although risk and benefit are not directly comparable i.e. every child tested faces pain while prevention of serious morbidity is extremely rare, physicians and parents may have different values for both the risks and the benefits of testing. Parents emphasize the short-term risks of testing and the possibility of diagnostic error, whereas physicians give greater weight to rarely-occurring serious sequelae (*Kramer et al. 1994*). Therefore the decisions of even the most capable physicians might not be optimal for the children and their families.
- Physicians may obtain diagnostic tests because they believe that testing may protect them from litigation. However, there is no evidence to support this belief and an aggressive approach is not necessarily in the best interests of the patient or the community (*Stamos et al. 1997*).
- The use of tests is largely influenced by practice background and the experience of the physician; ancillary tests are used more frequently by less experienced clinicians (*Zolkowski-Wynne. 1986, Procop et al. 1997*).

A cornerstone of the evaluative algorithm the guideline recommends is blood culture. The value of this diagnostic test has been questioned on several grounds:

- The majority of febrile children who remain febrile do not have persistent bacteraemia even if they did not receive antibiotic treatment at the first visit (*Jaffe et al. 1987, Rothrock et al. 1997*).
- Many children who develop serious infections, such as meningitis, would have done so by the time the blood culture result is available (*Kramer et al 1997*).
- Serious sequelae were mostly due to Haemophilus influenzae bacteraemia (*Barraf. 1993, Shapiro et al. 1993*), which has become rare in the era of routine immunization with Hib conjugate vaccine (*Lee et al. 1998*).
- The prevalence of bacteraemia is at least as high in children with otitis media or pneumonia as in those without a bacterial focus of infection, so testing of unknown infections while not testing of known focal infections is hardly justifiable (*Schutzman et al. 1991*).
- Use of blood culture does not lead to lower rates of subsequent outpatient visits, laboratory tests or treatment (*Kramer et al. 1989*). Furthermore, liberal ordering of blood cultures may necessitate unnecessary re-evaluations and hospitalizations for many false positive results (*Hammerberg et al. 1992*). The sensitivity of blood culture is also not perfect and can be largely affected by the volume and number of blood samples drawn (*Durbin et al. 1978, Kaditis et al. 1996*), by the culture technique (*Kramer MS, 1997*), and by prior antibiotic use (*Pichichero ME, 1979*). Decision analysis of diagnostic management strategies have shown that a "no blood culture" strategy has the highest risk-benefit utility (*Kramer et al. 1989*).

Similarly, it can be argued that catheterization or suprapubic aspiration for urine are unnecessary invasive procedures which could be avoided in the majority of these children (*Dagan et al. 1991*). The specificity and sensitivity of urine analysis is reasonably high (*Kramer et al. 1994*), and can be further improved by enhanced urinalysis (WBC count in a haemocytometer and Gram stain of un-spun urine) (*Hoberman et al. 1996*). However, pyuria might occur in febrile children without a urinary tract infection (*Turner et al. 1995*), and it can be absent in the presence of bacteraemic pyelonephritis (*Kumar et al. 1996*). Children with positive urine culture

with a normal urinalysis might be at little risk of pyelonephritis (*Kramer et al. 1997*). Hence, a negative urine analysis on a clean bag specimen may prove sufficient to obviate the need for urine obtained by a more invasive technique. Although a urine culture from a bag specimen is associated with a high risk of contamination or inconclusive results (*Crain et al. 1990*), a negative result is strong evidence against a UTI. It has been shown that urine culture has a low yield in early onset neonatal sepsis (*Visser et al. 1979*), therefore the risk of suprapubic bladder tap in this group of patients may not be justifiable.

Following the guideline (*Figure 1 & 2*), it was estimated that up to 72% of febrile children will have WBC done for risk-stratification purposes alone (*Green et al. 1999*). Because viral infections are far more prevalent than bacterial infections, the WBC, which is a key determinant in the guideline, has very low predictive powers (8-15%) for occult bacterial infection. Even among the true positives, i.e. those with a high WBC count who do have an occult bacterial infection, most will have an infection that is likely to clear spontaneously (bacteraemia) or to respond to treatment without serious sequelae even if diagnosis is delayed (pneumonia, UTI, cellulitis). Because of their rarity, the positive predictive value for more serious infections (meningitis, osteomyelitis, septic arthritis) is at least several orders of magnitude lower (*Kramer et al. 1997*). Some argue that young children with serious infection can often be accurately identified by the initial clinical evaluation, and that the simplicity of clinical judgment alone might be favoured over laboratory-intensive slide-rule algorithms (*Manger et al. 1993, Long et al. 1994, Green et al. 1999*). It emerges from the above discussion that the role of most tests currently used to identify serious illness in febrile children, and endorsed by the guideline, is questionable.

II. 1. c. **Antibiotic treatment**

Whether to administer antibiotic treatment is one of the major controversial issues in the management of febrile children without a focus of infection. It can be argued that antibiotic treatment empirically administered to these patients is justifiable:

- Non-toxic children with untreated pneumococcal bacteraemia still develop serious sequelae including meningitis (*Bratton et al. 1977, Carrol et al. 1983*).
- Early sterilization of CSF that follows the high serum and CSF levels obtained by a single dose of i.m.ceftriaxone can balance the risks of adverse effects of this therapy (*Bauchner et al. 1997*).
- Expectant antibiotic therapy eradicates bacteraemia, prevents the development of focal complications and reduces fever and improves the clinical condition of the patient. Hence, it is a cost-effective strategy in the management of febrile children at the emergency department (*Carrol et al. 1983, Baskin et al. 1992, Bass et al. 1993, Fleisher et al. 1994, Browne et al. 1997*). The antibiotic efficacy in preventing complications has been estimated to be 75% for meningitis, 95% for persistent bacteraemia, 70% for pneumonia, and 25% for soft tissue infections (*Lee et al. 2001*).
- With a low negative predictive value of the risk criteria that identify serious bacterial infection (*Manger et al. 1993*), a strong case can be made for the use of outpatient antibiotics.
- The lack of previous contact of the physician with the child and his/her family, with consequent uncertainty about the adequacy of parental surveillance and compliance with follow-up may necessitate aggressive treatment in the accident and emergency department.

On the other hand, many claim that antibiotic treatment is unwarranted for the majority of febrile children for the following reasons:

- The estimated risk of bacterial meningitis following bacteraemia is approximately 10% and is predominantly pathogen related, being lowest with S. pneumoniae, intermediate with H. Influenzae and highest with N. meningiditis (*Long et al.1994*) (*Table 3*). The Hib vaccine has virtually removed the worry of occult bacteraemia due to this organism. Streptococcus pneumoniae, which now causes more than 95% of occult bacteraemia, is an unusual cause of meningitis in childhood, and affected patients rarely had a history of febrile "well" period when intervention might have taken place (*Long et al. 1994*).

- The efficacy of expectant single dose or short-course antibiotics in prevention of meningitis, and other serious infective sequalae, is not proven (*Rohrock et al. 1998*).
- When antibiotics are administered empirically, it is no longer possible to judge the child's clinical state, need for more therapy or evolution of a partially masked focal infection. Interpretation of the results of CSF analysis can be particularly difficult. All urinary tract infections would be mistreated, masked, or missed.
- Compliance among children given oral antibiotics in an outpatient setting is poor (*Sunakawa et al. 1995*).
- In addition to the substantial financial and human costs, antibiotics have predictable as well as idiopathic side effects.
- The routine use of antibiotics selects for more resistant organisms and is an important risk factor for infection with such organisms, including salmonella and penicillin-resistant pneumococci (*Edwards et al. 1996*).
- The practice of treating fever with antibiotics encourages fever phobia and the substitution of automated management for thoughtful assessment, individualized management and close follow-up.

It is thought that the influence of studies of febrile children seen in emergency departments that reported on occult bacteraemia was a contributing factor in the liberal use of antibiotics and the emergence of resistant organisms (*Long et al. 1994*). Consumer satisfaction is also a factor as families may choose practitioners who readily prescribe antibiotics. With time constraints on practitioners, it takes less time to prescribe than to explain the differences between bacterial and viral infections. Nevertheless, performing no tests and giving no antibiotics for the febrile young child is perfectly defensible, and preferable (*Long SS, 1994*) and emphasis should be on individual clinical assessment and close follow up. Telephone follow-up or return visits, repeated observation over time at the initial visit or admission for a short period are likely to be more helpful than diagnostic testing and blind treatment. Nonetheless, the decision to observe should be made on a positive basis; if a suitable combination of characteristics in the illness, infant, care giver, and physician does not permit a confident decision to observe, then treatment is the appropriate choice (*Roberts et al. 1983*). It

would appear that rejection of expectant therapy for children without focal disease is as extreme as the recommendation for all febrile children to be treated expectantly (*McCarthy et al. 1994, McCarthy et al. 1997, Bauchner et al. 1997*).

Several changes in the epidemiology and management of occult bacteraemia and its complications have prompted the reevaluation of these guidelines (*Figure 1&2*). These include; i) the near elimination of Hib as a paediatric pathogen by immunization, ii) the rising incidence of penicillin-resistant pneumococci, iii) additional studies on the incidence of bacteraemia and utility of diagnostic tests, iv) increased concerns over testing and empiric treatment, v) continued variability in the use of the guidelines, vi) ongoing controversy among experts regarding the optimal approach to the febrile child, and vii) the introduction of the conjugate pneumococcal vaccine. Nevertheless, the controversy surrounding the management of febrile young children still continues (*Stoll et al. 2004*).

It is evident that the management of the young child with fever remains a formidable challenge and comprehensive evaluation of such a common problem can be costly, invasive, and not without risk. From the several strategies that have been proposed for the management of febrile young children, none has been universally adopted. The controversy in management has been fuelled by studies involving varying populations with respect to age, definition of fever, and varying clinical and laboratory criteria defining high or low risk for SBI. Different definitions of fever without source and SBI have further complicated the selection of children for various management strategies. Large studies have demonstrated the futility of relying on the appearance of the young child in determining the source of infection, and there is no single laboratory test that has been shown to reliably identify young children at high or low risk of having serious bacterial illness. Establishing early and accurate markers of infection could help rationalize antibiotic practices. Diagnostic tests may be characterized by their cost, sensitivity, and specificity. Initially, age and other clinical features define prevalence or a prior probability of SBI. The test result then divides patients into a higher or lower probability of the illness. This probability depends not only on the sensitivity and specificity of the test, but also on the prior probability of SBI among the children being

tested. Furthermore, management strategies will vary depending on the epidemiology of illnesses in the population. For instance, if the rate of bacteraemia in a population is >1.5%, then performing a diagnostic work-up at the initial visit may be considered cost-effective. At a lower rate of bacteraemia of 0.5%, clinical judgment, for instance using YOS, can be a cost-effective option and strategies that use empiric testing and treatment could be avoided (*Lee et al. 2001*). Because of the variation in prevalence of SBI between different settings, generalization of study findings may not be appropriate. However, knowing the local prevalence (pre-test probability) of disease enables a clinician to estimate the post-test probability using likelihood ratio of a test (which is independent of prevalence) generated by well designed studies. It is therefore essential to determine the prevalence of SBI in the population in whom the test is intended to be used.

Figure 1: Algorithm for the management of a previously healthy child (3 to 36 months) with FWS.

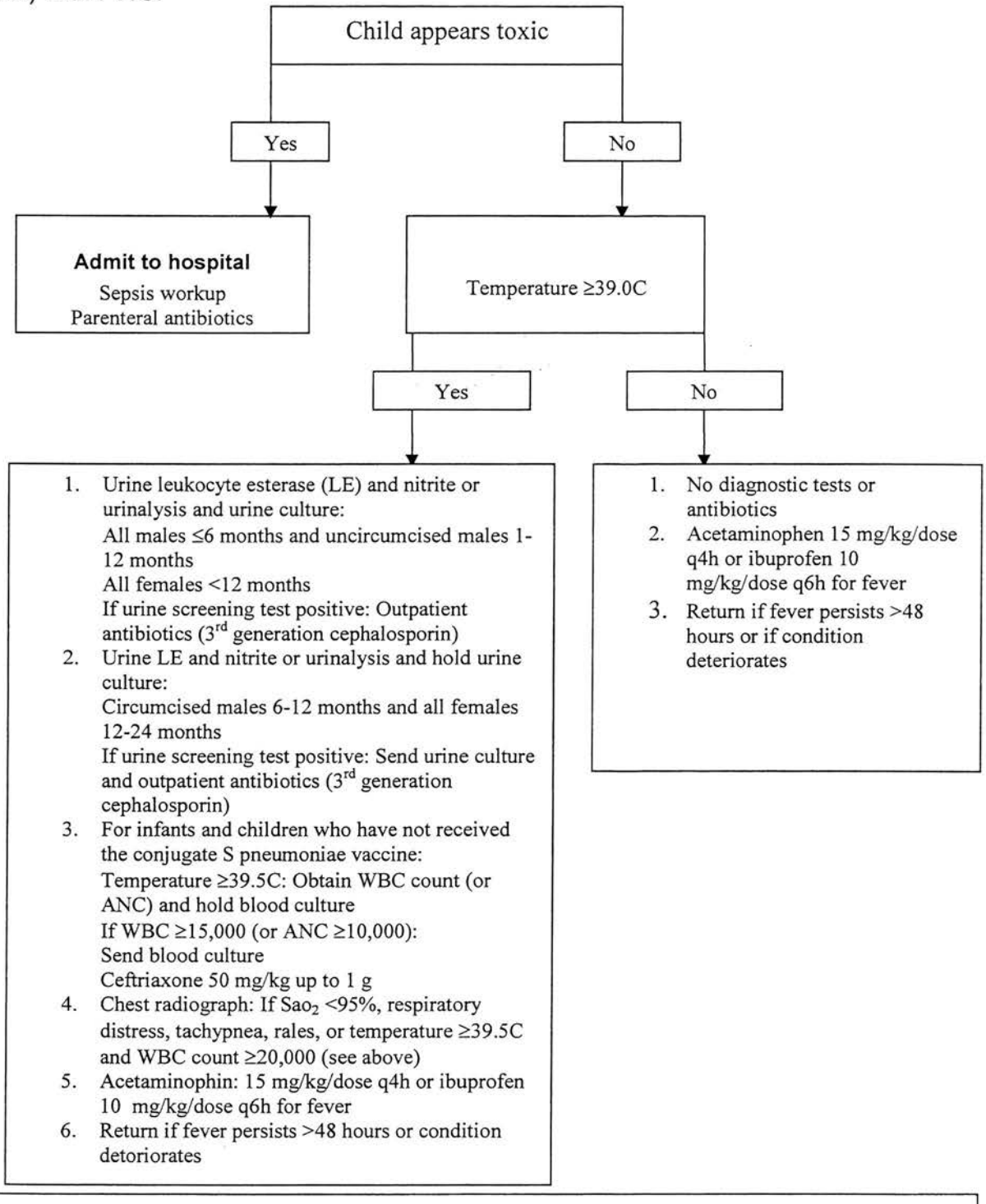
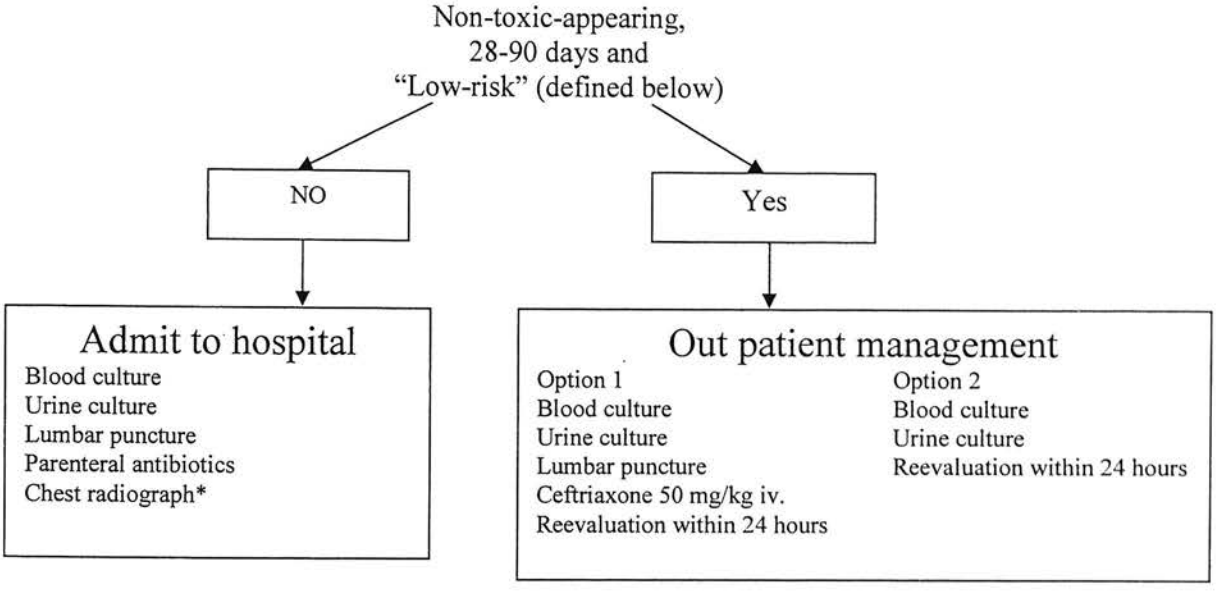


Figure 2[ϕ]: Algorithm for the management of a previously healthy infant (birth to 90 days) with FWS with a temperature of 38.0°C (100.4°F) or greater.



*Chest radiograph if signs of pneumonia: respiratory distress, abnormal breath sounds, tachypnoea, pulse oximetry <95%.

Follow-up of low-risk infants treated as outpatients with positive culture results:

Blood culture positive (pathogen): Admit for sepsis evaluation and pareneteral antibiotic therapy pending results.

Urine culture positive (pathogen): Persistent fever: Admit for sepsis evaluation and parenteral antibiotic therapy pending results.

Outpatient antibiotics if afebrile and well.

Low-risk criteria for febrile infants:

Clinical criteria:

- Previously healthy, term infant with uncomplicated nursery stay
- Nontoxic clinical appearance
- No focal bacterial infection on examination (except otitis media)

Laboratory criteria:

- WBC count 5-15,000/mm^3, <1,500 bands/mm^3, or bands/neutrophil ratio <0.2
- Negative Gram stain of un-spun urine (preferred), or negative urine leukocyte esterase and nitrite, or <5 WBCs/hpf
- When diarrhoea present: <5 WBCs/hpf in stool
- CSF: <8 WBCs/mm^3 and negative Gram stain (option 1 only)

[ϕ]Adapted from Baraff LJ, Management of Fever without Source in Infants and Children. Ann Emerg Med. 2000; 36(6): 602-615.

Chapter III

The Evaluation of the Febrile Child

Clinical Evaluation

One simple approach in the assessment of a febrile child is to pay careful attention to history and examination, meanwhile observing the child's responses to the environment. A systematic approach (*Figure 3*) can then be used in the clinical and laboratory evaluation of such a child.

III. 1. Observation Scales

History and physical examination are essential steps in the evaluation of the febrile child. However, findings from the initial evaluation can be of limited value or even misleading, for the following reasons:

1. Many children present with fever as the sole manifestation. In a private paediatric practice, a clinically identifiable source of infection, mostly otitis media, could be found in only half (52%) of toxic appearing highly febrile infants (*Schwartz et al. 1982*).
2. A febrile illness in a young child may be attributed, mistakenly, to a mild abnormality such as redness of the tympanic membrane due to crying (*Schutzman et al. 1991*). Furthermore, acute otitis media and upper respiratory tract infection

are frequently associated with underlying serious bacterial infection including meningitis and bacteraemia. (*Teele et al. 1975, McCarthy et al. 1976, Schutzman et al. 1991*)

3. Many children with serious illness, such as meningitis, do not always look seriously ill i.e. toxic. In one study, an attending-level physician judged that 67% of infants with SBI appeared to be well, including 63% and 82% of bacteraemia and UTI respectively (*Bonadio et al. 1994*). A review of 10 years experience in a large hospital revealed that nearly one half of episodes of meningococcaemia occurred in children who were not suspected of having serious, invasive disease (*Dashefsky et al. 1983*). Furthermore, attributes many physicians take as signs of well being, such as the presence of a social smile, have not been found useful in ruling out serious bacterial infection such as bacteraemia or meningitis (*Bass et al. 1996, Rogers et al. 1995*).
4. Classic symptoms and signs of disease, which are usually derived from more ill inpatients, are the exception rather than the rule. For instance, fever and earache, two symptoms described in paediatric textbooks as classical manifestations of otitis media, were absent in 31% and 40%, respectively, of 302 children younger than 4 years with proven otitis media (*Heikkinen et al. 1995*).

It would, therefore, be useful to derive a set of sensitive and specific, preferably objective, clinical criteria for predicting serious bacterial infections in febrile children at the time of initial evaluation in order to focus invasive and costly efforts at diagnosis, treatment and aggressive follow up appropriately and economically. Because a young febrile child with a serious illness often does not manifest classical physical examination findings suggestive of that illness, physicians usually rely on their instinctive judgment in assessing the seriousness of a child's illness. Because overall judgments, such as ill, questionably ill, or well, are considered oversimplifications and too vague, attempts were made to quantify this instinctive judgment from observational data gathered in an objective manner. One of the most widely used scales is the Acute Infant Observation Scale developed at the Yale-New Haven Hospital in New Haven Connecticut.

III. 1. a. Yale Observation Scale

McCarthy et al developed and applied a 6-point clinical assessment system for febrile children. The system, based on a series of studies on children ≤36 months with a fever ≥38.3°C, has come to be known as the Acute Infant Observation Scale, or the Yale Observation Scale (YOS). History and observation variables on which physicians based their instinctive clinical judgment of overall degree of illness of a febrile child, i.e. toxicity, were identified. These variables were scored on a 4-point ordinal scale, from 1 for normal to 7 for severe impairment. Pairs of house officers and attending paediatricians assessed and scored the patients. The sensitivity, specificity, and predictive values of the scoring system in detecting serious illnesses were, 57%, 76%, and 20% respectively (*McCarthy et al. 1980*). Observation rather than history was found to be more important in a paediatrician's judgment.The inter-observer agreement in scoring was "only fair". The limited usefulness of the scale in identifying serious illnesses was, in part, related to:

- A single one-off evaluation, for which either serial clinical evaluation or supplementation by screening laboratory tests were recommended.
- Poor inter-observer agreement, possibility due to:
  1. Ill-defined terminology of the variables especially those with complex behavioural components such as playfulness.
  2. Varied levels of training and experience.
  3. Scoring on a seven-point scale instead of dichotomous scoring.

McCarthy and his colleagues further perused the observation variables that physicians used to assess the overall severity of illness in a febrile child. Variables relating to eye function, response to stimuli, and motor activity correlated best with overall assessment and the occurrence of serious illness. The sensitivity, specificity and predictive value of the scale, based on these well-defined variables, were 70%, 79% and 29% respectively. There was more inter-observer agreement (88%) than in the previous study, possibly related to a gain in familiarity with the use of the scoring system (*McCarthy et al. 1981*). In their third report, the authors defined and validated the Yale Observation Scale. Observation items associated with serious illness in the previous studies were selected to

construct a three-point scale (*Appendix* 1). A model based on these items had 88% specificity, 77% sensitivity, and a positive predictive value of 92.3% for serious illnesses. The sensitivity of the model combined with history and physical examination was 92%. The inter-observer agreement (97%) was greater than before (*McCarthy et al. 1982*). This improvement was due to the use of more defined scale points in place of vague terms, and to a higher prevalence of serious illness in the later population (15.8% vs. 6.1-9.1%). The model was cross-validated by applying it to different subgroups of the same population and comparing its performance to that in the whole study population. This study showed that the chance of serious illness in an ill-appearing child (YOS >10) was 40%, and 2-3% in a well appearing (YOS<10) febrile child.

The YOS was used as a means of determining the likelihood of serious illness before physical examination and/or investigations (*a priori probability*) in a probability reasoning to aid clinical decision making in the management of febrile children. A cut-off point of 10 on the scale classified the patients as ill or well appearing. History and physical examination were scored as to whether they did or did not suggest a serious illness. Ill-appearing patients had greater occurrence of physical examination findings suggesting a serious illness (64%) than well appearing children (15%). The probability (PPV) of serious illness in ill-appearing children rose from 40% to 79% and from 3% to 25% in well-appearing ones, by the addition of physical examination. History findings did not have a significant contribution (*McCarthy et al. 1985*). The same authors studied the efficacy of YOS, combined with history and physical examination, in identifying serious illness in febrile children. The sensitivity of history and physical examination for serious illness was 78%. This was increased to 92% by the addition of the observation scale. The false negative rate for the items combined, was 14% (*McCarthy et al. 1987*).

The Yale Observation Scale has been developed as a means of adding accuracy to the more traditional methods of history and physical examination, in detecting serious illness in young febrile children. However, the scale failed validation when applied to different populations and, therefore, has not enjoyed wide acceptance as an aid in clinical decision making. When applied to a group of infants 4-8 weeks old, the scale had a sensitivity, specificity, and predictive value of 46%, 78, and 45%, respectively, in

predicting serious illness. 57% of infants with culture proven illness appeared well, and 12% of well-appearing infants had either a culture-proven bacterial illness or pneumonia (*Baker et al. 1990*). In a large number (6611) of a selected group of young febrile children who were non-toxic looking and had no identifiable focus of infection, the sensitivity, specificity, and positive and negative predictive values for a YOS score >10 in detecting occult bacteraemia were 5.2%, 96.7%, 4.5%, and 97.1% respectively. The difference in YOS score between bacteraemic and non-bacteraemic children was not clinically useful as the overlap in appearance between the two groups was too great (*Teach et al. 1995*). In the series reported by Baker et al, 66.2% of children with serious illness scored 10 or less, placing them in low-risk category on the basis of appearance alone (*Baker et al. 1993*). More recently, a study of young febrile children revealed that 69% of bacteraemic patients had the lowest possible score of 6 in YOS (*Kuppermann et al. 1998*). The differences in the findings between these studies are in part due to:

1. Differences in the prevalence of serious illness.
2. Inclusion, in some studies, of patients with overt focal infection, or toxic appearance.
3. Systematic observer bias resulting from assessment by several physicians, in different institutions, with different levels of experience.
4. Failure to allow for the use of antipyretics before assessment.
5. Small sample size of some studies.

III. 1. b. Other Scoring Systems

A different scoring system, based on functional status and clinical judgment, was used by another group of workers to predict bacteraemia in young febrile children. The functional status was obtained from the parent's record of the child's eating, drinking, sleeping, and play activity. It was scored on a 0-2 scale resulting in a composite score of 8 that indicated optimal function (*Appendix* 2). After physical examination, the physician predicted the existence of bacteraemia using an arbitrary scoring system: The physician's assessment was the most predictive factor, with a predictive value 14%, specificity 83%, and sensitivity 47%. Both functional status and WBC were poorly

predictive. Combination of findings did not improve the utilized values. It was concluded that history and physical examination should determine the need for further tests in febrile young children (*Waskerwitz et al. 1981*). Schwartz et al used yet another different scoring system utilizing both historical and observational variables to gauge the degree of toxicity (*Appendix 3*). Children with a score of 2+ or higher were considered toxic. 12% of toxic-appearing febrile children had occult bacteraemia, compared to none of the non-toxic appearing children. All the six bacteraemic children were identified as toxic. The sample size of this study was too small to make conclusions more than recommend obtaining a WBC and a blood culture in a toxic-appearing child (*Schwartz et al. 1982*). A severity index using a 0-1-2 point score for the variables (respiratory effort, skin colour, activity, temperature and ability to play) that were predictive of serious illness was derived and applied to a group of febrile children. The index had a predictive accuracy for non-severe illness of 98.7% with a false positive prediction rate of 15.8%. The index when used as a triage tool could be helpful in selecting febrile children who should receive blood cultures (*Nelson et al. 1980*). These later scales have not been validated by further studies.

III. 2. SBI risk (Rochester) Criteria

A major goal in the evaluative process of febrile children is to maximize the sensitivity and negative predictive value of the outpatient evaluation while minimizing the cost and risk of testing, hospitalization and antibiotics. Therefore, studies have stressed the importance of selected criteria in evaluating febrile children (*McCarthy et al. 1976*). Many risk factors have been examined, including age, temperature, existence of a chronic illness, the presence of focal signs of infection and preliminary laboratory tests e.g. WBC, erythrocyte sedimentation rate, CRP and a variety of methods of clinical evaluation (*Jaffe et al. 1991*). Based on these data, the physician can make informed decisions about the need for more definitive laboratory tests, therapy, or advisability of hospital admission. One widely accepted approach is the risk stratification method whereby patients are categorized into those with high or low probability of having a serious illness.

In 1982, Dagan et al introduced the Rochester Criteria that identified young children unlikely to have a serious bacterial infection (*Dagan et al. 1985*). These criteria were based on the experience of the authors and earlier studies (*Figure 4*). Using these criteria, 62% of the patients seen at the emergency department would be at low risk of having a serious illness. Less than 1% of these patients will have a SBI compared to 25% of the high risk group. The incidence of bacteraemia would be negligible compared to the 10% incidence in the high risk group. The negative predictive value of the low risk criteria for SBI was estimated to be 99.3%, far beyond that of the traditional risk factors, such as age, sex, and temperature, clinical symptoms and signs, or laboratory findings. These criteria were successfully applied to select young infants for outpatient treatment (*Dagan et al. 1988, McCarthy et al. 1990, Baker, et al. 1993*). However the Rochester criteria were not always successful when applied to different populations. The negative predictive values were found too low for clinical use, which meant that several infants with SBI would be assigned to the low risk group (*Anbar et al. 1986, Avner et al. 1993*). Others reported enhanced predictive power when other laboratory tests, such as ESR and CRP, were added to the criteria (*Chiu et al. 1994*). The discrepancies in the findings of these studies can be attributed to differences in:

- The study design, either retrospective or prospective.
- Inclusion criteria:
  1. Perinatal events.
  2. Previous health and recent antibiotic treatment.
  3. Hospitalized infants and infants managed as outpatient.
  4. Eligibility in terms of parental ability to provide home care.
  5. Appearance of the infant, whether ill or well. Application to a population including ill appearing infants (with a higher prevalence of SBI) would result in a lower negative predictive value (*Jaskiewicz et al. 1994*).
- Definition of serious bacterial infection:
  1. Microbiological definition of meningitis and other infections.
  2. Inclusion of superficial skin infection and otitis media.
- Definition of low risk criteria:

1. Spectrum of the septic screen done, e.g. CSF examination.
2. Inclusion, and definition, of stool WBC count.
3. Radiological infiltrates.

- Differences in patient referral pattern.
- Small sample sizes.
- Different geographic sites, e.g. the absence of group B streptococcal infection in the group reported by Dagan et al from Israel.
- Non-uniformity in subjective assessment.
- Differing aspects of health care delivery, exemplified by a lower rate of hospitalization in Rochester compared with other hospitals in the same county.

As discussed earlier, a variety of methods of clinical evaluation of febrile children have been developed but it remains that unremarkable or minimally abnormal clinical findings do not rule out serious bacterial disease (*Procop et al. 1997*). Therefore, various combinations of clinical and laboratory parameters, particularly haematological variables, have been used as rapid ancillary tests in the diagnosis of serious bacterial disease. Bonadio et al found a higher incidence of SBI with higher temperatures and higher WBC count. Although the physical appearance was insensitive in detecting bacteraemia and UTI, all cases of meningitis were predictable on clinical assessment alone (*Bonadio et al. 1994*). In their study of febrile children younger than three years of age, Kupperman et al reported that the absolute neutrophil count, temperature and age younger than 2 years were predictive of occult pneumococcal bacteraemia (*Kupperman et al. 1998*). Other workers found that CRP, duration of fever, clinical impression score, history of diarrhoea, and the presence of focal signs were independent predictors of SBI (*Berger et al. 1996*). Banya et al reported that a constellation of clinical findings that included a high temperature, dehydration, grunting, and physical signs of consolidation were good predictors for a positive blood culture. The authors concluded that use of these criteria in selecting children for obtaining blood culture would have reduced the number of blood cultures taken by 55.6% (*Banya et al. 1996*). In a more recent study, Bachur et al reported a 26% incidence rate of occult pneumonia in children <5 years with a temp>39C and WBC>20^9/L. The authors recommended routine CXR in this

group of children even in the absence of symptoms or signs of pneumonia (*Bachur et al. 1999*). Leventhal et al used a constellation of physical signs to categorize patients with fever, or respiratory symptoms, into high and low risk groups for pneumonia. These criteria were more reliable than the classical signs of pneumonia i.e. fever, cough, and rales, in detecting pneumonia (*Leventhal et al. 1982*). A predictive model consisting of age, height of temperature, urinalysis and WBC had a negative predictive value of 98.3% and 99.6% for SBI and bacteraemia and/or meningitis (*Bachur et al. 2001*). Some other studies found that the development of bacterial meningitis was strongly associated only with the species of bacteria but not with any other clinical or laboratory characteristics identifiable at the initial visit (*Shapiro et al. 1986*).

Figure 3: Clinical evaluation of the young febrile child*.

1. Assessment of the urgency of the situation: In certain circumstances, management decisions and actions need immediate institution before establishment of definitive diagnosis. Signs of severe life-threatening infections should be sought early in the evaluation of the febrile child. For instance, the complaint or observation that a child's crying increases with parental attempts to comfort or fondle (paradoxical irritability) is an important sign of meningitis in infancy (*Manger et al. 1993*). Also altered sensorium and focal neurological signs are indicators of CNS infections. Drooling and a sniffing position are signs of upper airway obstruction. Dyspnoea, cyanosis and pallor, tachycardia and hypotension are signs of cardiopulmonary compromise as seen in septicaemia. The characteristic haemorrhagic rash of meningococcaemia should be actively searched for.
2. Observational assessment: In less urgent cases, a period of careful observation, with the child comfortably placed on the parent's lap, helps the physician gauge the overall toxicity of the child. Observation should include the child's alertness, his / her responsiveness to persons and objects, respiratory status and colour, feeding, and age-appropriate motor function.
3. Looking for a possible focus of infection: Sites of common childhood infections e.g. ears, throat, joints etc. should be thoroughly examined. Gentle palpation of the anterior fontanelle and abdomen and auscultation of the chest should be performed.
4. Assignment to a low or high risk for a serious infection: Because of the acknowledged difficulty in the outpatient evaluation of young febrile children, a commonly adopted approach is to classify febrile children into toxic and non-toxic looking. Toxic-appearing children are automatically admitted for sepsis work up and observation and/or parenteral antibiotic therapy. Non-toxic looking patients are stratified into high or low risk for serious infection. Factors that help in categorization include; age, height of temperature, and the total and differential white blood cell count (*see text*).

*Adapted from Henretig, 1998.

Figure 4: The Rochester criteria identifying children at low risk for SBI.

- Patient appears generally well
- Patient has been previously healthy:
    1. Born at term
    2. Did not receive perinatal antimicrobial therapy
    3. Was not hospitalized longer than the mother
    4. Was not treated for unexplained hyperbilirubinaemia
    5. Had not received and was not receiving antimicrobial agents
    6. Had not been previously hospitalized
    7. Had no chronic or underlying illness
- No evidence of skin, soft tissue, bone, joint, or ear infection
- Laboratory values:
    1. Peripheral blood WBC count (5.0 to 15.0×10^9 cells/L)
    2. ≤10 WBC /hpf on microscopic examination of a spun urine sediment
    3. ≤10 WBC /hpf on microscopic examination of a stool smear only if diarrhoea.

Chapter IV

The Evaluation of the Febrile Child

Laboratory Evaluation

IV. 1. **Ancillary tests**

Tests commonly performed as part of the evaluative process of febrile children include chest X-Ray and urine and CSF analysis, especially since urinary tract infection and pneumonia can be surprisingly "silent" in young children (*Krober et al. 1985*). However, there can be difficulties in their interpretation, and the sensitivity and specificity of each test are far from perfect (*McCarthy et al. 1977*). Chest radiograph interpretation, in particular, is hardly an exact science as it is a clinical judgment that may result in disagreement among radiologists. The inter-observer variability of radiologists is considerable and can be biased by the clinical information and the preliminary interpretation of the treating physician (*Leventhal et al. 1982, Green et al. 1999*). The commonly reported pulmonary infiltrates are not helpful in deciding upon a causative organism, whether viral or bacterial. They are also neither specific nor sensitive for positive blood culture. It has been demonstrated that children with otitis media are more often bacteraemic than those with pneumonia (*McCarthy et al. 1976*). Examination of CSF is routinely performed in febrile young infants and in older children who manifest signs of meningeial inflammation. Because microbiological examination

of CSF is not always instantly available, initial diagnosis of meningitis usually rests on the cell count of CSF. Aseptic meningitis with isolation of virus from CSF often occurs in the absence of CSF pleocytosis which, if present, can vary widely in numbers and can initially be predominantly polymorphic (*Krober et al. 1985*). On the other hand, some bacteraemic children can have aseptic lymphocytosis in the CSF (*McCarthy et al. 1976*). CSF culture is more sensitive than the cell count as a test for bacterial meningitis in patients who have not been taking antimicrobials (*Shapiro et al. 1986*). Furthermore, a significant association between the performance of lumber puncture at initial presentation and the subsequent development of meningitis has been demonstrated (*Woods et al. 1990*). The pros and cons of urinalysis in febrile children have been extensively reviewed in the medical literature (*Krober et al. 1985, Bonadio et al. 1994, Jaskiewicz et al. 1994, Chiu et al. 1994*) and will be discussed further in subsequent sections. Apart from these organ and organism-specific tests, there are other tests in clinical use, which indicate the presence of tissue injury and inflammation and may help in the diagnostic or prognostic evaluation of febrile illness. These tests largely represent various components of the acute phase reaction.

IV. 2. **WBC and Differential Count**

The peripheral white blood cell (WBC) and differential WBC counts have been used for over a hundred years to help discriminate acute infectious and non-infectious disease (*Todd et al. 1974*). These simple and relatively inexpensive laboratory tests have become a routine part of the evaluation of unexplained fever and they are widely used as part of health supervision in paediatric patients (*Moyer et al. 1990*). The neutrophil is the leucocyte most intimately involved in the host defence against bacterial infection. During many systemic infections the bone marrow overcompensates to replenish the marginating neutrophil pool, which has migrated to tissues, and results in neutrophilia. This neutrophilia is often accompanied by increased numbers of immature neutrophils which is called a left shift. This shift is quantified by determining the absolute band count, band/segmented neutrophil ratio, band/total neutrophil ratio, and immature/total neutrophil ratio. If the infection is extremely severe or the host is already compromised,

the bone marrow fails to compensate for the loss in central and marginal neutrophil pool and neutropenia results. The characteristic WBC pattern that often accompanies bacterial infection consists of neutrophilia and eosinopenia with or without a left shift. As the infection resolves, the number of neutrophils decreases and the number of monocytes increases. This is followed by relative or absolute lymphocytosis and return of eosinophils (*Weitzman et al. 1975*).

The WBC count is an inexpensive, easily available test. However, its usefulness in diagnosis of serious bacterial illness is limited for many reasons:

1. Several physiologic and non-pathologic factors can strongly influence the interpretation of the WBC. (*Table 7*).
2. Leucocytosis, with or without left-shifted myelopoiesis, is a non-specific response to almost any disease. For instance, WBC counts in excess of 50,000/ mm^3 have been demonstrated in vasculitides and connective tissue disorders and profound leucocytosis has also been described in some viral infections (*Morens et al. 1979*).
3. WBC fails to respond as expected in some instances of bacterial disease (*Procop et al. 1997*).
4. There is no uniformity in criteria used for the proper interpretation of abnormal values, due partly to the difficulty in making a definitive aetiologic diagnosis in many cases of apparently infectious illness (*Stein et al. 1972*).
5. It has been demonstrated that 50-75% of the variance in leucocyte differential counts is not attributable to disease, but to variability in sampling, in counting, and in observers (*Moyer et al. 1990*).
6. The differential count must be performed manually in many laboratories, and even when automated, 30-40% of the counts must be repeated manually because of suspected abnormalities. This undermines the cost-effectiveness of this test because the potential for cost-savings by reduction in laboratory use is greatest for labour-intensive tests (*Moyer et al. 1990*).
7. The published normal ranges for WBC may not be clinically relevant. Benuck et al demonstrated a whole range of sensitivities (29%-94%) of published neutrophil criteria in identifying a set of 34 culture proven neonatal sepsis (*Benuck et al. 1983*).

8. The WBC, and its differential, is a multi-part test, which results in up to 30% of any group of patients to have at least one value outside the 95% range.
9. WBC counts from different vascular sources may make the interpretation of these numbers difficult. It has been demonstrated that venous and arterial blood counts were 82% and 77%, respectively, of capillary blood value (*Liu et al. 1985*).
10. Normal ranges are relatively broad, and different values have been cited for different days of life (*Akenzua et al. 1974*).
11. An abnormal WBC and differential count does not usually have inherent diagnostic meaning that is independent of clinical findings at the time of evaluation at the emergency department (*Crain et al. 1982*).
12. Several factors relating to the infectious process may modify the accompanying WBC picture:
    i. The infecting micro-organism: pyogenic bacteria often result in a more pronounced neutrophilia than other bacteria. Typhoid fever, pertussis, and tuberculosis are typically not associated with neutrophilia. Scarlet fever and brucellosis are associated with eosinophilia. WBC may not be elevated in gram negative infections (*Lee et al. 1998, Kuppermann et al. 1998, Dashefsky et al. 1983*).
    ii. Localisation of infection tends to result in greater WBC count than septicaemia.
    iii. Severity of infection: with many exceptions, the more severe the infection, the higher the neutrophil count.
    iv. The age of the patient: neutrophil counts are less reliable indicators of infection in the first few days of life (*Kunnamo et al. 1987*).

The correlation between both serious bacterial disease and culture positivity and an abnormal haematological profile is well established (*Table 8*). Some authors have even extrapolated the etiologic agent, its virulence, and the severity of disease from the type of haematological abnormality and therapeutic decisions are frequently made on the basis of WBC count. However, while many of these studies show acceptable positive predictive values for both leucocytosis and differential WBC count, others have emphasized the drawbacks to the use of haematological variables to predict serious

bacterial disease. It is difficult to rely on criteria developed from these studies for many reasons. The subjects in these studies were a heterogeneous group, with different disease prevalence and clinical severity. The definition of serious bacterial disease was not uniform across the studies. The interpretation of WBC morphology and degenerative changes and the cut-off values of neutrophil and band cell counts and ratios were also different (*Stein et al. 1972, Todd et al. 1974, Morens et al. 1979, Chiu et al, 1984, Jaffe et al. 1991, Bonadio et al. 1994, Berger et al. 1996, Procop et al. 1997, Lee et al. 1998, Gombos et al. 1998*).

A prototype of studies evaluating the role of WBC in detecting occult bacterial infection was that done by Kramer et al who studied a large cohort of febrile young children seen in a paediatric emergency room. The gain in the probabilities of meningitis, UTI and pneumonia attained by a high WBC (>15,000/mm^3), was negligible. It was argued that the incidence of meningitis in a child without the symptoms and signs of this disease was so low that even doubling its probability by a high WBC was unlikely to alter clinical management. The predominantly viral origin of most pneumonias, the lack of morbidity associated with its gross under-detection and the relative ease of obtaining a CXR undermine the value of a WBC in the detection of pneumonia. The increased probability of UTI resulting from a high WBC is also obviated by the less invasive, equally sensitive, and much more specific urinalysis. It was argued that persistence of fever and the development of new signs or symptoms have far greater differential diagnostic value than WBC (*Kramer et al. 1993*). Other leucocyte-associated tests used for detection of bacterial infection are nitroblue tetrazolium test and acridine orange leucocyte cytospin tests (*Kite et al. 1988*). It would seem that most of these parameters would add little to the post-test probability in situations of diagnostic uncertainty. Furthermore, in patients with a high pre-test likelihood of serious illness, a haematologic profile would be of little use because an abnormal result would be expected and a normal result would be ignored.

IV. 3. The Acute Phase Response

Patients with inflammation, such as infection, develop a number of systemic changes collectively called the acute phase response, even though these changes accompany both acute and chronic inflammatory disorders. Acute phase changes may be divided into changes in the concentrations of many plasma proteins, and a large number of behavioural, physiological, biochemical, haematological, and nutritional changes. This response is mediated by cytokines, such as interleukin 6 (IL-6), interleukin-1 (IL-1) and tumour necrosis factor (TNF), which are synthesized by macrophages and lymphocytes, that accumulate at the site of tissue damage. Neuroendocrine changes reflecting action of inflammation-associated cytokines include the production of ACTH and AVP (which account for hyponatraemia during some inflammatory disorders) by IL-6. Behavioural changes that often accompany inflammation, including anorexia, somnolence, and lethargy, are similarly induced by cytokines. Nutritional effects include anaemia and cachexia induced by cytokines in chronic disease. The presence of any of the components of the acute phase response provides unequivocal evidence of tissue injury. However, a wide range of stimuli can produce cellular or tissue injury, or stimulate macrophages, resulting in the systemic response. Examples of these stimuli are:

1. Chemical or physical trauma
2. Chemical, toxic, or allergic inflammation
3. Infection; bacterial, viral, fungal or parasitic
4. Ischaemic necrosis
5. Malignant neoplasia.

Measurement of the acute phase response may thus be used for either detecting organic disease, assessing the extent or activity of that disease, monitoring response to treatment, predicting outcome, or detecting intercurrent infection. In paediatrics, the main value of tests for detecting and monitoring the acute phase response is as an early indicator of bacterial infection in circumstances where a microbiological diagnosis is impossible or delayed (*Stuart et al. 1988*).

IV. 3. a. ***Fever***

Fever is the representative of the neuroendocrine changes that characterize the acute phase response. The hypothalamic thermo-regulatory centre maintains normal core body temperature at a set point of 37°C (98.6°F) with a narrow range of 1-1.5°C. Fever is an elevation of body temperature mediated by an increase of the hypothalamic heat regulatory set-point. Body temperature follows a circadian rhythm; early morning temperature is low, with the highest level occurring between 16.00 and 18.00 hrs. This circadian rhythm is apparent from early infancy and is most prominent in mid childhood. It follows that defining fever in children is a complex issue but most would agree that any rectal temperature of 38°C or higher constitutes a fever (*Manger et al, 1993, Barraf. 1993*). The increased heat production associated with fever increases oxygen consumption, carbon dioxide production, and cardiac output. Thus it may exacerbate cardiac insufficiency in patients with heart disease or chronic anaemia, pulmonary insufficiency in those with chronic lung disease, and metabolic instability in children with diabetes mellitus or inborn errors of metabolism. Furthermore, children between 6 months and 5 years are at increased risk of febrile seizures and those with idiopathic epilepsy may have increased frequency of seizures as part of a non-specific febrile illness. Fever by itself, except under unusual circumstances, is not beneficial to the host response to infection (*Barraf. 1993*).

Fever occurs when various infectious and non-infectious processes react with the host defense mechanisms. Non-infectious causes of fever include:

1. Vaccines.
2. Tissue injury (burns, infarction, i.m. injections).
3. Malignancy.
4. Drugs (drug fever, cocaine, amphoteracin B).
5. Immunologic-rheumatologic disorders (SLE, rheumatoid arthritis).
6. Inflammatory diseases (IBD).
7. Granulomatous diseases (sarcoidosis).
8. Endocrine disorders (thyrotoxicosis, phaeochromocytoma).
9. Metabolic disorders (gout, uraemia, Fabry`s disease).

10. Unknown or poorly understood entities (familial Mediterranean fever).
11. Factious (self-induced): Either by intentional manipulation of the thermometer or injection of pyrogenic material. The diurnal variation of temperature is usually preserved in patients with febrile illnesses. When this circadian rhythm is maintained and is associated with tachycardia, chills (rigors), and sweating, a true rather than factitious fever should be suspected.

Regardless of the aetiology, the final pathway of most common causes of fever is the production of endogenous pyrogens, which then directly alter the hypothalamic temperature set-point resulting in heat generation and heat conservation. Cytokines, especially IL-1, IL-6 and TNF, released in response to immune and non-immune stimuli, are the main endogenous pyrogens. The sequence of cytokine generation in response to exogenous pyrogens, with subsequent hypothalamic prostaglandin E2 production, may take 60-90 min (*Manger et al. 1993*). However, cytokines are not the sole inducers of fever; the observation that subdiaphragmatic vagotomy blocks fever after intraperitoneal (but not intramuscular) injection of lipopolysacharide implicates neural transmission in the febrile response (*Franklin et al. 1999*).

Fever is the most common sign of illness in infants and young children, and highly febrile (103.0°F rectal) children represent about 20% of all sick patient visits (*Wright et al. 1981*). In most children, fever is either due to an identifiable microbiologic agent or subsides after a short time and it may be categorized as follows:

1. Fever of short duration with localizing signs for which the diagnosis can be established by clinical history and physical examination, with or without laboratory tests.
2. Fever without localizing signs, for which the history and physical examination do not suggest a diagnosis but laboratory tests may establish an aetiology.
3. Fever of unknown origin (*Arvin et al. 1996*).

In most infectious processes the fever pattern is of little diagnostic importance, although the fever associated with malaria, Hodgkin's disease and cyclical neutropenia may indicate the underlying condition. In some instances, such as diarrhoea (*Victoria et al. 1990*) and bronchiolitis (*El Radhi et al. 1999*) the presence of fever could be an

indicator of the severity of illness not necessarily associated with bacterial infections. The most important role of high fever in children is its role as a sign of serious infectious illness. Mild elevations of temp (37.7-38.3°C) are common in the first few (6) months of life, accounting for approximately 20% of infant visits. Higher degrees of fever (temperature >38.3 °C) are uncommon in this age group but are seen more frequently with each succeeding month (*Pantell et al. 1980*). There seems to be an emergency regulatory mechanism in fever that sharply limits the temperature at levels of about 41.1°C (106°F), hence the rarity of hyperpyrexia in infections. In one study, only 0.048% of emergency room patients had such a temperature (*Dolan et al. 1976*). However, fevers in this range and higher indicate not only bacteraemia, but also possible CNS infection, or pathologic hyperthermia (*McCarthy PL, 1997*). A positive correlation between the height of fever and the rate of serious bacterial infection has been established (*Teele et al. 1975, McCarthy et al. 1977, Leventhal et al. 1982, Banadio et al. 1990, Schutzman et al. 1991, Bonadio et al. 1994*). The incidence of bacteraemia increases in a stepwise fashion with increasing temperature (*Table 9*). The lack of response to antipyretics, such as acetaminophen, has also been found to indicate an increased risk of serious bacterial infection (*Mazur et al. 1989*). This has been challenged by other workers who found that neither a fall in temperature, nor changes in clinical appearance after fever reduction could predict the absence of serious bacterial infection (*Baker et al. 1987, Tiller et al. 1988, Baker et al. 1989*).

IV. 3. b. **The acute phase plasma proteins**

An acute phase protein (*Table 10*) has been defined as one whose plasma concentration increases or decreases by at least 25% during inflammatory disorders (*Pepys et al. 1983*). The magnitude of the increases varies from about 50% in the case of ceruloplasmin and complement components to as much as 1000 fold in the case of CRP and serum amyloid A (*Gabay et al. 1999*). Inflammation causes substantial changes in the plasma concentrations of acute-phase proteins while moderate changes occur after strenuous exercise, heatstroke, and childbirth, and small changes occur after psychological stress and in several psychiatric illnesses (*Gabay et al. 1999*). The

changes in the concentration of these proteins are largely due to changes in their production by hepatocytes. Several cytokines are capable of inducing acute phase production in hepatocytes. IL-6 seems to be the most important because of the number of acute phase proteins whose synthesis it influences as well as the magnitude of the changes it produces (*Geiger et al. 1988*). Studies have shown that the synthesis of CRP by human hepatocytes is dependent on IL-6, but not on IL-1 or TNF, and that CRP is a direct indicator of IL-6 levels in humans in vivo (*Bataille et al. 1992).*

These plasma protein responses presumably have beneficial overall functions concerned with restricting injury and promoting resolution and repair. For instance, caeruloplasmin scavenges superoxide produced by activated neutrophils and fibronectin non-specifically promotes phagocytosis. The antioxidants haptoglobin and hemopexin protect against reactive oxygen species. Both α_1-protease inhibitor and α_1- anti-chymotrypsin antagonize the activity of proteolytic enzymes and inhibit the generation of superoxide anions.

The variety of tests available to monitor the acute phase response have led to controversy over which tests should be used. Individual acute phase proteins vary not only in the rate at which their blood concentration rises after the onset of inflammation, but also in the half-life and rate of catabolism in different forms of inflammation. CRP, SAAP and α_1- anti-chymotrypsin rise within 6-10 hours of injury, show large incremental increases of 10-1000 fold over their normal values, have short half-lives, and behave consistently in different forms of inflammation. On the other hand, orosomucoid, α_1-anti-trypsin, haptoglobin, and fibrinogen rise after 24-48 hours, rarely increase more than two fold, have long half-lives and, with the exception of orosomucoid, show variable catabolism in some diseases. Haptoglobin and α_1-anti-trypsin have common genetic variants which result in their deficiency. C3, C4 and caeruloplasmin generally show poor and inconsistent acute phase responses. Different organisms may initiate different patterns of response in acute phase proteins. For instance, E. coli gives the most reliable increase in CRP, whereas the response in early onset group B streptococcal infection is less predictable (*Philip et al. 1984*).

IV. 3. b. i. C-reactive protein (CRP)

Of the useful acute phase proteins, CRP is the most easily measured and commercial assay kits are widely available. CRP was discovered in 1930 and derives its name from its calcium-dependent reactivity with pneumococcal C-polysaccharide. CRP belongs to the pentaxin family, so named because of their pentagonal appearance in the electron microscope. This group of proteins also includes serum amyloid A protein which shares a common structural configuration and considerable homology with the CRP molecule. Human CRP has a molecular weight of 110 kDa and consists of 5 subunits each with 187 amino acids, with considerable α- helix. This differs from immunoglobulins, which do not contain α- helix. CRP specific human DNA has been isolated and its gene has been mapped to chromosome 1 (*Philip et al. 1984*).

CRP is synthesised by hepatocytes (*Aleksander et al. 1984*) and there is no evidence for its production by any other cell type, although surface CRP has been found on certain lymphoid and phagocytic cells (*Nudelman et al. 1983*). Cytokines, especially IL-6 and IL-1 are the main inducers of hepatic synthesis of CRP, and prostaglandins may also be involved. In serum, CRP is associated with VLDL and LDL fractions. Following production in the liver, CRP localizes at the site of tissue injury or inflammation. For instance, CRP has been detected in CSF of patients with meningitis and in the joint fluid of patients with rheumatoid arthritis (*Nudelman et al. 1983*). Local deposition has also been noted in chronic vasculitis. The in-vivo half-life of CRP is 19 hours, the shortest among most human plasma proteins, and its fractional catabolic rate is independent of the plasma CRP concentration. The synthesis rate of CRP is thus the only significant determinant of its plasma level, confirming the validity of serum CRP measurements as an objective index of disease activity in disorders associated with an acute-phase response. In contrast, the concentration of other acute phase proteins, such as the clotting factors, complement proteins, proteinase inhibitors, and transport proteins, is dramatically affected by the activation and/or complex formation involved in their physiological functions (*Vigushin et al. 1993*). After the onset of inflammation, CRP synthesis increases within 4 to 6 hours, doubling every 8 hours thereafter, and

peaks at 36 to 50 hours. Levels remain elevated with ongoing inflammation, but with resolution they decline rapidly with a half-life of 4 to 7 hours (*Jaye et al. 1997*). The mechanisms of its clearance are not known, although 90% of injected radioactive CRP has been recovered in the urine (*Vigushin et al. 1993*). It was shown that its peak levels correlate with the severity of tissue damage and that this level depends on the duration of increased CRP production rather than upon the initial rate of rise. Anti-inflammatory drugs lead to a decrease in CRP production.

The ligand that CRP binds best is phosphorylcholine, which is a constituent of C-polysaccharide. CRP, bound to target ligands, can activate the classical complement system and facilitates phagocytosis by opsonising target substances and it also enhances NK cell activity (*Pepys et al. 1981*). CRP and complement components are the only acute phase proteins directly involved in the elimination of microorganisms. Although CRP has proinflammatory properties, its net effect is anti-inflammatory. CRP can prevent the adhesion of neutrophils to endothelial cells by decreasing the surface expression of L-selectin. It can also inhibit the generation of superoxide by neutrophils and stimulate the synthesis of IL-1R antagonist by mononuclear cells (*Gabay et al. 1999*). It efficiently blocks phosphocholine-containing inflammatory mediators, such as platelet activating factor, and may block phospholipase activity which provides substrates for the cyclo- and lipooxygenase pathways (*Vigushin et al. 1993*). There is some evidence that the prognosis of neonatal infection is better if CRP level is initially elevated (*Nudelman et al. 1983*). Hence, CRP has been suggested as a potential tool for therapy and prophylaxis (*Philip et al. 1984*). Binding of CRP with heparin, histones and myelin basic protein suggests that it has a direct role in clearing necrotic host tissue (*Jaye et al. 1997*).

Methods of measuring CRP were originally based on the ability of serum to precipitate the C-fraction polysaccharide (*Quellung* reaction*)*. Then followed the c*apillary precipitin and latex fixation agglutination.* These semi-quantitative tests are easy to perform, economical and rapid. However, they are less sensitive, with a limit of detection greater than 10 mg/l. The capillary test is difficult to read if the serum has a high lipid content or is haemolysed. In the latex test, undiluted serum with high CRP can

result in a false negative result (prozone effect) and high titres of rheumatoid factor also can give false positive results. After biochemical characterization of CRP in 1978 and development of specific monoclonal antibodies, a wide array of sensitive quantitative methods evolved. As much of the value of measuring CRP lies in serial monitoring of the onset or resolution of inflammation, sensitive quantitative assays are much more useful. Techniques that give the required rapid results include *Laser-beam nephelometry* (1.5 hour assay time), or *rate immuno-nephelometry, and turbidometry* (0.25-0.5 hour): These sensitive (~0.04 mg/l) assays depend on the Tyndall's phenomenon of light scattering property of particles in suspension. Lipaemic serum may interfere with CRP determination. Nephelometry requires only 50 µl of serum, which is obtainable by finger prick. Even more sensitive and specific assays are the immune assays which include *Enzyme immuno-assay* (0.25 hour), *Radial immunodiffusion* (60-72 hours) and *electro-immunodiffusion* (60-90 hours) (*Komoroski et al. 1987*). These detect as little as 0.3 ug/dl and correlate well with rate immuno-nephelometry (*Gill et al. 1981*). A most sensitive test is a solid phase ligand-binding, *radiometric monoclonal antibody immunoassay,* which is capable of reliably detecting concentrations in the normal range not usually detectable by conventional methods (*Wasunna et al. 1990*). A *fluorescent antibody technique* detects CRP localized in tissues. Automated chemistry analyzers are beginning to offer CRP in their test menus. CRP is normally present in trace amounts in the blood of healthy individuals (*Nudelman et al. 1983*). Using sensitive assays, CRP was identified in the serum of normal, healthy individuals in paediatric and adult populations, at ≤10 mg/l (*Jaye et al. 1997*). CRP shows quantitative differences in different individuals, which may be genetically determined (*Pepys et al. 1983*). Different cutoff values were used in research. Quoted upper limits of CRP varied from 8.0 mg/l in newborns (*Felix et al. 1966*), to 29 mg/l in adults (*Wasunna et al. 1990*). Each laboratory should establish its own normal ranges for CRP, based on specific patient populations and the method used.

CRP response in disease

CRP is the classical and dramatic acute phase reactant with levels rising from a median normal of <1 mg/l to as much as 300 mg/l or more within 24-48 hours of an acute stimulus.

- Conditions associated with *major* elevations of serum CRP include:

1. Infections
2. Allergic complications of infection: Rheumatic fever, erythema nodosum leprosum
3. Inflammatory disease: Rheumatoid arthritis, systemic vasculitis, psoriatic arthritis, Crohn's disease
4. Ischaemic necrosis: Myocardial infarction
5. Trauma: Surgery, burns, fractures

- Conditions associated with *minor* elevations of serum CRP include:

1. Systemic lupus erythematosus
2. Scleroderma
3. Dermatomyositis
4. Sjögren's syndrome
5. Ulcerative colitis
6. Leukaemia

Clinical applications of CRP

- Screening for organic disease
- Monitoring the extent and activity of disease: Infection, inflammation, and necrosis
- Detection of inter-current infection.

The non-specific nature of the acute phase response coupled with the relatively crude and poorly standardized semi-quantitative assays have caused clinical CRP tests to fall out of favour. Earlier methods of CRP assay were either insensitive (e.g. latex agglutination slide test), required long assay time (e.g. radial immunodiffusion), or were technically difficult (e.g. electro-immunodiffusion). With the advent of precise immuno-chemical assays there has been renewed interest in the value of CRP measurements in clinical practice. CRP is a precise reproducible test, and it is a sensitive objective index of disease activity. Although a high CRP is not necessarily due to the same process for

which the patient is being investigated, an elevated serum CRP concentration is unequivocal evidence of an active tissue-damaging process, and thus provides a simple screening test for organic disease. Since CRP is a non-specific response, a CRP value on its own can never be diagnostic. However, in conjunction with other clinical data, it can make a valuable contribution to important clinical decisions.

Compared to ESR, CRP has many advantages:

1. CRP levels respond much more rapidly with a much wider incremental range (100 μg/l to >300 mg/l in 2-3 days) while ESR changes within a much smaller range over a period of days or weeks.
2. ESR does not show the same sensitive response to infection nor the same discriminant difference between diseases like rheumatoid arthritis and SLE.
3. ESR is often not performed correctly so that results may not be reproducible.
4. There is a significant diurnal variation in ESR associated with food intake (*Pepys et al. 1983*).
5. In afebrile children with different forms of malignancies, CRP levels are within the same range of that observed in healthy children (*Santolaya et al. 1994*). Hence, any rise indicates an intercurrent infection.
6. CRP can be run quickly and requires a small aliquot of serum, obtainable by fingerprick.

Opinions vary on which serum CRP levels should be used as cut-off index in categorizing the severity, and aetiology, of inflammatory responses. Typical serum concentrations suggested are; mild inflammation and severe viral infection, 10-40 mg/l; active inflammation and bacterial infection, 40-200 mg/l; and severe bacterial infections and burns, 200-400 mg/l. However, there is no absolute serum concentration of CRP that is diagnostic of bacterial infection, although 40 mg/l has been claimed to give higher specificity, with acceptable sensitivity, than 20 mg/l. Sensitivity values vary between studies according to the level of CRP considered, the definition of infection used, and the time at which blood was taken in the course of the disease. Specificity also depends on whether patients with obvious non-infective pathology were excluded and on whether serial measurements were made. CRP peaks within 48 hours and with remission of

disease it falls at the same rate. When clinical history is less than 24 hours, CRP is the acute phase protein of choice for its rapid response (6-10 hours) and large incremental increase. The elevated circulating levels of CRP are expected to fall by up to 50% per day when the acute phase stimulus resolves, thus it is useful in monitoring the resolution of inflammation (*Vigushin et al. 1993*).

CRP is used as a diagnostic aid in a variety of clinical conditions:

1. In conditions where it is not usually significantly raised such as leukaemia, systemic lupus erythematosus atosis and scleroderma, CRP can be a sensitive sign of inter-current infection.
2. Serial CRP determination may be of help in the post-operative follow up of children requiring shunts for hydrocephalus. It has been shown that an absence of a fall in CRP levels, 7-10 days post-operatively, provides an early warning sign of complications, such as infection or thrombophlebitis (*Fischer et al. 1976*).
3. Serum CRP levels can aid in the differential diagnosis of arthritis in children (*Kunnamo et al. 1987*) and its levels in joint fluid may be useful in distinguishing inflammatory from degenerative or traumatic arthritis (*Nudelman et al. 1983*). CRP was also found useful in the identification of concurrent septic arthritis in children with acute haematogenous osteomyelitis (*Unkila-Killo et al. 1994*).
4. CRP is helpful in differentiating Crohn's disease from ulcerative colitis.
5. CRP was found to be the most consistently abnormal acute-phase reactant in the presence of rheumatic fever activity, particularly carditis. It has been used as a guide in ambulating clinically inactive patients with rheumatic fever since the ESR can be elevated 1-8 weeks after CRP has returned to normal *(Nudelman et al. 1983*).
6. Slightly elevated concentrations of CRP, within the range (2-10mg/l) in normal subjects, have been found to predict subsequent coronary events (*Gabay et al. 1999*).
7. It has been claimed that CRP can be helpful in differentiating pyelonephritis from cystitis (*Wientzen et al. 1979*). These findings have not been confirmed (*Hellerstein et al. 1982*) and this distinction may not be of clinical use.

8. CRP was found to be a reliable and early predictor of infectious morbidity, and thus aiding in the selective management of patients with premature rupture of membranes (*Evans MI, 1980*).
9. Measurement of CRP is of particular value as an aide in differentiation of invasive bacterial infection from viral infection. In general, CRP elevations in invasive bacterial disease tend to be in the range of 150 to 350 mg/l. Invasive bacterial infection without marked CRP elevation is unlikely, but may be encountered if an early sample is taken. In contrast, CRP values in most acute viral infections tend to be much lower, <20-40 mg/l. The greater CRP response in bacterial compared to viral infections may be related to the extracellular existence of bacteria, resulting in more tissue damage, and as a non-specific response to the components of bacterial cell wall. However, this distinction is not absolute, as values of >100 mg/l can occur in certain viral infections, such as adenovirus (*Clarke et al. 1983*). Although CRP cannot be used to classify the precise microbial aetiology of an infection, it may be of some diagnostic value in conjunction with other tests and physical findings in certain clinical settings:
    i. In the neonate, CRP has been found to be a useful discriminative test for bacterial infection (*Corral et al. 1981, Schouten-Van Meeteren. 1992*). However, because there may be a lag between the onset of infection and the demonstration of a rise in serum levels of CRP, some investigators have dismissed this test as unhelpful in neonatal infection (*Mathers et al. 1987*). Some workers found that the majority of infected babies had a delayed rise in CRP concentration, 12-24 hours after an abnormal immature/total neutrophil ratio was first detected (*Pulliam et al. 2001*). Furthermore, other neonatal problems not resulting from infection, such as asphyxia, foetal distress, PROM, maternal fever and meconium aspiration, can also be associated with elevated levels of CRP (*Ainbender et al. 1982*).). It is also difficult to apply normal ranges in newborn babies because of the varying rates of maturity of CRP synthesis after birth (*Kebler et al. 1994*). Another difficulty is that some infants seem incapable of responding with an acute phase response (*Philip et al. 1984*). Because these infants usually died, it

was thought that CRP may have a prognostic value in neonatal infection, with a low CRP implying a poor prognosis (*Pulliam et al. 2001*). Nonetheless, CRP has been found useful in decreasing antibiotic use in neonatal intensive care units (*Philip et al. 1981*), and in guiding the duration of antibiotic treatment in newborns with suspected infection (*Stephan et al.* 1982). Serial measurements can optimise sensitivity and enhance the negative predictive value in deciding to stop or withhold antibiotic therapy (*Massroor et al. 1993, Pourcyrous et al. 1993*). Sensitivity of CRP elevation in early detection of neonatal sepsis varied from 47 to 100%, with specificity from 6 to 97% (*Jaye et al. 1997*). Such wide ranges are the result of using different patient selection, sample timing and method, test methodology, cut-off values and infection definition.

ii. CRP was found predictive of lobar infiltration and discriminated viral from bacterial pneumonia (*McCarthy et al. 1978*). However, high serum CRP concentrations are frequently recorded in viral respiratory infections, especially those due to adenovirus (*Ruuskanen et al. 1985)* and little value of CRP is found in differentiating pneumococcal or other bacterial pneumonia from viral pneumonia (*Korppi. 2004*).

iii. CRP was found useful in the rapid differentiation of acute epiglottitis from spasmodic croup and acute laryngotracheitis (*Peltola et al. 1983*). CRP could differentiate children with streptococcal pharyngitis from those who were carriers (*Nudelman et al 1983*).

iv. High levels of serum CRP differentiated bacterial from viral acute otitis media (*Komoroski et al. 1987, Tejani et al. 1995*), and predicted recurrence of otitis (*Del Beccaro et al. 1992*).

v. CRP levels completely separated septic meningitis from aseptic (viral) meningitis and helped follow the course of the disease (*Peltola et al. 1982, Clarke et al. 1983*). CRP levels in CSF were also useful in the initial work up of children with suspected meningitis (*Corral et al. 1981*). In other studies however, CSF levels of CRP failed to add to the information routinely obtained

(i.e. Gram stain, cell count, glucose and protein) in predicting the presence of bacterial infection (*Alistair et al. 1983*).

vi. In febrile neutropenic children, CRP clearly discriminated bacterial from non-bacterial infections (*Santolaya, 1994*).

vii. Quantitative CRP concentration is a valuable laboratory test in the evaluation of febrile young children who are at risk for occult bacteraemia and SBI, with a better predictive value than the WBC or ANC (*Pulliam et al. 2001*). Other workers claim that CRP was costly and offered no advantage over WBC and ANC in screening febrile children for occult bacterial infection (*Isaacman et al. 2002*).

IV. 3. b. ii. Erythrocyte Sedimentation Rate (ESR)

This indirect test of acute phase response measures the distance in millimetres that erythrocytes fall during one hour. It depends on the aggregation of red blood cells and formation of rouleaux, which is determined by: i) red cell characteristics, ii) plasma viscosity, and iii) the bridging forces of macromolecules, such as plasma proteins, which lower the negative charge on the red blood cells. Different plasma proteins, mainly fibrinogen and immunoglobulins, contribute to this phenomenon. The bridging force of a plasma protein is proportional to its molecular weight; the heavier a protein, the more red cells aggregate. Thus, fibrinogen is the strongest aggregator, gamma globulins with half the aggregating strength, and albumin the weakest. The relative contributions of different plasma proteins to ESR are; fibrinogen 55%, α-2-macroglobulin 27%, the immunoglobulins 11%, and albumin 7% (*Stuart. 1988*). Direct measurements of fibrinogen are unsatisfactory because of considerable overlap of value between diseased and healthy persons (*Powell et al. 1995*).

Many methods are used to measure ESR, the Westergren method being the earliest and the gold standard. A standard 200 mm glass tube is used and the normal values are taken as less than 16 mm for men and less than 25 mm for women. The second most commonly used method is the Wintrobe method, in which a 100 mm tube is used. The normal rates are <6.5 and <16 mm for men and women, respectively. This

method is more sensitive than the Westergren method in the mildly elevated range of the sedimentation rate. However, it is relatively insensitive in the high ranges and may therefore give misleadingly low results. Both methods can be affected by mechanical, environmental, and technical factors; i) tube dimensions (a large bore tube or a tall column will lead to an increase in ESR); ii) temperature, maximum rouleaux occurs at 37°C; iii) a tilted surface can increase the rate; iv) preformed rouleaux in a blood sample that is not well mixed will increase the rate, v) a clotted blood sample will consume fibrinogen and lower the rate, vi) low rate in long kept blood sample (>2 hours), and vii) vibration, e.g. from a centrifuge, will inhibit settling and lower the rate. ESR is also strongly influenced by anaemia (increases ESR) and polycythaemia (false low rate) and this cannot be corrected.

Almost any disease can lead to an elevation of the rate, so that its value is considered more as a *sickness index* than as a diagnostic tool. Diseases commonly associated with a highly elevated ESR include malignancy, collagen vascular disease, and infection. Any infection may raise the ESR, but bacterial infections do so more commonly than viral infections. Some infectious diseases, such as pelvic inflammatory disease, meningitis, and infective endocarditis, are characteristically associated with a highly elevated ESR. On the other hand, there are many disease states which are associated with a low ESR: i) abnormal cell shapes (e.g. hereditary spherocyctosis) and haemoglobinopathy, such as sickle cell disease, ii) abnormality of plasma protein such as hypofibrinogenaemia (e.g.neonatal, herediatry, DIC), hyperproteinaemia (e.g. Waldenstrom macroglobulinaemia), iii) Drugs such as anti-inflammatory agents, corticosteroids and asparaginase, and iv) congestive heart failure.

Because it is a manual procedure, ESR quality control is unsatisfactory. Measuring ESR takes at least one hour and should be performed within two hours after blood collection, and it requires relatively large blood volume so is not suitable for paediatric use. Plasma viscocity is analogous to ESR as it reflects the concentration of the same proteins as ESR. Plasma viscosity can be measured within minutes and on stored samples. Unlike ESR, its result is independent of anaemia, red cell characteristics (low ESR in sickle cell anaemia), or gender and is largely independent of age (*Stuart.*

1988). Plasma viscosity can be done from a finger/heel prick and is therefore more suited for paediatric use but automated viscometers are not widely available. An increase in plasma fibrinogen concentration takes 24-48 hours and the concentration subsequently decreases with a half-life of 4-6 days. Thus measuring ESR, and plasma viscosity, is not valuable for monitoring rapid changes in the onset or resolution of the acute phase response. They do however also respond to increases in immunoglobulins and immune complexes and other proteins and thus cast a broader net for the detection of disease than does any other acute phase protein alone. They are less affected by acute events, such as intercurrent viral infections, that cause a transient increase in sensitive acute phase proteins such as CRP.

ESR is probably the most widely used index of acute phase response and has served as a simple laboratory test for decision making since it was introduced in the 1920s (*Stuart J, 1988*). It is cheap, easy to perform, and does not require a power supply, equipment or much technical skill. It is especially attractive for small laboratories, primary health clinics, and countries with limited resources. Nonetheless, compared to CRP, ESR has several disadvantages as a measure of the acute phase response. ESR is an indirect measurement of plasma acute-phase proteins and can be greatly influenced by the size, shape and number of erythrocytes, as well as other plasma proteins such as immunoglobulins. As the patient's condition worsens or improves, ESR changes relatively slowly, whereas plasma CRP concentrations change rapidly. The range of abnormal values for CRP is broader than that of ESR changes, with accompanying clinical implications. Furthermore, ESR increases steadily with age, while plasma CRP concentrations do not. Before the advent of sensitive quantitative CRP tests, ESR performed best in predicting bacterial infection in febrile paediatric patients and was thus recommended in the evaluation of these patients (*McCarthy et al. 1978*). Compared to the more traditionally used screening tests (e.g. WBC indices), ESR was favoured as an indicator of bacteraemia in febrile children (*Bennish et al. 1984*). Some studies have demonstrated that an elevated ESR can also be consistent with non-invasive bacterial infections, such as acute otitis media (*Del Beccaro et al. 1992*).

IV. 3. b. iii. Other acute phase proteins

Examples of acute phase proteins evaluated as diagnostic tools for detecting bacterial infections include:

Elastase-α_1- proteinase inhibitor complex: Elastase, a proteinase secreted by polymorphic neutrophils, is neutralised by complexing with α_1- proteinase inhibitor (α_1-anti-trypsin). Elastase-α_1- proteinase inhibitor complex was demonstrated to be a sensitive marker of sepsis in the newborn. However this test lacked specificity, as both obstetric and neonatal complications were associated with high levels (*Kebler et al. 1994*). Reference ranges of E-α_1-PI have been developed for the neonatal period (*Rodwell et al. 1992*). Elastase plasma levels in whole blood from patients with sepsis, incubated with lipopolysacharide, were increased by up to 188% above normal, while the release of TNF, IL-1 and IL-8 was markedly decreased. This suggests that other mediators or mechanisms may be involved in the up-regulation of detrimental protease release during sepsis (*Ertel et al. 1994*).

Complement products: The complement cascade is activated directly by bacteria and antigen-antibody complexes, and the degree of complement activation could, therefore, provide early and specific evidence of bacterial infection. C3d, the end product of the C3 complement component activation via both the classical and alternative pathways, was found to be as reliable as CRP in the diagnosis of neonatal bacterial infection, with a sensitivity and specificity of 70% and 97.7% respectively (*Guillois et al. 1994*). The value of Ba, (activated factor B from the altenative pathway), C4d, and C3d in the early detection of neonatal infection has been studied. The highest sensitivity (47.1%) and specificity (92%) were those of Ba. Complement C9 was also reported to be more sensitive than CRP (*Peakman et al. 1992*).

Orosomucoid (α_1-acid glycoprotein): Some workers have found normal serum levels of orosomucoid in healthy full-term and pre-term infants and no elevation in infants with non-infectious disease. There was a marked increase from the early stages in infants with bacterial infection, levels returning to the normal range with clinical improvement (*Gotoh et al. 1973, Sann et al. 1984*).

Fibronectin: This is a large opsonic glycoprotein, which promotes reticulo-endothelial system clearance of bacteria, and its levels are reduced in sepsis. An acute reduction in the concentration of plasma fibronectin has been found to be a valuable marker of sepsis in neonates and young children (*Gerdes et al. 1983, Koenig et al. 1988*).

Haptoglobulin: Contradictory results on the usefulness of haptoglobulin in detecting bacterial infection have been reported (*Speer et al. 1983*).

These acute phase proteins have certain disadvantages that hamper their routine clinical use as indicators of infection; overlapping values for normal and diseased persons, prolonged doubling time and/or half-life, problems with standardization of assays, and elevations in response to trivial inflammation, such as the common cold. Some proteins, such as haptoglobin and alpha-1-antitrypsin can be actively catabolised in some disease states and may be deficient in some inheritable conditions.

IV. 4. Other Markers

Other markers used to detect bacterial infections include:

Leucocyte alkaline phosphatase activity: Low levels of this enzyme have been reported in infected newborn infants and this was suggested as a helpful aid in the diagnosis of neonatal bacterial infections (*Donato et al. 1974*).

Leucocyte lactate dehydrogenase: Elevated levels of leucocyte LDH were found in infants with bacterial meningitis and it was suggested that this test could also be helpful in detecting serious bacterial infection (*Powers et al. 1974*).

MxA protein: It has been demonstrated that elevated levels of MxA protein (an interferon inducible gene product) in blood lymphocytes could discriminate between viral and bacterial infections in febrile children (*Halminen et al. 1997*).

Polymerase chain reaction (PCR): DNA sequences that are highly conserved among most pathogenic bacteria were amplified by PCR and found useful in the early diagnosis of systemic bacterial infection (*McCabe et al. 1995*).

Pro-calcitonin: The specific function of this protein is unknown, but it is thought to sustain inflammatory reactions after its production in hepatocytes is stimulated by cytokines, especially IL-6 (*Hsiao et al. 2005*). It is known to rise slightly in viral

infection and enormously in bacterial infection. High serum pro-calcitonin levels were found in the plasma of septic patients (*Assicot et al. 1993*). This protein was found a useful marker for the early diagnosis of neonatal infection (*Gendrel et al. 1996*) and was recently shown to be superior to leucocyte count and CRP in predicting severe bacterial infection in children (*Hatherill et al. 1999, Galetto-Lacour et al 2003*).

Neopterin: This is a pteridine bi-product of activated monocytes/macrophages released into blood upon immune activation. Raised systemic levels of this substance correlated with bacterial sepsis and plasma levels differentiated between bacterial and viral infection in preterm infants (*Jurges et al. 1996*).

IV. 5. Cytokines

Plasma concentrations of cytokines and their receptors have been studied in patients with inflammatory conditions. Measurement of cytokines in plasma is difficult, partly because of their short half-lives and the presence of blocking factors. The high cost, limited availability, and the absence of standardization of their measurement argue against its use in clinical practice. This is discussed in further detail in the following chapters of the thesis. Table 11 summarizes some of the newly introduced diagnostic tools for predicting bacterial infection.

IV. 6. Neural Networks

This classification technique has been used for many purposes. In the medical field, its main uses were in radiology and in constructing management algorithms for cardiac emergencies. Its role in the detection of serious bacterial infection is not fully evaluated.

Table 7: Non-infectious influences on the WBC picture

Factor	Comments
Adrenaline and strenuous exercise	Occurs within minutes, no left shift, e.g. asthma
Corticosteroids	Occurs within 4 hours, left shift with eosinopenia
Diabetic ketoacidosis	Can be as high as 25,000/mm^3, with left shift
Tissue injury	Burns, crush injury, fracture, neoplasm; neutrophilia with left shift
Acute haemorrhage	More with internal bleeding
Acute haemolysis	Limits use of WBC in diagnosing infection, e.g. in sickle cell anaemia
Myeloid reaction	WBC >50,000/mm^3, in severe infections, miliary TB, bacterial meningitis, staphylococcal pneumonia, and in JRA

Table 8: Relation between the incidence of bacteraemia and the white blood cell count.*.

WBC ($\times 10^3$)	Incidence of bacteraemia (%)
<10	0.7
10-15	3.9
15-20	5.4
>20	10.7

*: Steele RW, 2007

Table 9: Correlation between the incidence of bacteraemia and the degree of fever.*

Temperature (°C)	Incidence of bacteraemia (%)
<38.9	0.5
38.9-39.4	4.1
39.5-39.9	8.7
40.0-40.5	8.1
40.5-41.0	13.0

*: Steele RW, 2007

Table 10: The acute phase plasma proteins

Protein class	Increased	Decreased
Proteinase inhibitors	α_1-antitrypsin	Inter α-antitrypsin
	α_1-antichymotrypsin	
Coagulation proteins	Fibrinogen	
	Prothrombin	
	Factor VIII	
	Plasminogen	
Complement proteins	$C1_s$, C2, B, C3, C4, C5, C9, C56, C1INH	Properdin (P)
Transport proteins	Haptoglobin	
	Hemopexin	
	Caeruloplasmin	
	Ferritin	
Lipoproteins		LDL, HDL
Miscellaneous	C-reactive protein	Albumin
	Serum amyloid A protein	Pre-albumin
	α_1-acid glycoprotein (orosomucoid)	Fibronectin
	Gc globulin	

Table 11: Clinical characteristics of modern diagnostic tests for neonatal sepsis*

Test	Laboratory Time Required	Volume of Blood Required	Type of test
Cytokine levels (e.g. IL-6, Il-8, IL-1 receptor antagonist, and TNF)	<2 h	200 μl	ELISA
Fibronectin level	2-3 h	1-2 ml plasma	ELISA
Neutrophil CD11b level	30-60 min	0.1 ml whole blood	Flow cytometry
Neutrophil elastase inhibitor	1 h	4 μl	Turbidimetric assay
PCR	1-4 h	200 μl	Nucleic acid extraction, amplification and analysis
Procalcitonin level	Quantitative, 2h; semiquantitative, 30 min	Quantitative, 20 μl; semiquantitative, 200 μl	Immunoluminometric assay
TNF receptor p55 & p75 levels	4-6 h	200 μl	ELISA
sICAM-1	2 h	200 μl	ELISA

* Adapted from Malik et al. 2003.

Part Two

The cytokines

Chapter V

Cytokines; an overview

V. 1. Nomenclature

Cytokines were originally thought to be produced only by lymphocytes and to communicate with other cells of the immune system, hence the original name lymphokines. However, it is now known that neither the production of cytokines nor their effects are restricted to lymphoid cells, therefore the term cytokines was introduced. Cytokines were originally named according to their biologic effects, e.g. tumour necrosis factor, B-cell stimulating factor, etc. However, these descriptive names were misleading because most cytokines possess more than one biologic property. It was therefore agreed that a new cytokine would be named according to its biologic property, but once its amino acid sequence was established, it would be called interleukin and assigned a specific number. For example, human B-cell stimulating factor 1 was called interleukin-4 after its complementary DNA was cloned. Nevertheless, some cytokines retain their original names, e.g. interferons, the colony-stimulating factors, and the thymus derived hormones. The term interleukin is somewhat misleading, because interleukins are synthesised by and act on leukocytic and non-leukocytic cells (*Whicher & Evans, 1990*).

V. 2. Chemical structure

Cytokines are polypeptides that participate in a variety of cellular responses, including the regulation of the immune system. In contrast to immunoglobulins, their chemical composition is not determined by the nature of the stimulating antigen. Most cytokines are glycated, giving rise to considerable molecular heterogeneity. The sugar moieties may be important for receptor binding or modulation. Cytokine receptors contain trans-membrane domains that interact with GTP-binding proteins, which regulate different enzymes in the signal transduction pathways, e.g. phosphoinositase C, adenylate cyclase, and tyrosinase kinase. The signals that link these changes to gene transcription are largely unknown. Many cytokines interact with each other at the cellular level. The effects of a cytokine on a cell depend on the milieu of other cytokines and hormones; therefore, measurements of single cytokines may reveal only part of the story. Advances in protein biochemistry and recombinant DNA technology during the 1980s have transformed our understanding of cytokines. Studies using their recombinant products have greatly extended our understanding of their many metabolic, endocrine, haematological, haemodynamic and immunological effects (*Whicher & Evans et al. 1990*).

V. 3. Effector & target cells

Cytokines are produced throughout the body. Many cytokines can act on the same cells producing them (autocrine function). In localized areas, such as joint spaces or lymphoid tissue, they can act on other types of cells in the immediate vicinity exerting a paracrine effect. Cytokines that are produced in large amounts and gain access to the circulation act in a hormonal (endocrine) fashion and can have profound systemic effects. Cytokines have several properties relevant to the immune response as opposed to the inflammatory response. Cytokines that primarily effect an immune response are locally active (paracrine) and tend to be of T cell origin, e.g. IL-2, IFN-γ, and IL-4. The more systemically active (endocrine) cytokines are involved more in inflammation and are produced mainly by macrophages (*Whicher & Evans 1990*).

V. 3. a T-cells

In contrast to the relatively antigen-non-specific inflammatory response, the immune response is a highly specific reaction effected by lymphocytes, which recognize specific antigens. The initial event in the immune response is the uptake and processing of the antigen by phagocytic cells (monocytes and macrophages). Processed antigens are presented in association with class I or II MHC antigens on the phagocytic cell surface. T cells with T cell receptors that recognize the antigen-MHC complex can respond by synthesizing and secreting various growth and differentiation factors; the cytokines. These cytokines themselves are antigen-non-specific and, in contrast to antibodies, the structure of the stimulating antigen does not determine their chemical composition. However, they are responsible for the rapid expansion and differentiation of the antigen-specific lymphocyte population.

The antigen receptor on the surface of the T cell that interacts with the antigen-DR (or MHC) complex consists of a complex of five peptides. Of these, three are invariant from cell to cell while the other two are unique to each T- cell clone. The latter two peptides account for the antigen specificity of the clone. The invariant three-peptide complex is called CD3. When an antigen is presented in the appropriate configuration to the complementary antigen receptor, the T-cell becomes activated. As a consequence of this activation, the T-cell synthesizes a variety of proteins, of which some are secreted and others become integral components of the cell membrane. The former include IFN-γ and interleukins-1, 2, 3, 4, and 6, whereas IL-2 R is an example of a membrane-associated protein generated during antigenic challenge. The cytokines thus produced are independent of the antigen and function to amplify the response to antigen in a non-specific fashion. Gamma-interferon released from activated T-cells enhances DR expression on the macrophage thereby increasing the T-cell response to the antigen. Interleukin-1 augments the synthesis of interleukins-1, 2, 3, 4 and 6, gamma-interferon, and interleukin-2 receptors. Interleukin-2 binds to specific receptors on activated T-cells, giving rise to the expansion of the activated clone. Interleukins 4 and 6 provide the critical signals for the growth and maturation of antigen-primed B cells. Two distinct T helper cell types have been described: activated T_H1 cells which secrete IL-2 and IFN-γ,

and T_H2 cells which produce IL-4 and IL-5. Both types of cells produce IL-3 and GM-CSF. IL-1β is an accessory signal that is required for the proliferation of T-cells in response to IL-2 and IL-4. However, not all T-cells respond to IL-1. Reportedly, T_H2, but not T_H1, respond to IL-1. Naïve T cells stimulated by lectin require an accessory signal that cannot be replaced by IL-1. This accessory signal is thought to be IL-6, because resting T cells express high affinity receptors for this cytokine (*Whicher & Evans 1990*).

V. 3. b. **B-cells**

The principal steps leading to the production of antibodies are i) specific activation of the virgin or memory B-cell, ii) proliferation (clonal expansion), and ii) differentiation into antibody-producing plasma cells. Cytokines act in concert to regulate proliferation and differentiation of B-lymphocytes. A small resting cell is converted to a large B-cell on binding of its surface immunoglobulin to a specific antigen. Interleukin-4 stimulates the proliferation of this large cell. Interleukin-6 drives this proliferating cell to become a specialized immunoglobulin-secreting plasma cell. As mentioned earlier, interleukin-1 stimulates the synthesis of IL-6 by the activated T-cell. Cytokines that participated at different stages of B-cell stimulation also play a part in determining what type of antibody is secreted by the plasma cell. IL-4 and IL-5, produced by T_H2 cells, influence the switch towards IgE and IgA. On the other hand, T_H1 cytokines, IFNγ and IL-2, induce production of IgG. The macrophage-derived IL-6 acts on activated B cells stimulating the general synthesis of immunoglobulin. It therefore seems likely that the nature of antibody response is determined by the cytokine profile associated with the particular immune response (*Whicher & Evans 1990*).

V. 3. c. **The macrophage**

The pro-inflammatory cytokines include IL-1, IL-6, IL-8, TNF-α, and interferon gamma (IFN-γ). Most nucleated cells appear able to produce these cytokines, especially IL-1, but macrophages produce the largest amounts and are thus key producers of inflammation. These cytokines have a wide range of target cells that possess the

corresponding receptors. However, some cytokines, e.g. IL-1, can induce prostaglandin synthesis without discernible receptor interaction. The inflammatory responses mediated by macrophage-produced cytokines include:

1. Increase in vascular permeability by release of PGE_2 from connective tissue
2. Procoagulant production by endothelial cells
3. Production of proteases from fibroblasts and mesenchymal cells
4. Induction of respiratory burst and production of oxygen radicals and hydrolytic enzymes by neutrophils and monocytes.
5. Chemotactic attraction of monocytes and neutrophils to the site of inflammation. This latter effect is induced by IL-8, while IL-1, TNF and interferon- γ mainly mediate the other processes (*Whicher & Evans 1990*).

V. 3. d. **Polymorphs**

The neutrophil is the primary cell of the acute inflammatory response. The diverse anti-microbiological mechanisms of the neutrophil can be broadly divided into non-oxidative (release of cidal enzymes and other proteins by degranulation) and oxidative (release of reactive oxygen intermediates). Cytokines play a pivotal role in each of the steps of neutrophil function:

1. Stimulation of myelopoeisis
2. The release of PMNs from marrow reserves
3. Adhesion to endothelium, which is vital in the transportation of neutrophils to sites of inflammation.
4. Chemo-attraction to sites of inflammation and micro-organisms. This is mediated by chemokines which are released in response to the action of many cell products, such as leukotriene B4, PAF, C5a, and the bacterial peptide formyl-Met-Leu-Phe.
5. Induction of integrin expression on the surface of neutrophils. Adhesion via the integrin CD11b/CD18b plays a critical role in priming the neutrophil for both microbicidal mechanisms and in providing an environment that protects the killing mechanisms from the inhibitory effects of tissue fluids and plasma.

6. Stimulation of the micro-organism-killing activities of polymorphs. (*Saez-Liorens & McCracken. 1993*).

V. 3. e. The endothelium

Under normal circumstances, peripheral blood polymorphs are distributed between the circulating pool (in the central, axial stream of the vessel) and the marginating pool (along the endothelial surface of the blood vessels). The half-life of these cells in the circulation averages 6 to 7 hours. In patients with sepsis, an initial transient neutropenia develops and is followed several hours later by an increase in the peripheral blood PMN count. This initial neutropenia results from the activation by cytokines of leucocyte-endothelium adhesion receptors that promote margination of polymorphs.

Initially, the neutrophils start to roll along the wall of the post-capillary venule, at a rate much slower (30 μm/s) than the progress of erythrocytes flowing past (1000 μm /s). The progress of some cells becomes arrested and they become flattened and elongated. These cells begin to move by a slow crawling action and to emerge through the intact endothelial wall. Distinct proteins expressed on the surfaces of the cells involved mediate these steps. These proteins are referred to as adhesion molecules and bind specifically to receptor structures on the surface to which adherence is occurring, which is usually another cell or elements of the extra-cellular matrix. Endothelium-leucocyte interaction represents a critical event in the generation and amplification of the inflammatory cascade and results from cytokine-induced activation of both cells to up-regulate synthesis and surface expression of adhesion molecules. Adhesion is controlled through the amount, distribution and adhesiveness of these molecules brought about by signals from within the cells expressing them, these latter being mediated by cytokines. Examples of such molecules expressed on polymorphs include the integrin (CD11 / CD18) and the selectin group of receptors (*Whicher & Evans 1990*).

V. 3. f. Selectins

The selectins are a family of three adhesion molecules which are vital in the initial rolling stage of leukocyte-endothelium adhesion and are expressed on both cell types. Leukocytes roll on stimulated endothelium before migrating to sites of inflammation or vascular injury. This initial and essential step is mediated by the selectins and their ligands. There are three types of selectins. L-selectin is abundantly expressed on unstimulated neutrophils and mediates the homing of lymphocytes to lymph nodes. E-selectin is expressed only by endothelial cells relatively slowly in response to stimulation by cytokines such as TNF and IL-1 and aids in migration of leukocytes. P-selectin is stored in α-granules of platelets and Weibel-Palade bodies of endothelial cells and is expressed on these cells after stimulation by rapidly acting agents such as thrombin or histamine. All three selectins mediate the rolling type adhesion of leucocytes. These proteins are mobilized to the surface of the cells by transport of intracellular stores contained in granules. All three proteins share a similar structure with a terminal lectin moiety, a protein structure that specifically binds to carbohydrate structures, facing outwards from the cell surface. The fucose-containing neutralizing de Lewis^x (Le^x) antigen, a blood group antigen with an added sialic acid residue (sLe^x), is one of the carbohydrate ligands for these selectins.

The binding of the selectins to their ligands makes the leukocytes decelerate by rolling on endothelial cells. Rolling greatly increases the affinity of the leucocyte β_2 integrin adhesion receptors for ligands (the adhesion molecules) on activated endothelium. The same types of receptors also mediate the extravasation of leukocytes. Leukocytes migrate, using their β_1 integrins, toward a chemotactic stimulant produced by bacterial invasion. Vascular injury immediately induces endothelial cells to release the contents of their storage granules (Weibel-Palade bodies), including P-selectin and von Willebrand factor (vWF). VWF is quickly deposited on the exposed extracellular matrix, where it enhances adhesion of platelets (Ib-α) to the damaged site. Degranulation of platelets and activation of the platelet integrin $\alpha_{11b}\beta_3$ (Iiib/IIIa) induce further accumulation of platelets and recruit neutrophils and monocytes to aid in repair (*Lorant et al. 1995, Frenette et al. 1996*).

V. 3. g. Integrins

The integrins are a family of cell-surface receptors involved in almost every aspect of cell signaling and mobility. All members are [illegible] eutral, consisting of two dissimilar protein chains designated α and β, non-covalently linked together. The members of the subfamily of integrins that share the β_2 (CD18) chain are found only on leukocytes, e.g. $CD11_a$/CD18 (LFA-1), CD 11_b/CD18 (Mac 1), and $CD11_c$/CD18, all of which are expressed on neutrophils. The integrins mediate the firm adhesion and subsequent crawling of the flattened bipolar neutrophils. These proteins are also mobilized from granular stores. While LFA-1 varies little between cells or in response to stimuli, Mac1 expression increases rapidly in response to a wide variety of stimuli, including C5a and IL-8. ICAM-1, expressed on endothelium, is the ligand for these integrins (*Frenette et al. 1996*).

V. 4. Cytokine modulation

Cytokines are powerful molecules with the potential for auto-stimulation; therefore equally powerful regulatory processes must also exist. Negative control exists at many levels: gene transcription / translation, binding of the protein, either specifically or non-specifically, thus increasing catabolism, and finally competition for receptor occupation. Additional control is provided with the opposing action of other cytokines that are released simultaneously:

1. A group of related cytokines are released in acute inflammation that appear to provide a negative feedback to cushion the effects of the inflammatory process. Inflammatory stimuli activate the production of counter-inflammatory cytokines that can modify the host inflammatory response. For instance, IL-10 and IL-4 have multiple down-regulating effects on specific immune responses, including down-regulation of TNF release, and induction of IL-1ra and IL-1 receptor type 2.
2. IL-6 induces the release of acute phase proteins, such as CRP, fibrinogen, some complement components and many anti-proteases, from the liver.
3. Many cytokines have soluble binding proteins. Theses are either shed from the membrane or separately translated as a truncated protein. Soluble receptors have

been described for IL-1, IL-2, IL-4, IL-6, TNF-α, and IFN-γ. Certain receptors, e.g. p55 IL-2R, have very little affinity for their cytokines, so their functional role is not clear. Soluble receptors retain the ability to bind their respective cytokines. Free and bound cytokine exists in equilibrium, and this balance may determine the bioactivity of a given cytokine concentration. With regards to sepsis, sTNF-R has been most extensively studied. Plasma sTNF-R concentrations are present in normal humans and are released in excessive amount into the circulation in response to endotoxin. Endotoxin injection results in a 4-5-fold increase in sTNF-R concentration by 3 hours, with peak plasma concentrations 10 times that of TNF. In human sepsis, sTNF-R is increased and correlates with severity of illness and mortality, but its pathophysiologic role is incompletely understood. TNF-sTNFR complex may serve as a slow release reservoir of TNF, which could perpetuate the inflammatory response.

4. Cytokines also bind to non-specific binding proteins e.g. IL-6 binding to α_2-macroglobulin. These may act as carriers regulating cytokine breakdown, or as regulatory proteins inhibiting their biological functions.
5. Autoantibodies for cytokines have been described not only in disease, but also naturally, e.g. anti-IL-1α and anti-TNF-α.
6. A natural inhibitor for IL-1 has been described. A 22 kDa protein, with 26% homology for IL1, binds to IL-1 receptors on target cells and blocks the actions of IL-1. This IL-1 receptor antagonist binds only to type 1 receptors. It does not immunologically cross-react with IL-1. This protein has been purified from the urine of febrile patients. It has been observed that as monocytes mature into macrophages, the production of IL-1 decreases and that of IL-1ra increases. Paradoxically, sTNF-R and IL-ra are released into the circulation in 10-100-fold excess compared with TNF and IL-1. In patients with sepsis syndrome, prognosis may be determined by the balance of cytokines and cytokine □ eutralizing molecules (*Whicher & Evans et al. 1990*).

Chapter VI

Individual cytokines

VI. 1. Tumour necrosis factor-α (TNF)

TNF was first described, in the serum of mice challenged with endotoxin, as a factor that caused the necrosis of some tumours in vivo. It was later cloned as a 17 kDa protein, and retained the name TNF-α. TNF-β, a lymphocytotoxin derived from T-cells, has 28% homology, and shares the same receptor, with TNF-α. Genes coding for both molecules are on chromosome 6, near the region coding for MHC. There are two independent cell surface receptors for TNF-α, with MW of 65 and 75 kDa. All somatic tissue, except erythrocytes, possess TNF-α receptors, but with great variation in density. This is reflected on the ubiquitous effects of TNF-α (*Emery et al. 1991*).

TNF-α is synthesised by cells of the monocyte-macrophage lineage, notably astrocytes, microglial cells, and kupffer cells-, and by lymphocytes. Synthesis is induced by bacterial products such as endotoxin, toxic shock protein-1, mycobacterial proteins, fungal antigens, viruses, and C5a. This underlies the importance of this cytokine in infection and inflammation. Following synthesis, it becomes widely distributed in the tissues and then rapidly degraded. Pre-treatment of macrophages with dexamethasone effectively inhibits its synthesis upon exposure to endotoxin. Pre-treatment with anti-

TNF serum also protects against the lethal effects of injected endotoxin (*Bellomo et al. 1992*).

TNF is a primary mediator of inflammation with pleiotrophic effects on target cells (*Morrison et al. 1987*). TNF is distinct from other cytokines by being strikingly cytotoxic. TNF was initially known as cachectin because of its ability to induce lipolysis and glycogenolysis, and to induce anorexia, weight loss, and anaemia if given in sublethal doses to animals. Effects of TNF established by in vitro studies include neutrophil adherence to endothelium, induction of the coagulation cascade, production of free radicals and stimulation of osteoclast bone resorpotion. TNF also enhances the production of other cytokines, such as IL-1, IL-6, IL-8 and PGE2. Clinical effects attributed to TNF include; fever, lactic acidosis, hyperglycaemia, diuresis, capillary leakiness, and haemodynamic features of shock. Infusion of recombinant TNF results in systemic inflammatory response syndrome (SIRS) with fever, haemodynamic abnormalities, leucopenia, elevated liver enzymes and coagulopathy. TNF is capable of causing end organ dysfunction, which occurs in severe sepsis. After i.v. administration of endotoxin in humans, TNF concentrations increase rapidly to peak at 4 hours, coinciding with the peak of fever, rigors, myalgia, headaches and nausea. In contrast to IL-1, TNF seems to have no direct effect on lymphocyte activation, but both induce the same spectrum of acute phase changes (*Whicher & Evans et al. 1990*). The two cytokines act synergistically in a variety of biologic responses, including tissue damage, metabolic abnormalities and neuro-endocrine changes. TNF-α has a major amplifying effect on most of the diseases characterised by increased IL-1 production, e.g. malignancy. In meningitis, cerebral malaria and the acute respiratory distress syndrome (ARDS) the excessive production of TNF-α is of primary pathological importance.

Increased levels of TNF were first demonstrated in plasma of patients with meningococcaemia in 1987. TNF can be detected in the plasma of many patients with sepsis, and concentrations generally correlate with severity of illness and outcome (*Peakman et al. 1992*). Persistently elevated levels of TNF might be more determinant of prognosis than peak levels (*Thijs LG, 1995*). There are wide variations among studies in the reported proportions (16-100%) of septic patients with elevated TNF levels

(*Dinarello et al. 1987*). Raised TNF-α levels also appear to be a feature of certain healthy people (*Emery et al. 1991*).

VI. 2. **Interleukin-1**

There are two structurally related forms of this cytokine; IL-1α and IL-1β. Both activate the same IL-1 receptor, exerting the same biological effects, with little homology (26%) in their structure. In contrast to IL-1β, IL-1α is rarely found in body fluids in soluble form. IL-1β is the predominant form produced by endotoxin-stimulated monocytes. Interleukin-1 exerts effects through its two receptors by inducing signal transduction, which may involve G proteins and serine kinase or cAMP and protein kinase. Monoclonal antibodies directed against type 1 (80kDa), but not type 2 (60kDa) receptors, block signal transduction. IL-1 can act by inducing prostaglandin synthesis without discernible receptor interaction (*Whicher & Evans et al. 1990, Morrison et al. 1987*).

IL-1 is produced by nearly all cell types, including T & B lymphocytes, natural killer cells, skin keratinocytes, astrocytes, microglia, epithelial cells, mesangial cells and vascular tissue. However, macrophages produce the largest amounts. Antigens, toxin, injury, and inflammatory processes induce the production of IL-1. Endotoxin is one of the most potent, at picogram levels, inducers of IL-1. Antigens may induce IL-1 directly by cell/cell contact or indirectly by release of other cytokines from activated T cells, e.g. IL-2, IFN-γ and MCSF. Factors known to down-regulate IL-1 production include steroids, IL-4, IFN-β, and IL-1, PGE2, fever and malnutrition (*Emery et al. 1991*).

The physiological role of IL-1 is not known, but its levels in the CSF are raised during sleep. IL-1 induces slow-wave sleep and the synthesis and release of hepatic acute-phase proteins, ACTH, cortisol, and insulin. IL-1 has effects on a wide variety of tissues: i) T-cell activation and production of cytokines such as IL-6, IL-8 and TNF. ii) Leukocyte adhesion to endothelium, prostaglandin release and production of a procoagulant state. iii) Induction of proteases and bone resorption, and iv) Induction of the acute phase response. Infusion of IL-1 causes fever, haemodynamic abnormalities,

anorexia, malaise, arthralgia, headache, and neutrophilia. IL-1 has been implicated in the pathogenesis of Crohn`s disease, sunburn, burns, psoriasis, endometriosis, silicosis, immune complex glomerulonephritis, scleroderma, acute arthritides and rheumatoid arthritis (*Bellomo et al. 1992*).

IL-1 is not normally present in human plasma (*Blackwell et al. 1996*). IL-1 production can be detected in the circulation within a few hours after the onset of infection or injury. Peak levels are reached around 4 hours after endotoxin (*Morrison et al. 1987*). Detectable levels of IL-1β have been reported in 0-100% of patients, and its correlation with outcome remains unclear (*Ozdemir et al. 1994, Blackwell et al. 1996*). Markedly elevated levels of IL-1ra were detected in plasma of septic patients, and persisted for longer periods than IL-1 (*Thijs et al. 1995*) Measurements of IL-1 in biological fluids has been confounded by many factors. Preparations were often contaminated with LPS thus confusing the specificity of any response seen. Production of IL-1 is induced by other cytokines, and its actions overlap with those of different cytokines suggesting redundancy of function (*Emery et al. 1991*). Nevertheless, the high recovery of IL-1β added to normal plasma or serum from septic patients suggests that the presence of serum inhibitors of IL-1 could not have impaired the detection of IL-1 in the study patients (*Peakman et al. 1992*).

IL-1 receptor antagonist (IL-1ra)

IL-ra was first identified as a 23 kDa protein from the urine of patients with monocytic leukaemia. The amino acid sequence of this protein is 26%, and 19% homologous to IL-1β and IL-1α respectively. IL-1ra blocks the activity of IL-1 by competing for binding to type 1 and type 2 IL-1 receptors without causing signal transduction. IL-1ra can attenuate endotoxin effects in animal models of sepsis. In human volunteers, plasma IL-1ra concentrations peak 3-4 hours after endotoxin injection. In humans with sepsis, IL-1ra is present in plasma at markedly higher concentrations than IL-1, but its function in this setting is uncertain (*Dinarello et al 1987*).

VI. 3. Interleukin-6 (IL-6)

Biologically identified in 1981 and molecularly cloned in 1986, IL-6 has been known by various designations. Originally it was labelled IFN-2 when found as a secretory product of fibroblasts after induction for IFN (*Emery et al. 1991*). It was also known as BSF2/BCDF and HPGF when found to stimulate B-cell differentiation and hybridisation, respectively (*Assicto et al. 1993*). It was then called hepatocyte stimulatory factor for stimulating hepatic synthesis of acute phase proteins (*Calandra et al 1991*). IL-6 exists in five or six different forms, with molecular masses between 21.5 and 28 kDa, which are the result of post-translational changes such as glycation and phosphorylation. These post-translational modifications may play a role in tissue-specific functions and/or in plasma half-life. A high-molecular-mass form of 42-45 kDa has also been described in plasma, possibly a complex formed with a carrier protein (*Kishimoto et al 1989*). Three regulatory regions on chromosome 7(p21) are involved in its gene expression.

Chromosomal abnormalities of human chromosome 7 and 5 (the site of murine IL-6) have been found to be associated with a variety of haematopoeitic disorders, among them multiple myeloma. Initially, a 1.3 kb mRNA is translated into a 212-amino acid precursor protein with a MW of 26 kDa (*Heinrich et al. 1990*). After removal of a 28-amino acid signal peptide, the resulting 184-amino acid protein, containing two possible N-glycosylation sites, is N- and O-glycosylated and subsequently secreted. Human IL-6 shows significant homology with G-CSF and has a homology of 65% at the DNA level and 45% at the protein level, with murine IL-6 (*Kishimoto et al. 1989*). The kinetics of IL-6 clearance is biphasic, consisting of a rapid initial elimination corresponding to a half life of 3 minutes, and a second slower decrease corresponding to a half life of 55 minutes. IL-6 binds to α_2-macroglobulin, retains its biologic activity and becomes resistant to proteases (*Heinrich et al. 1990*).

IL-6 is synthesised and released in response to inflammatory stimuli by monocytes, macrophages, endothelial cells, and fibroblasts. T and B cells produce it under non-physiological conditions. Tumours are also potent producers of Il-6 (*Emery et al. 1991*). Virus-infected microglial cells and astrocytes have produced IL-6, which was

also detected in synovial fluid during bacterial and viral infection (*Kishimoto et al. 1989, Heinrich et al. 1990*). Monocytes/macrophages are preferentially stimulated by bacterial LPS, whereas fibroblasts and endothelial cells respond better to the endogenous cytokines IL-1 and TNF, which are abundantly synthesised by monocytes, suggesting that these cytokines play a key role in the production of IL-6. IL-1 and TNF act via two second messenger systems: protein kinase C and cAMP. The production of IL-6 by T cells requires the presence of monocytes, while monocytes produce IL-6 in the absence of any apparent stimulus in in-vitro cultures. In addition, it was shown that the peak of IL-6 mRNA in monocytes was achieved in five hours, while that from T cells was at 24-48 hours. This suggests that IL-6 produced by monocytes and T cells, with different kinetics, may exert distinct effects at different phases of the immune response. A variety of cytokines, including IL-1, TNF, PDGF, and IFN-β, as well as serum, poly (I) poly (C) and cyclohexamide also enhance the expression of IL-6 gene in different cell types. Viruses induce Il-6 production in fibroblasts or in the CNS. Dexamethasone inhibits IL-6 production and oestradiol-17β impairs its formation in human endometrial stromal cells. The glucocorticoids increase the stimulatory effect of monokines on the synthesis of acute phase proteins, but also inhibit the synthesis of monokines themselves. The effects of abnormal expression of IL-6 gene have been studied in IL-6 transgenic mice following the mapping of the genomic gene of human IL-6 on chromosome 7. Several potential transcription control elements, such as glucocorticoid responsive element (GRE), a serum responsive element (SRE), cAMP responsive element (CRE), and a NF-kB binding site, were identified within the IL-6 promoter. Three regulatory regions have been shown to be involved in the regulation of IL-6 gene expression; multi-responsive element, NF-IL6, and NF-kB. These mediate induction, or inhibition, by different regulators such as IL-1, TNF, LPS etc. Glucocorticoids negatively regulate the IL-6 gene expression in various tissues and cells. Studies on the regulation of IL-6 gene expression and its action through its receptor will provide information on the molecular mechanisms of a variety of diseases and may provide novel diagnostic and therapeutic approaches (*Whicher & Evans et al. 1990*).

The receptor for IL-6 belongs to the immunoglobulin supergene family. It is a glycoprotein with a MW of 80 kDa, consisting of 449 amino acids. The cytoplasmic domain of 82 amino acids lacks a tyrosine kinase, unlike other growth factor receptors, and it is not essential for signal transduction (*Emery et al 1991*). The underlying mechanisms of signal transduction are still unknown. The action of IL-6 appears to be independent of protein kinase C, ITP and adenylate cyclase activity. A possible signal transducer (gp130) with a MW 130 kDa could associate with IL-6 receptor after the interaction of IL-6 with the 80 kDa receptor. Polymorphism in this 130 kDa gp can explain the multiple functions of IL-6 on various tissues. On the other hand, the redundancy in cytokine function may result from several cytokine receptors sharing the same signal transducer (*Heinrich et al. 1990*). IL-6 receptor is expressed on both lymphoid and non-lymphoid cells. Whereas expression of IL-6R by monocytes is down regulated by binding to Il-6, hepatocytes express it only after prior treatment with IL-6 or IL-1. The liver seems to be the major target for IL-6. In the rat, 80% of injected radiolabelled IL-6 localizes on the surface of the liver parenchymal cells, suggesting the existence of IL-6 receptors on the plasma membrane of hepatocytes. The number of cytokine receptors is usually in the order of 10^2 to 10^3 per cell, which is less than that for hormones or growth factor receptors. In monocytes, the synthesis of IL-6 receptor mRNA is strongly inhibited by IL-6, IL-1 and LPS. Thus, during inflammation, a switch from monocytes to hepatocytes and activated B cells as target cells for IL-6 may occur. Dexamethasone stimulates IL-6 receptor mRNA in human hepatocyte cultures. A soluble IL-6 binding molecule can be found in the serum of healthy individuals. Unlike all known soluble cytokine receptors, the soluble receptor (sIL-6R) has no antagonist role but, on the contrary, enhances the response to IL-6 (*Kishimoto et al. 1989, Lehrnbecher et al. 1995*).

IL-6 is a pleiotropic cytokine exerting growth-inducing, growth-inhibitory and differentiation-inducing effects, depending on the nature of the target cells:

1. The first identified function of IL-6 was the induction of the terminal B-cell differentiation and immunoglobulin production. IL-6 acts directly on B cells activated by IL-4 and IL-5 and induces immunoglobulin M, G, and A production.

Normal resting B cells do not express IL-6 receptors; hence IL-6 does not exert any growth-promoting activity on these cells. IL-6 augments the primary and mainly the secondary antibody responses in mice. IL-6 is an important helper in primary antigen-receptor-dependent T cell activation and subsequent proliferation of activated T cells. This is synergised by IL-1 and is said to be dependent on IL-2. These two cytokines induce the production of IL-2 and IL-2 receptor expression on T-cells. IL-6 enhances the action of IL-2 in the late phase of cytotoxic T cell generation and plays a role in the expression of class I histocompatibility antigens in human fibroblasts. IL-6 also primes neutrophil and monocyte oxidative burst response (*Heinrich et al. 1990*).

2. IL-6 has been strongly implicated as an autocrine growth factor for several tumours, such as myeloma and cardiac myxoma, and it stimulates proliferation of lymphatic leukaemia and lymphoma cells. On the other hand, IL-6 inhibits the growth of certain myeloid leukaemic cell lines and induces their differentiation into macrophages. It also exerts a strong inhibition on the growth of human fibroblasts, breast carcinoma cells and endothelial cells in culture (*Calandra et al. 1991*).
3. IL-6 synergies with IL-3, M-CSF, GM-CSF to enhance the growth of haemopoietic progenitor cells. IL-6 shortens the G_0 period of stem cells and up-regulates IL-3 receptors on bone marrow stem cells. It was therefore suggested that IL-6 might have a role in bone marrow transplant (*Whicher & Evans et al. 1990*).
4. Induction of mesangial cell growth and neural cell differentiation. IL-6 also induces the secretion of nerve growth factor by astrocytes, hence it may be involved in repair mechanisms in the course of viral infections (*Kishimoto et al. 1989*).
5. IL-6 can activate the coagulation system (*Blackwell et al. 1996*).
6. IL-6 may induce fever, and its synthesis by endometrial cells has been suggested as a mechanism for the temperature fluctuations during the menstrual cycle (*Blackwell et al. 1996*).
7. IL-6 is probably the major physiological inducer of acute-phase protein synthesis in the liver, with IL-1 and TNF playing only subsidiary roles (*Calandra et al. 1991*).

8. IL-6 may have an important role in the release of corticosteroids during inflammation. IL-6 triggers the release of ACTH from murine pituitary glands. IL-6 shows a synergistic effect with glucocorticoids on the induction of acute phase proteins, thus IL-6 induced secretion of ACTH may have a positive feedback loop on acute phase reaction. On the other hand, glucocorticoids are potent inhibitors of IL-6 production in various cells. Therefore, the interaction of IL-6 with the neuro-endocrine system may regulate positively and negatively acute phase reactions and immune responses (*Kishimoto et al. 1989*).
9. IL-6 was first described as an additional factor released along with fibroblast IFN-β, thought to have antiviral activity, so was called IFN-β_2. However, it is doubtful if IL-6 has any antiviral activity.
10. The relative lack of toxicity of IL-6 argues against a major causative role in septic shock.

The multiple effects of IL-6 contribute to a co-ordinated response of the body to aggression. IL-6 is induced by other cytokines such as IL-1, PDGF, and GM-CSF. It is synergistic with others in inducing many of its actions, e.g. induction of fever, acute phase protein synthesis and thymocyte growth. This underlines the complexity of the cytokine network (*Bellomo et al. 1992*).

The role of Il-6 in disease is complex. Its association with disease states and its causative role of certain disease manifestations are undoubted. However, there is no consensus on whether it is a harmful or a protective molecule although it is regarded anabolic in some situations:

1. The first example of a disease associated with abnormal IL-6 production was cardiac myxoma. Patients with cardiac myxoma are hypergammaglobulinaemic, display various kinds of autoantibodies and increased acute phase proteins and have high plasma IL-6 levels. These features disappear with myxoma resection. Furthermore, myxoma cells contain increased amounts of IL-6 and its mRNA (*Kishimoto et al. 1989*).
2. Patients with Castleman`s disease, benign hyperplastic lymphadenopathy, show hypergammaglobulinaemia, increased acute phase proteins and platelets, and high

plasma levels of IL-6. Some patients develop multiple myeloma. The germinal centre of the hyperplastic lymph nodes in Castleman`s disease produces large amounts of IL-6 with no significant production of other cytokines. Thus abnormal production of Il-6 may be the primary event in the pathogenesis of this disease (*Kishimoto et al. 1989*).

3. Macrophage-derived IL-6 may also be involved in the in vivo growth of Lennert`s T-cell lymphoma, which is characterised by massive infiltration of macrophage-derived histiocytes (*Kishimoto et al. 1989*).
4. Patients with rheumatoid arthritis characteristically show polyclonal plasmacytosis, autoantibodies, and increased acute phase proteins and platelets. Elevated levels of Il-6 can be detected in synovial fluid and sera of patients with active disease. T-cells, B-cells, synovites and chondrocytes have been identified as sources of IL-6. Patients with Felty`s syndrome treated with GM-CSF showed simultaneous induction of IL-6 release and flare up of arthritis. It is not known whether the abnormal expression of these inflammatory cytokines is a primary event in the disease process, or a secondary phenomenon (*Whicher & Evans, 1990*).
5. In mesangioproliferative (MPG) GN, IL-6 is found in urine, and its levels in urine correlated with the progressive stage of MPG (*Whicher & Evans, 1990*).
6. IL-6 is involved in the polyclonal B-cell activation in HIV infection, which induces IL-6 production in monocytes. Its levels in the sera of patients with this disease is increased, and anti-sense of the IL-6 gene blocks the in vivo proliferation of Kaposi`s sarcoma (*Whicher & Evans, 1990*).
7. Diseases characterised by a marked acute phase response and sensitivity to steroids, such as giant cell arteritis and rheumatoid arthritis, have increased levels of IL-6 (*Emery et al. 1991*).
8. IL-6 contributes to the thrombocytopenia seen in diseases with marked acute phase response, e.g. SLE, and inflammatory bowel disease (*Emery et al. 1991*).

The biologic activities of IL-6 suggest that, like TNF and IL-1, interleukin-6 may be an important mediator involved in host defense against infection. In vitro, endotoxin has been shown to induce gene expression and secretion of IL-6 by

monocytes/macrophages, human fibroblasts, and human endothelial cells. In healthy volunteers, an intravenous bolus infusion of endotoxin is followed by a transient increase of circulating levels of IL-6. IL-6 was also detected in the serum, CSF, and synovial fluid of patients with bacterial infections. The exact role of IL-6 in sepsis is uncertain, although it is an integral part of the cytokine cascade. Infusion of IL-6 does not result in a sepsis-like state (*Emery et al. 1991*)

After endotoxin infusion, IL-6 peaks in 4 hours, after TNF and IL-1which synergise to increase IL-6 production. IL-6 exhibits a very short half-life due to its rapid binding to skin and hepatic receptors where it is degraded (*Castell et al. 1988*, *Whicher & Evans, 1990*). The half-life of IL-6 was calculated to be 70 ± 11 min, compared to 103 ± 27 min for TNF (*Waage et al. 1989*). Hence, serum IL-6 levels only reflect a minority of the IL-6 produced in vivo, i.e. the portion not absorbed by either cell surface receptors or the kidneys (*Bataille et al. 1992*). In vitro studies have shown that non-stimulated monocytes do not produce IL-6 (*Schindler et al. 1999*). However, IL-6 is detectable in the plasma, but not other fluids, of normal individuals (*Van Oers et al. 1988*). In other tissues, detectable levels of IL-6 were predictive of a variety of morbid states. For instance, increased amounts were found in the urine of renal transplant recipients and persisting high levels were predictive of rejection (*Van Oers et al. 1988*). IL-6 is also significantly elevated in synovial fluid from patients with rheumatoid arthritis (*Houssiau et al. 1988*). An elevated amniotic fluid IL-6 was found to be a sensitive and specific marker for the identification of intrauterine infection in women with preterm labour (*Greig et al. 1993*) and reliably predicted perinatal morbidity and mortality (*Yoon et al. 1995, Singh et al. 1996*). IL-6 in the cervical secretions was also predictive of preterm labour (*Rizza et al. 1996*). IL-6 is most consistently elevated in patients with sepsis, reported proportions of high levels varying between 64-100% of cases (*Schindler et al. 1990*). The highest levels were detected in patients with shock and a clear correlation with mortality was observed. IL-6 levels were highest on admission and showed a rapid decline, though it was detectable for up to 36 hours (*Thijs et al. 1995*). There are, however, wide individual variations in IL-6 levels reported in different studies (*Van Oers et al. 1988*), due in part to different methods of assay. IL-6 concentrations correlate

more closely than other cytokines with severity and outcome of human sepsis, and its levels predicted bacteraemia (*Blackwell et al. 1996*).

VI. 4. Interleukin-8 (IL-8)

IL-8, also called neutrophil attractant/activation protein-1 (NAP-1), is a 72 amino acid small basic protein with a MW of 10 kDa (*Blackwell et al. 1996*). It is a heparin-binding member of the α-chemokines (C-X-C) family of proteins (*Miller et al. 1996*). Originally isolated from supernatants of LPS-stimulated monocytes, IL-8 is now known to be produced by a variety of cell types, including polymorphonuclear phagocytes (PMN), endothelial cells, fibroblasts, hepatocytes, and keratinocytes in response to several inflammatory stimuli, including LPS, TNF and IL-1, but not IL-6. Bacterial products, such as endotoxin, are potent inducers of IL-8 (*DeForge et al. 1991*). IL-8 production is also stimulated by several other products of both G+ve and G-ve bacteria (*Miller et al. 1996*). IL-8 is one of the main cytokines produced by PMN. Cytokine (IL-1, IL-6, IL-8 and TNF) concentrations are unrelated to leukocyte counts and markers of neutrophil (elastase) or monocyte (neoptrin) activation (*Remberger et al. 1997*). This suggests that cytokine release associated with evolving septic shock may not depend on circulating leucocytes. The cytokines IL-10, IL-4, IL-13 and TGF-β can repress IL-8 release by activated PMN (*Dinarello et al. 1987*). Compared with healthy controls, the release of IL-8 by PMN from septic and non-septic SIRS patients is significantly reduced whether activated by LPS or heat-inactivated bacteria. This dampening of PMN reactivity is not thought to be due to endotoxin tolerance, but may be due to other LPS mediators, such as IL-10 (*Marie et al. 1998*). Basal cytokine production is not demonstrable in primary amnion culture, whereas incubation with group B streptococcus leads to a significant increase in IL-6 and IL-8, but not of IL-1 or TNF (*Klaus et al. 1991*). In LPS-stimulated human whole blood, TNF and IL-6 mRNA and protein peak at 2-4 hours and 4-6 hours, respectively. IL-8 mRNA and protein plateau between 6 and 12 hours then rise again in a second wave which continues to escalate until the end of 24 hours (*DeForge et al. 1991*). This suggests that the early mediators may augment continued expression of IL-8 to recruit and retain neutrophils at the site of inflammation.

Similarly, in human volunteers, IL-8 could be detected after LPS or TNF administration. After incubation with Pseudomonas aeruginosa supernatant, bronchial epithelial cells and monocytes show a marked increase in the level of IL-8 gene expression. Infection of mucosal cell monolayers by invasive bacteria result in expression and up-regulation of IL-8, MCP-1, GM-CSF, and TNF. These same cytokines are up-regulated after mucosal stimulation by TNF or IL-1. Non-invasive bacteria, and Giardia, fail to produce the same effect. Coagulation also efficiently induces IL-8 production (*Johnson et al. 1996*). Following endotoxin injection, IL-8 plasma levels peak in 2-3 hours, and its kinetics mirror those of IL6 (*Chae et al. 1996*). IL-8 has a short (± 8 minutes) half life in the circulation (*Terashima et al. 1998*).

The primary function of IL-8 is to activate and chemo-attract neutrophils to sites of inflammation and to help in the process of marginalization and migration of polymorphs (see below). In addition, basophils and T-lymphocytes are attracted by nanomolar concentrations of IL-8. IL-8, has the ability to bind to the endothelial cell surface and the extracellular matrix, and hence has both haptotactic and chemotactic effect. IL-8 is also a potent activator of polymorphonuclear leucocytes and mediates the release of granule contents. This latter function is enhanced by TNF-priming of neutrophils (*Rot et al. 1993*). Intravenous injection of IL-8 causes no haemodynamic abnormalities, but causes transient granulocytopenia followed by granulocytosis (*Terashima et al. 1998*). IL-8 does not stimulate monocytes (*Whciher & Evans, 1990*).

IL-8 has been implicated as a critical cytokine in mediating the early phase of tissue injury and organ dysfunction. Its production is increased in the lung following hypoxia-hyperoxia and in the setting of neutrophil alveolitis (*Blackwell et al. 1996*). Bronchoalveolar lavage (BAL) IL-8 concentrations correlate with BAL neutrophil count and increased mortality in patients with ARDS (*Dinarello et al. 1987*). High concentrations of IL-8 are found in the sputum of CF patients (*Bellomo et al. 1992*). Elevated plasma levels of this cytokine have also been found in septic shock, with higher levels in survivors suggesting a protective role (*Blackwell et al. 1996*). Elevated IL-8 levels have also been reported in a number of other inflammatory diseases, such as rheumatoid arthritis and alcoholic hepatitis (*Whciher & Evans. 1990*). IL-8 has been

identified in the amniotic fluid of patients with preterm labour and IU infection (*Cherouny et al. 1993*).

VI. 6. Intercellular adhesion molecule-1 (CAM-1)

ICAM-1 belongs to a group of cell-surface proteins called the immunoglobulin superfamily. These include the T-cell receptors CD3, and the co-receptors CD4 and CD8 and their respective counter-receptors MHC 2 &1. All these proteins share the immunoglobulin domain structure, and they play a critical role in the specific immune response. ICAM-1 is a sialylated glycoprotein with tissue specific differential glycosylation resulting in a wide molecular weight range of 70-120 kDa. It has five extracellular domains, a single transmembrane region, and a short cytoplasmic tail (*Sessler et al. 1995*). ICAM-1, like E-selectin, is expressed by the endothelial cells only following exposure to stimuli such as TNF and IL-1. It serves as a counter-receptor for the leucocyte beta 2-integrins; LFA (CD11a/CD18) and Mac-1 (CD11b/CD18). The latter may also adhere to proteins of the endothelial extracellular matrix such as fibrinogen. ICAM-1 has a restricted tissue distribution and is constitutively expressed at low levels on sub-populations of haematopoeitic cells, fibroblasts and various epithelia. It is however, highly inducible on various cells during inflammation (*Haung et al. 1996*). Endotoxin, TNFα, IL-1 and interferon-γ all induce a large increase in the expression of ICAM-1, but not ICAM-2, by endothelial cells. E-selectin expression is also increased, although more transiently than ICAM-1. These changes occur relatively slowly (in hours) as they involve transcription and translation of mRNA. Thus these cytokines induce receptors for both the rolling and subsequent firm steps in neutrophil adhesion (*Seth et al. 1991*). A proportion of ICAM-1 (sICAM-1) is released into the circulation upon activation of the endothelium by cytokines. ICAM-2 is a shorter, two domain protein that is expressed by unstimulated endothelial cells and also binds to CD11a/CD18. ICAM-3, also called ICAM-R, is expressed by leucocytes but not by endothelial cells and is involved in lymphocyte homotypic adhesion. It is a five-domain protein with extensive homology to ICAM-1 (*Baines et al. 1998*).

VI. 6. a. Polymorph adhesion and margination

These are the processes by which polymorphic leucocytes are attracted to and transported to areas of injury and inflammation. IL-8 has a major regulatory role in the process of neutrophil extravasation. IL-8 induces cleavage of the extracellular portion of L-selectin, up-regulation of CD11b/CD18 expression and adhesiveness on the surface of the neutophil. It also induces the formation of the bipolar shape that characterises crawling neutrophils. IL-8 then forms fixed (haptotactic) rather than soluble (chemotactic) gradients, by binding to glycosaminoglycan heparan sulphate of the endothelial matrix, and neutrophils migrate up this gradient. Other members of this family appear to have similar effects on other leukocyte types, although there is considerable overlap and redundancy in the system. The early phase of neutrophil migration involves rolling of neutrophils on P-selectin and some increase in vascular permeability induced very rapidly by histamine or thrombin. During the late phase, 1-2 hours later, endotoxin, TNF and IL-1 induce endothelial E-selectin and ICAM-1 expression, and IL-8 release, which results in neutrophil L-selectin activation. This results in a prolonged period of neutrophil rolling, activation, and migration out of the blood vessels into the tissues. TNF induces a rapid increase in the adhesiveness of L-selectin, reduces deformability of neutrophils, and induces degranulation and respiratory burst, but only in cells adherent to endothelium. Extravasation of neutrophils during acute inflammation occurs mostly through the endothelium of postcapillary venules, for reasons which relate to the relatively slow blood flow and associated shear stresses as well as the specialized function of endothelium at this site. Cytokines, such as TNF, can directly induce a reversible increase in permeability of endothelium, and addition of neutrophils can further increase this permeability. Leucocyte adhesion and signaling are important steps in the development of inflammatory and immune responses. ICAM-1 (CD54) promotes leucocyte adherence, antigen presentation and subsequent immune-cell proliferation by binding to the leukocyte integrin adhesion receptors CD11a/CD18 and CD11b/CD18. Invasive bacterial infection results in increased expression of ICAM-1 in various immune and non-immune cells and this is followed by increased neutrophil migration and adhesion to these cells (*Sessler et al. 1995, Haung et al. 1996*).

VI. 6. b. Adhesion molecules in disease

Two inherited diseases illustrate the importance of adhesion molecules for the transit of leukocytes. In leukocyte adhesion deficiency type 1 (LAD-1) β_2 integrin subunit (CD18) is lacking. Patients have severe leucocytosis and recurrent infections, and leukocytes from these patients have defective adherence, chemotaxis, and phagocytosis. In LAD II, there are developmental abnormalities, leucocytosis, and recurrent infections. Neutrophils from these patients do not roll *in vivo*. There is impaired synthesis of fucosylated carbohydrates (sLe- X), which include the ligands of the selectins. Neutrophils from LAD II patients do not bind to E-, or P-selectin, although they have normal levels of L-selectin and CD11b/CD18 integrin. This shows that integrins and selectins adhesion molecules have distinct but interdependent roles in neutrophil adhesion during inflammatory response. Experimentally, ischaemic brain damage in mice lacking ICAM-1 is less severe than in normal mice. It has also been noticed that up-regulation of several vascular adhesion receptors occurs in rejected transplanted organs and in the synovium of patients with rheumatoid arthritis. This demonstrates the pivotal role of adhesion molecules in the immune and inflammatory processes and in the pathogenesis of many diseases. Administration of antibodies against ICAM-1 prolongs the survival of murine cardiac allografts, suggesting a potential for anti-adhesion therapy.

Corticosteroids have been shown to decrease the expression of E-selectin and ICAM-1 by stimulated endothelium, and colchicine appears to decrease the adhesiveness of endothelial cells to neutrophils by changing the distribution of E-selectin molecules on the surface of the endothelial cells. Adhesion molecules also have a primary role in platelet homeostasis. $\alpha_{11b}\beta_3$ (IIb/IIIa) glycoprotein on resting platelets adheres to fibrinogen and is defective in Glanzmann's thromboasthenia. Platelets adhere to matrix vWF by their glycoprotein Ib-α receptors. Glycoprotein Ib complex of platelet adhesion receptors is deficient in Bernand-Soulier syndrome. Cancer cells may also use adhesion molecules and receptors to form metastases (*Phillips et al. 1995, Frenette et al. 1996*).

VI. 7. Interleukin -2

IL-2 is a protein with a molecular weight of 15,000 D (103 amino acids) coded on chromosome 4. The main cellular source of IL-2 is activated lymphocytes. IL-2 has various immunologic functions, the most important being the ability to initiate proliferation of activated T-cells. High-affinity receptors for IL-2 are absent from resting T-cells but appear within hours of activation. The IL-2 receptor consists of two chains, α (CD25) and β (TAC). Both chains are required to generate a functional IL-2 receptor. The binding of IL-2 to these receptors gives rise to the clonal expansion of T-cells activated by the specific antigen. Withdrawal or elimination of the antigen leads to the involution of the IL-2 receptor and the cessation of T-cell proliferation even in the presence of IL-2, thus limiting the extent of the clonal expansion. IL-2 may have a role in T-cell maturation, suggested by the presence of immature thymocytes bearing IL-2 receptors. IL-2 markedly enhances the cytolytic activity of a population of natural killer cells capable of killing tumour cells, hence its use in clinical trials in patients with cancer. IL-2 is also critical in clonal expansion of cytotoxic T-cells. Antibodies to its receptors show promise as immunosuppressive agents. No measurable increase in its concentration has been found in sepsis (*Bellomo et al. 1992*). Soluble interleukin2 receptor levels were found useful in facilitating the diagnosis of sepsis in preterm babies (*Spear et al. 1995*).

VI. 8. Interleukin-4 (IL-4)

IL-4 is a glycoprotein of 19 kDa MW. Its gene is located on chromosome 5. A high affinity IL-4 receptor has a MW of 140 kDa. IL-4 is principally made by T-helper cells. IL-4 was originally described as a co-stimulator of B-cell proliferation. It also functions as an important growth factor for T cells. IL-4 is responsible for IgE production. It also has some inhibitory effects on some B cell responses and proliferation induced by IL-2, and can suppress the macrophage-induced TNF, IL-1 and IFN-γ production. It can block the immune response against Leishmania major and schistosomiasis in experimental animals, leading to progressive disease. IL-2 and IL-4 appear to have complementary but subtly different roles by activating distinct

populations of T cells. Preferential stimulation of particular T cell subsets may contribute to the pathology of disease. One mouse strain with high IL-4 and IgE but low IFN-γ when infected with leishmania, shows poor delayed hypersensitivity responses, suggesting overactive T_H2 cells, and develops a severe generalized form of disease. Another strain with low IL-4 and IgE has a milder, localised, infection. Injection of T_H1 clones into infected mice clears infection, whereas injection of T_H2 cells exacerbates infection (*Bellomo et al. 1992*).

VI. 9. **Interleukin-5 (IL-5)**

IL-5 is a T cell derived glycoprotein of 45k Da (123 amino acids) which induces B cells to differentiate into immunoglobulin-secreting cells following antigenic stimulation. IL-5 was originally described as a B-cell-differentiating antigen and was called T-cell-replacing factor. It induces the growth of bone marrow cells committed to the eosinophilic lineage, hence the term eosinophil-differentiating factor (EDF). Cytokines from both types of T cells and from antigen processing cells (APCs) act in concert to regulate the proliferation and differentiation of B lymphocytes. T_H2 cells produce IL-4 and IL-5, which together influence the switch towards IgE and IgA. T_H1 cytokines, IFN-γ and IL-2 acting together, induce production of IgG and IgM. (*Bellomo et al. 1992, Whicher & Evans, 1990*).

VI. 10. **Interleukin-7 (IL-7)**

IL-7 is a 25 kDa protein which acts as a stromal cell factor capable of inducing the growth and proliferation of B cell precursors. It also induces the proliferation of immature thymocytes (*Bellomo et al. 1992*).

VI. 11. **Interleukin-9**

IL-9 is a growth factor for lymphoid and myeloid lineage. There is no information on the role of these cytokines in human disease (*Bellomo et al. 1992*).

VI. 12. Interleukin-10 (IL10)

IL-10 was first described as a product of T_H2 lymphocytes that inhibits cytokine production by activated macrophages. IL-10 is active on T cells, mast cells and B cells. IL-10 inhibits in vitro production of TNF, IL-1, and IL-6 by monocytes and has been shown to be protective in animal models of sepsis. IL-10 levels peaked during the first 48 hours, and elevated levels of this anti-inflammatory cytokine have been reported in sepsis (*Thijs et al., 1995, Dinarello et al. 1987*).

VI. 13. Interferons

Interferons were initially identified for their ability to interfere with viral replication in infected cells. This family of cytokines may be natural negative growth regulators for several different types of cells. Interferons are a multi-gene family of proteins, with MW between 17 to 25 kDa. On the basis of amino acid sequence, three families of these cytokines are identified; IFN-α (with 15 different functional proteins), IFN-β and IFN-γ. Alpha and beta interferons are structurally related polypeptides induced by viral infection and have potent antiviral, antiproliferative and immunologic properties. Their production is induced by bacteria, viruses and double stranded DNA. IFN-γ is induced by mitogens and antigens. Whereas endotoxin directly induces IFN-α and -β from B cells and macrophages, the induction of IFN-γ requires IL-1 and IL-2. IFN-α is a mononuclear cell product, while IFN-β comes primarily from fibroblasts and epithelial cells, and IFN-γ from T-cells.

Despite only 29% homology, IFN-α and IFN-β use the same receptor. They are anti-viral, inducing (2`, 5`)-oligoadenylate synthetase which causes mRNA breakdown. In addition, they are anti-proliferative for many transformed cell lines and cancers. These two cytokines are potent stimulators of natural killer and cytotoxic T cells and up-regulate MHC class I antigens, enhancing antigen recognition. One of the most dramatic therapeutic applications of all cytokines has been the complete remission obtained by IFN-α in hairy cell leukaemia. IFN-α also is effective in chronic myeloid leukaemia, has some activity in myeloma and lymphomas but the response of solid tumours was

disappointing (*Whicher & Evans, 1990)*. IFN-α has been extensively used in the management of various malignancies and recently in the treatment of chronic active hepatitis of viral aetiology (*Bellomo et al. 1992*). IFN-γ has a wider role in inflammation, is a much more potent immunomodulator, and interacts more closely with cytokines (*Morrison et al. 1987*). It participates in the cytokine network by inducing synthesis of IL-1 and TNF, resulting in subsequent production of IL-2 and IFN-γ by T-cells, and thus a positive feedback loop. IFN-γ induces TNF receptors on many cells and enhances the cytolytic effects of TNF. Gamma interferon induces class I & II MHC molecules and is a potent activator of macrophages. It enhances macrophage oxidative metabolism (which results in increased bactericidal and tumouricidal activity) and stimulates their phagocytic activity by inducing the expression of Fc receptors. This cytokine augments B-cell stimulating cytokines and increases antibody production, but antagonizes IL-1 effects on connective tissue by inhibiting the synthesis of collagens. IFN-γ has been shown to be protective in leprosy, to aid in vaccination against malaria, to partially correct the phagocytic defect of chronic granulomatous disease, and to increase survival and reduce sepsis in mice with simulated surgical wound infection (*Bellomo et al. 1992*).

VI. 14. **Haematopoeitic cytokines**

Cytokines affecting haematopoeisis are called colony-stimulating factors because of their ability to induce the formation of colonies when marrow is suspended in vitro. Interleukin -3 and granulocyte-monocyte colony-stimulating factor (GM-CSF) are two well-characterised examples. These substances probably play no part in base-line haematopoeisis but they are responsible for myeloid hyperplasia and peripheral leucocytosis. Some cytokines, such as gamma-interferon and TNF, antagonize CSFs and may contribute to aplastic anaemia (*Dinarello et al. 1987*).

VI. 14. a. **Interleukin-3 (IL-3)**

IL-3, also known as multi-colony stimulating factor, is a glycoprotein produced by activated T cells. It is encoded on chromosome 5 (5q23-31) and has a MW of 28 kDa

and 140 amino acids. It is involved in regulating the growth and differentiation of haematopoeitic progenitor cells. It is synergistic with IL-6 and erythropoeitin. IL-3 has the widest range of targets, stimulating proliferation of macrophages, neutrophils, eosinophils, megakaryocytes, early erythroid cells, and mast cells.

VI. 14. b. Colony Stimulating Factors (CSF)

CSF defines a collection of glycoproteins that regulate the proliferation of bone-marrow-derived precursor cells into functionally active cells and modify the effector functions of the mature cells. Three CSFs have been described; GM-CSF, G-CSF and M-CSF. There is no significant homology between these proteins. CSFs are produced by many cell types including fibroblasts, macrophages, endothelial cells, T- and B-cells, and some epithelial cells. Endotoxin is a potent inducer of CSFs from these cells. The precise role of this endotoxin-induced production of CSFs is not clear, but they appear to play a key role in the development of endotoxin tolerance, and thus may serve as a host defense mechanism (*Morrison et al. 1987*). CSFs affect mature cells at lower concentrations than required to enhance proliferation of precursor cells. CSF act on macrophages and monocytes to stimulate their phagocytic activity, synthesis of prostaglandins, secretion of proteases, and expression of cytolytic activity. They induce granulocyte production of superoxide, and produce other cytokines. Clinically, they reduce the duration of chemotherapy-associated neutropenia in BMT and may be of use in overwhelming sepsis (*Bellomo et al. 1992*). GM-CSF acts on virtually the same cells as IL-3, except mast cells, and is encoded on the same chromosome 5 (5q23-33). It has a MW of 35 kDa and 127 amino acids. GM-CSF exerts its primary effects later in the maturation sequence supporting cells committed to becoming myelocytes and monocytes. GM-CSF also increases the bactericidal competence of granulocytes and the numbers of receptors for chemoattractants. G-CSF gene is located on chromosome 17 and encodes a 20-kDa glycoprotein. G-CSF is produced by stromal cells, macrophages, neutrophils, and endothelial cells. Unlike the other CSFs, which are produced as single chain polypeptides, G-CSF is a dimer. Each of these CSFs acts on its own receptor, and different CSF receptors can be expressed on the same cell at the same time, allowing

different CSFs to act in concert. IL-3 would act on pluripotent cells producing a wide range of cell types, but GM-CSF and G-CSF would act on cells committed to certain lineage. All CSFs can induce production of IL-6 in normal myeloid cells, and the latter in turn induces the production of GM-CSF and expression of receptors for GM-CSF, IL-3 and G-CSF (*Whicher & Evans, 1990*).

VI. 14. c. Monocyte chemoattractant protein-1 (MCP-1)

MCP-1 is a 76 amino acid polypeptide capable of activating and attracting monocytes. It can be produced by lymphocytes, fibroblasts, or monocytes stimulated by IL-1, TNF, PDGF, or LPS (*Bellomo et al. 1992*).

VI. 15. Platelet derived growth factor (PDGF)

PDGF has a MW of 30 kDa. Other cells, such as monocytes, fibroblasts, and endothelial cells, can also produce it. It is a growth factor acting on a broad spectrum of cells. It is likely to be important in the generation of granulation tissue during wound repair, in fibroproliferative inflammation, and in atherosclerosis. It has been implicated in the development of pulmonary fibrosis, in the pathogenesis of scleroderma, and in synovial cell proliferation in rheumatoid arthritis. PDGF also participates in the release of pro-inflammatory cytokines (*Bellomo et al. 1992*).

v. 16. Transforming growth factor (TGF)

TGF-α and TGF-β are distinct peptides with unique biological activities and separate receptors. TGF-α is made by keratinocytes and monocytes and is produced by cancer cells. It is a powerful growth factor. It may function as an autocrine stimulator of growth in malignancy. TGF-β is released by the alpha granules of aggregating platelets. It is powerfully chemotactic for monocytes. It induces production of IL-1, TNF, PDGF, and therefore amplifies the phlogistic response. TGF-β also inhibits B- and T-cell proliferation at femtomolar concentrations, being one of the most potent biological lymphocyte suppressers known. Hence it may play an important immunoregulatory role in inflammatory states (*Bellomo et al. 1992*).

Chapter VII

Pathophysiologic Role of Cytokines

No direct causative role of a cytokine has been established in any disease. However, the excessive or insufficient production of cytokines may contribute to certain disease states, particularly those of infectious and autoimmune origins. For instance, decreased production of IL-2 from the leucocytes of patients with AIDS, autoimmune disease, type-I diabetes mellitus, burns and cancer, has been reported. The production of gamma interferon is reduced in patients with lepromatous leprosy and connective tissue disease, such as SLE and rheumatoid arthritis, as well as in newborns. The production of alpha interferon is reduced in patients with myeloproliferative disorders and chronic hepatitis B. It remains to be seen whether these defects could be corrected in vivo by the systemic administration of cytokines or cytokine inducers. Defects have also been identified in cytokine signaling pathways, such as the defect in IL-2 receptor that is associated with recurrent or severe atypical mycobacterial or BCG infection.

Overproduction of TNF-α protects against mycobacterial infection, but causes overwhelming septic shock in meningococcal infection. Absence of the common cytokine receptor γ chain, which is essential for -2, -4, -7, -9 and-5 receptor function, leads to T-, B+, NK- ve severe combined immunodeficiency (SCID). Absence of Jansus-associated kinase 3 (JAK-3), a cytokine-receptor-associated signaling molecule, also leads to a similar form of SCID. In arthritis, synovial cells spontaneously produce high

levels of IL-1. Intra-articular injection of IL-1 produces a transient arthritis, which becomes chronic on repeated injections. TNF-α produces less joint inflammation than IL-1, but the two have additive effects. IL-6 is present in large amounts in the joint fluid and its levels correlate with systemic measures of disease activity. However, IL-6 does not stimulate either collagenase or PGE2 from synovial fibrobalsts or chondrocytes. It was postulated that abnormalities of cytokine production, or the expression of their receptors, might underlie some forms of malignancy. For instance, it was shown that human myeloma cells and Kaposi's sarcoma, respectively, respond to IL-6 and fibroblast growth factor in an autocrine fashion. Both cardiac myxoma and the hyperplastic lymph nodes of Castleman`s disease produce high quantities of IL-6. In the latter, multiple myeloma may result, and removal of involved lymph nodes results in complete recovery. On the other hand, patients with a large tumour burden have deficient IL-1 production. The interferons, IL-2 and TGF-β have growth-inhibitory properties and have been successfully used in the treatment of some malignancy, such as hairy cell leukaemia (*Gennery et al. 2001*).

Cytokines seem to have a prominent role in graft rejection. Both the immunoregulatory and the inflammatory cytokines appear to participate in this process. IL-2 is a key component in T cell priming for allograft rejection and high levels of IL2-R have been found in liver transplant recipients, levels rising 6 days before rejection. Acute renal allograft rejection is associated with high levels of TNF detectable 3-4 days before the clinical rejection episode. High levels of IL-6 are detected in plasma and urine of renal transplant recipients. Without complications, peak levels of this cytokine are reached 2-3 days after transplantation, falling to normal within 3 weeks. In rejection, IL-6 levels start to increase 2-3 days before clinical evidence of rejection, urinary levels being most marked. IL-8 may be of value in determining prognosis in bone marrow transplant (BMT). Patients who developed veno-occlusive disease showed a short, very high IL-8 peak 1-4 days after diagnosis of the liver disease. The highest levels of IL-8 was in those who developed acute GvHD, whereas IL-6 was highest in those who developed bacteraemia *(Remberger et al. 1997).*

VII. 1. Cytokines and sepsis

Cytokines are decisive factors in determining the pathophysiology of the sepsis syndrome. Sepsis syndrome seems to result from overwhelming systemic inflammation which is caused by excessive release of cytokines into the systemic circulation. Four cytokines, TNF-α, IL-1β, IL-6, and IL-8 have been most strongly associated with the sepsis syndrome. Cytokines are released in a sequential manner resulting in a cytokine cascade. TNF and IL-1 appear to mediate most of the physiological disturbances that are characteristic of sepsis. Proximal cytokines (TNF and IL-1), as well as endotoxin, stimulate the production of distal cytokines, such as IL-6 and IL-8. The latter seem to intensify and perpetuate the inflammatory response and might have a role in tissue repair. IL-6 may, however, down-regulate TNF and IL-1 production, which might be important in limiting the inflammatory response. IL-8 is a potent activator and chemoattractant for polymorphs and is thought to mediate neutrophilic tissue inflammation (*Blackwell et al. 1996*).

VII. 1. a. The acute phase response

The acute phase response is a systemic reaction to tissue injury induced by inflammation, infection and trauma. It is characterised by fever, leucocytosis and negative nitrogen balance and an increased synthesis of hepatocye-derived plasma proteins known as acute phase reactants (e.g. CRP and SAAP) and by the reduced synthesis of albumin and transferrin. IL-6, IL-1 and TNF have been implicated in the induction of the acute phase response. There is a synergistic interaction between these cytokines and glucocorticosteroids. IL-1 and TNF stimulate IL-6 and all three induce acute phase response reactant genes. IL-6 acts at the level of transcription with maximum mRNA response at 4 hours. The maximum response is increased by concomitant dexamethasone. IL-1 and IL-6 stimulate ACTH release with a subsequent increase in cortisol levels to inhibit cytokine and hence switch off the response. IL-6 is the most potent inducer of the acute phase response and it only can induce the full range of acute phase proteins. Diseases characterized by markedly high IL-6 levels, e.g. polymyalgia and myeloma, are also characterized by marked acute phase responses with

very high CRP and ESRs and by being very responsive to steroids. In trauma patients, IL-6 and IL-8 are elevated early and can identify those who develop multiple organ failure (MOF). Elevated levels of sICAM-1 take longer to develop (*Patrick et al. 1996*).

VII. 1. b. The systemic inflammatory response syndrome (SIRS)

High levels of cytokines are found in the plasma of patients with a variety of infectious diseases, such as meningococcemia and cerebral malaria. Cytokines mediate multiple effects in these conditions, including changes in vasomotor tone, procoagulant activity, and leucocyte adhesion, and their levels are associated with adverse outcome. Sepsis syndrome represents a spectrum of pathophysiology that results from an exaggerated host inflammatory response to a specific inciting event. The clinical spectrum of sepsis syndrome ranges from mild physiological derangements to severe multiple organ dysfunction and death. Clinically, four stages of the sepsis syndrome can be identified:

1. Systemic inflammatory response syndrome (SIRS) is a term used to define a constellation of clinical signs, which include tachypnoea, fever or hypothermia, tachycardia and leococytosis, or leucopenia, with a left-shifted differential white blood cell count. SIRS can result from either infectious or non-infectious conditions. Non-infectious conditions include trauma, burns, haemorrhagic or hypovolaemic shock and pancreatitis.

2. Sepsis is considered when there is a systemic response to a possible infection, which can be bacterial, parasitic, protozoan or viral. Evidence of bacteraemia or a focus of infection is not required. Septicaemia is sometimes used when an organism is recovered from blood culture or its toxic products are identified. Although most cases are related to gram-negative infections, full-blown septicaemia can result from Gram-positive infections.

3. Severe sepsis is defined as sepsis which is associated with specific or multiple organ dysfunction.

4. Septic shock is when there is systemic hypotension associated with tissue hypoperfusion and anaerobic metabolism.

This classification predicts mortality in a stepwise fashion; a mortality of 7% for SIRS, 16% for sepsis, 20% for severe sepsis, and 46% for septic shock. The severity of sepsis is proportional to the intensity of the host response. Usually, an invading micro-organism is efficiently cleared by the monocyte-macrophage system after opsonization by antibody and complement. Thus bacteraemia results only in short-lived illness. Sometimes, however, depending on the age of the patient, the virulence and number of bacteria in the blood, the nutritional and immunologic status of the host, and the timing and nature of therapeutic intervention, a systemic inflammatory response is established and can progress independently of the original infection. In many patients with sepsis, it is difficult to document a bacterial cause.

Endotoxin is a major factor contributing to the pathogenesis of bacterial infection and the host responses such as fever. The responses elicited by endotoxin are neither specific nor unique. All endotoxin components, lipid-A, protein and the polysaccharide, participate in endotoxin pathophysiology. The polysaccharide portion of endotoxin initiates the alternative complement pathway and acts as ligands for the receptors on monocytes and macrophages. The most unique feature of endotoxin is its ubiquitous capacity to elicit the entire spectrum of host effector molecules. The diversity of these molecules confound efforts to define a unifying concept of endotoxin action. Other microbial products, including those from gram-positive organisms, elicit similar pathophysiological responses. There is increasing experimental and clinical evidence that a number of cytokines play a major role in the response to injury and infection and in the development of organ damage in critically ill patients (*Aleksander et al. 1984*). TNF is a key mediator of organ injury during sepsis, its levels are elevated in early shock and high levels correlate with unfavourable outcomes (see above). Interleukin-1 induces leucocytosis and muscle catabolism, and causes hypotension and tachycardia. Interleukin-6 is a pyrogen and a lymphocyte activator and is the major stimulator of the acute phase reaction. Interleukin-8 is a powerful neutrophil activator and is involved in

the production of ARDS. High levels of IL-8 have been found in patients with septic shock. As a result of the interplay of microbial products and pro-inflammatory mediators, many biochemical and immunologic pathways are activated to control bacterial invasion and to regulate physiologic derangements. Thus, there is activation of the complement and coagulation cascades, the kallikerin-kinin system, and production of β-endorphins. If these physiologic responses surpass the host's tolerance, the disease progresses and can result in multiple organ dysfunction and death (*Bellomo et al. 1992*).

VII. 1. c. Anaphylatoxins

C3a and C5a are low MW cationic polypeptides generated following the activation of the complement system. They induce vasodilatation, smooth muscle contraction, and chemotaxis of neutrophils and monocytes (C5a). They are also effective immunoregulators of the humoural immune responses. Endotoxin can activate the complement system in the absence of any anti-endotoxin antibodies. Lipid-A activates the classical pathway while the polysaccharide region activates the alternative pathway. Both the core polysaccharide (l-glycero-d-mannoheptose) and O-antigen polysaccharide independently contribute to this activation. As a consequence, individual endotoxins from different gram-negative organisms vary widely in their capacity to mediate complement activation.

VII. 1. d. Arachidonic acid metabolites

The generation of arachidonic acid by the action of phospholipase A_2 on cell-membrane-localized phospholipids is an early event in the activation of most mediator cells. Subsequent metabolism of arachidonic acid by either the cycoloxygenase (to produce prostaglandins) or the lipoxygenase (to produce leukotrienes) pathway is critical to the development of host inflammatory responses. Endotoxin can induce both pathways. These products are essential intermediates in the triggering signal for macrophage activation. PGE2 is required for generation of collagenase and tissue factor activity. The leukotrienes are required in induction of IL-1 and tumouricidal activity. Arachidonic acid metabolites also act as regulatory modulators in the end-stage of

macrophage activation and have direct effector function such as vasodilatation, vasoconstriction, and chemotaxis.

VII. 1. e. Platelet Activating Factor (PAF)

PAF represents a family of cell-derived lipid mediators with acetyl-glycerol-ether-phosphorylcholine as their basic structure. PAF is produced in many cell types, including neutrophils, basophils, monocytes, platelets, and endothelial cells. It has diverse biological activities including platelet aggregation and secretion, neutrophil degranulation, smooth muscle contraction, increased vascular permeability, hypotension, and death. It is one of the most potent autocoid mediators yet described. It plays a role in anaphylactic shock and endotoxin shock. PAF induces neutrophil margination on endothelial cells. IL-1 is a potent stimulator of PAF production from endothelial cells.

VII. 1. f. Tissue Activity Factor (TAF)

Diffuse microvascular thrombosis is a significant clinical finding in acute endotoxaemia and gram-negative sepsis. Both the intrinsic pathway (by endotoxin mediated activation of FXII) and the extrinsic pathway (by expression of TAF by endotoxin-stimulated monocytes) participate in this coagulopathy. Tissue factor is a cell-surface-localised glycoprotein induced by endotoxin on monocytes and macrophages. This glycoprotein serves as a receptor for factor VII, which undergoes allosteric conformational change to an enzymatically active form upon binding to tissue factor. The active protease then cleaves factors X and IX. Tissue factor remains cell associated. The mRNA inhibitor cycolhexamide, indomethacin and hydrocortisone have inhibitory effects on the generation of tissue factor (*Morrison et al. 1987*).

VII. 1. g. Endorphins

Endorphins are endogenous opioids recently implicated as mediators of the hypotension of septic shock. Endotoxin can induce their synthesis and release from splenic adherent monocytes. Naloxone has a palliative effect on their hypotensive action although no demonstrable beneficial effect on survival in gram-negative sepsis. These

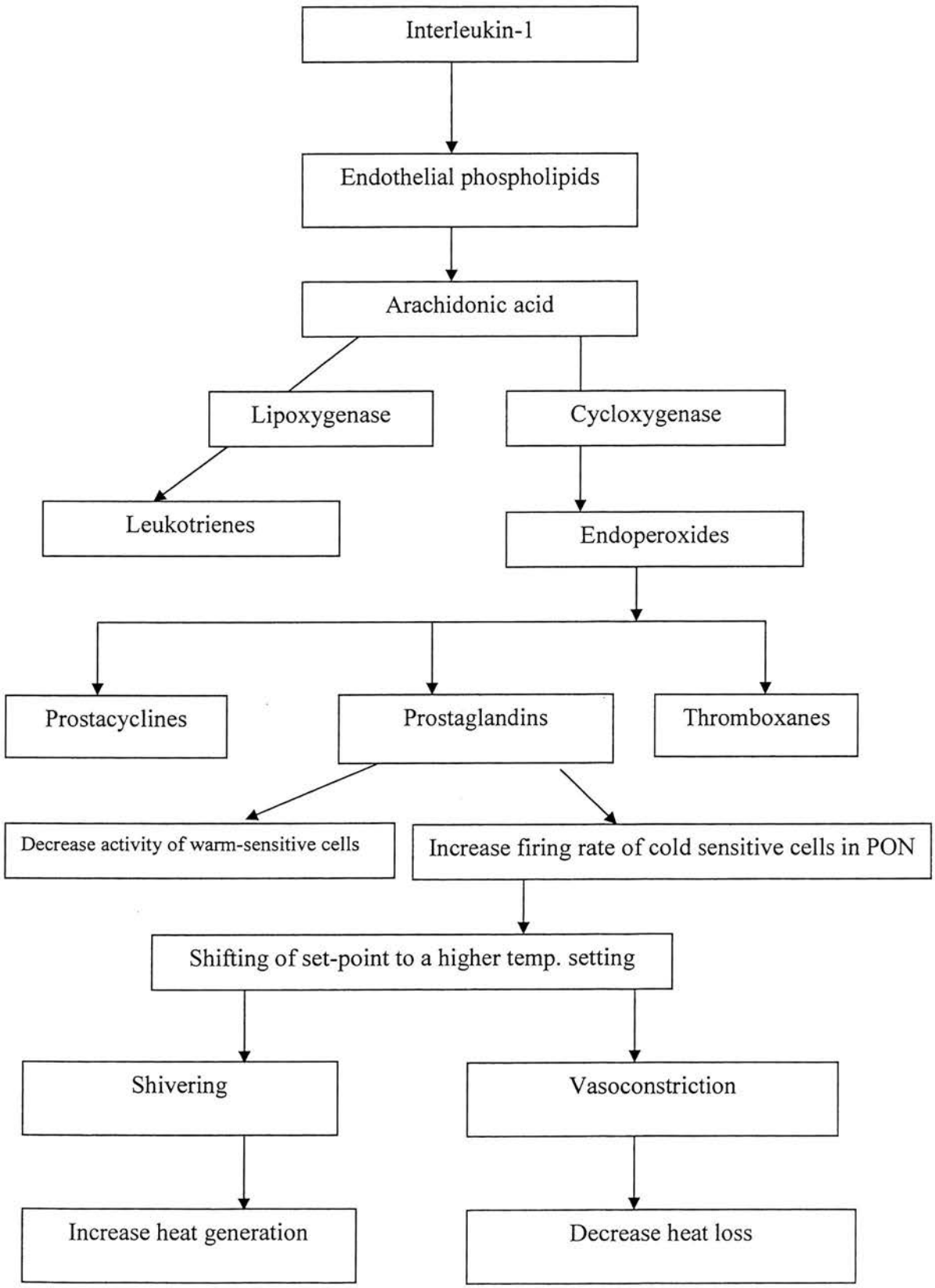
neurohormones have a variety of physiologic effects including analgesic and behavioural changes; in addition they have both enhancing and suppressive immunoregulatory effects. The pituitary gland is a major source of the 31kDa precursor molecule that contains sequences for both ACTH and β-endorphin. Modification of the latter yields α and γ endorphins, which represent the first sixteen and seventeen aminoacids of β-endorphin respectively. The pentapeptide metencephalin has also been shown to have immunoregulatory activity (*Morrison et al. 1987*).

VII. 2. Cytokines & fever

Fever is produced by three mechanisms; the first involves the raising of the set point in the hypothalamic thermostat (*Figure 5*). This occurs in infection, collagen vascular disease, and malignancies. This type of fever is lowered by antipyretics and physical removal of heat. A second type of fever results from heat production in excess of heat loss. Examples of this mechanism include salicylate overdose, hyperthyroidism, and malignant hyperthermia. The third type of fever is caused by defective heat loss, as seen with ectodermal dysplasia, heat stroke (excessive environmental temperature), and poisoning with anticholinergic drugs. Antipyretics are ineffective for the second and third types of fever. Fever occurs as a result of a number of complex biologic interactions. Exogenous pyrogens, including viruses, bacteria, fungi, antigen-antibody complexes, and drugs, are engulfed by phagocytic leucocytes. Phagocytic leucocytes, when activated by leukotrienes, prostaglandins, and calcium, synthesize the endogenous pyrogen interleukin-1. Monocytes and macrophages are particularly effective producers of interleukin-1, polymorphic neutrophils and eosinophils less so, and lymphocytes not at all. Interleukin-1 (also known as lymphocyte-activating factor), in conjunction with interleukin-2, is responsible for increasing the number of helper T cells and initiating the production of prostaglandins in the hypothalamus. Helper T cells play a key role in fighting infections, and prostaglandins are responsible for producing fever. Interleukin-1 acts on the arachidonic acid pathway, stimulating the production of prostaglandins from the phospholipids in the vascular endothelial cells of the hypothalamus. Prostaglandins increase the firing rate of cold-sensitive cells in the preoptic anterior nuclei (PON) and

decrease the activity of warm-sensitive cells, resulting in shifting of the set point to a higher temperature setting. The body responds to a perceived colder peripheral skin temperature by vasoconstriction, which decreases heat loss, and through increased shivering, which increases heat generation. Antipyretics lower the central set point, resulting in the periphery feeling hot at the skin surface. The body lowers its temperature by vasodilation, which increases heat loss, and lying quietly, which minimizes heat generation. Aspirin, paracetamol, and non-steroidal anti-inflammatory drugs exert their antipyretic effect through inhibition of the cyclo-oxygenase enzyme, thereby preventing synthesis of prostaglandins from arachidonic acid. Because they do not suppress interleukin-1, they do not diminish proliferation of helper T cells and, thus, do not adversely affect the body's ability to fight infection. Corticosteroids, on the other hand, decrease interleukin-1 release from monocytes and macrophages quantitatively. This activity is detrimental to the body's ability to fight infection. Aspirin, paracetamol, and non-steroidal anti-inflammatory agents all have excellent antipyretic and analgesic activity. Only aspirin and non-steroidal agents have anti-inflammatory activity.

Figure 5: Pathway of fever production



Chapter VIII

Clinical Uses of Cytokines

VIII. 1. Cytokines in therapy

Recombinant growth factors such as EPO and G-CSF are widely used for treatment of anaemia and neutropenia in cancer patients undergoing cytotoxic therapy and in severe sepsis. In vivo administration of IL-6 in primates increases platelet counts by 100% (*Kishimoto et al. 1992*). This observation could be of major practice importance and may lead to the use of various combinations of haematopoeitic cytokines, such as IL-6, IL-3 and stem cell factor (c-kit ligand) in new therapeutic approaches of thrombocytopenia. A number of theoretical strategies exist for reducing the actions or interactions of these cytokines and adhesion molecules. They include inhibiting their production or expression, e.g. the non-specific inhibition by steroids in meningitis, and blocking their actions once released. The latter include naturally occurring competitive antagonists, soluble receptors, antibodies and blocking agents. Examples of anti-cytokine treatment strategies that have been evaluated include:

1. Polyclonal or monoclonal endotoxin antibodies: Monoclonal antibodies are of large molecular weight and thus tend to induce an immune response in themselves. Anti-lipid A (of LPS) is the most extensively studied. A neutrophil

granule protein called bactericidal/permeability increasing protein binds and neutralizes the lipid A component of the LPS.

2. Anti-TNF antibodies or TNF receptor constructs: Two distinctive TNF receptors and a whole family of TNF-related proteins have now been described. Both TNF receptors exist in monomeric soluble form in plasma, as well as being expressed on the cell surface. TNF itself exists as a trimer that probably binds two receptors simultaneously on the cell surface. Monomeric soluble receptors appear to have little antagonistic activity. However, engineered dimeric receptors bound to the Fc portion of immunoglobulin appear to block the effects of TNF highly effectively.
3. Blocking the actions of IL-1 by IL-1ra: There are three members of the IL-1 family of cytokines - IL-1α, IL-1β, and IL-1 receptor antagonist. All three bind to the two recognized IL-1 receptors but the IL-1ra has no signaling effect and thus acts as a competitive antagonist. The type-2 IL-1 receptor also acts as a negative-feedback regulator as it does not transmit a signal on binding to IL-1. This type-2 receptor is shed from the cell surface and has been found in high concentrations in plasma of patients with septic shock and appears to have a selective high affinity for IL-1β. A more indirect approach is the inhibition of the enzyme that cleaves the IL-1β precursor into its active form.
4. Several disorders, including allograft rejection, are due to an undesired immune response in which cytokines play a part. Immunosuppressive agents such as glucocorticoids and cyclosporin are potent inhibitors of both cytokine synthesis and the response of cells to cytokines. Immunosuppression may also be induced with the use of cytokines fused to toxins (chimerism) which home activated cells bearing the cytokine receptors. Antibodies directed towards cytokines or their receptors are also potentially useful immunosuppressive therapy. Some cytokines are themselves immunosuppressive, e.g. IL-6 and interferons inhibit cellular responses and proliferation.

Human trials of anti-cytokine therapy have been disappointing for the following reasons:

1. The timing and dose may be critical
2. Blocking cytokines may inhibit host defenses
3. Such therapy might disturb the complex immunoregulatory balance with extremely unpredictable effects
4. Cytokines have redundant and overlapping functions and inhibition of a single cytokine is unlikely to provide a solution.

A better approach may be the use of counter-inflammatory molecules such as IL-10 or targeting more proximal points in the cascade e.g. NF-kB. Cytokines are not stored in intracellular compartments and are newly synthesized and released in response to inflammatory stimuli. This regulation occurs predominantly at the level of gene transcription with new expression of cytokine mRNA. Nuclear factor kB (NF-kB) is a transcription factor that plays a central role in regulating the cytokine cascade by binding to regulatory portions of the cytokine genes. NF-kB is activated by endotoxin, TNF and IL-1. Intervention to inhibit NF-kB activation could down-regulate systemic inflammation to a much greater extent than blocking the production or action of a single cytokine.

Ways to apply these new therapeutic strategies are being sought in numerous other disease processes including acute infection. In addition to chronic inflammatory conditions such as autoimmune arthritis and inflammatory bowel disease, processes as diverse as proliferation of leukaemic cells, tumour metastasis and destruction of pancreatic-β cell in diabetes mellitus, all appear to involve cytokines (*Finn et al. 1995*). Interferon therapy has been found to be beneficial in chronic myelogenous leukaemia, hairy-cell leukaemia and other cancers. Alpha interferon has some success in chronic hepatitis B and some neurologic disorders, and topically for herpetic keratitis, genital warts and upper respiratory tract infections. Beta interferon has also been used to treat juvenile laryngeal papilloma. IL-2 has received the most attention in cancer therapy, melanoma and renal cell carcinoma being the most sensitive (*Dinarello et al. 1987*).

VIII. 2. Cytokines in diagnosis

To enter the repertoire of the clinical chemistry laboratory, cytokine assays must either provide new information of use in diagnosis or management, or surpass existing assays of disease markers in sensitivity, specificity, or speed of response. Although new information is being obtained from studies of cellular cytokine production, none of these has given rise to useful diagnostic tests as yet. It is in the area of improved markers that cytokine assays are beginning to play a role. Most of the current markers of inflammation or tissue damage - such as the acute phase proteins, enzymes and tissue antigens - reflect either the effects of cytokines (e.g. acute phase proteins and fever) or the cellular damage caused by the inflammatory process (e.g. tissue enzyme release). Cytokine measurements are of fundamental interest because increases in their concentrations can be expected to precede the other changes. Indeed, it is this characteristic that so far suggests most of the potential clinical applications for cytokine measurement (*Whicher & Evans. 1990*). Cytokines are usually of low MWt (<80 kDa) and are extremely potent (acting at picomolar concentrations). They regulate both the amplitude and duration of the inflammatory response. The response of a cell to a ctyokine depends on the cytokine`s local concentration, the cell type, and the presence of other cytokines to which it is being concomitantly exposed. Cytokine interactions are also modulated by changes in receptor density and solubilisation of receptors, which may then act as inhibitors of cytokine function. The concept of an intricate network of polypeptides, gained from in vitro research, makes the interpretation of in vivo data very difficult to interpret. The measurement of cytokine concentrations in body fluids may or may not reflect anticipated intercellular interactions and cannot take into account the concomitant effects of other cytokines on effector or target cells. Hence, the inability to measure the presence of a cytokine in a biological fluid does not necessarily imply a lack of biological activity at a cellular level. Likewise, a markedly elevated level of a cytokine might reflect deficiency of inhibitors. Therefore, the interpretation of what we measure is, at best, controversial (*Bellomo et al. 1992*).

The inflammatory cytokines (IL-1, TNF, and IL-6) are increased in blood and tissue fluid during inflammation from causes as diverse as burns, graft rejection, and sepsis. If

measurements are to have a value in clinical practice, they must lead to a more rapid and precise diagnosis. IL-6 and TNF may have this potential in graft rejection and septicaemia. However, large inter-study variations in the proportion (16-100%) of patients with detectable plasma levels of TNF & IL-1, and correlation with outcome, exist. Possible explanations for this discrepancy include:

1. Peak levels of these cytokines occur rather rapidly after the challenge, and therefore the time of measurement after the onset of sepsis may have influenced the results. In patients admitted to intensive care units, TNF levels, when detectable, are usually higher at the time of admission than during subsequent sampling, especially in survivors (*Dinarello et al. 1987*).
2. The cytokine levels in plasma may not wholly reflect their synthesis by the cells. For instance, plasma levels of TNF do not correlate with monocyte-associated TNF in septic patients.
3. Differences in assay technique could also be responsible for the differences observed; Receptor-bound cytokines may not be detected by bioassays that measure unbound portions only and also by immunoassays that use monoclonal antibody against the receptor binding sites of the cytokine. Levels of both TNF receptors have been found elevated in sepsis. TNF/sTNF-R ratio at the time of admission has been found to be of predictive value for outcome.
4. Some cytokines, such as TNF, are detectable in plasma of healthy individuals. This is partly explained by non-specific reaction and, also, if heparin is used as an anticoagulant, it can stimulate TNF-α release. Raised TNF-α levels also appear to be a feature of certain healthy people (*Emery et al. 1998*).

In patients with sepsis, detectable or elevated levels of the pro-inflammatory cytokines TNF, IL-1, IL-6 and IL-8, as well as the anti-inflammatory cytokine IL-10, are found in the circulation. TNF-α levels are increased in most patients with sepsis, whereas IL-1 and IFN-γ are increased in fewer than 20% of the cases. Elevated IL-6 levels are found most consistently, but elevated levels of IL-8 are often detected as well. Whereas increased concentrations of soluble TNF receptors and IL-1ra appear in circulation, sIL-6 receptor levels seem to be reduced in sepsis. The immune cytokine IL-

2 may have a role in indicating activation of cell-mediated immunity as immunoglobulins indicate involvement of the humoural immune system. Patients with IL-1 detectable with increased concentrations of TNF have a poor outcome, but the association with mortality or severity of disease is most obvious for IL-6 (*Whicher & Evans 1990*). IL-6 may be the marker of choice, given the assay sensitivity and the massive incremental changes observed. Clinical implications of mean levels might be greater than those of peak levels, so measurements at regular intervals could be more helpful for many of these cytokines (*Thijs et al. 1995*).

VIII. 3. Cytokines as markers of bacterial infection

Attempts have been made to incorporate cytokines in the armamentarium used to detect or predict bacterial infection in patients presenting with the sepsis syndrome. Of the cytokines proved to be of value in this respect are; IL-6, IL-8, and sICAM-1.

IL-6 was most extensively studied as a marker of sepsis. Initial studies showed that the majority of patients admitted to intensive care units (ITU) with the sepsis syndrome had elevated levels of IL-6 (*Hack et al. 1989, Casey et al. 1993*). In a group of septic surgical patients, IL-6 was the only cytokine consistently elevated and its levels correlated with clinical severity and outcome (*Damas et al. 1992*). In children with fever and malignant disease, IL-6 was more sensitive than CRP in detecting sepsis. However, it failed to differentiate infected from non-infected patients and could not influence initial treatment decisions in patients with granulocytopenia (*Calandra et al. 1991, Abrahamsson et al. 1997*). Conditions other than sepsis are also associated with high plasma levels of IL-6. For instance, in patients with severe burns, IL-6 rose to 2-100 times the normal levels within hours of the injury (*Nijsten et al. 1987*).

IL-6 in umbilical cord blood has consistently been shown to be a sensitive marker for early infection in newborn infants (*Lehrnbecher et al. 1996, Mehr et al. 2000*). IL-6 was found more useful than CRP in predicting sepsis in very low birth weight babies (*Kuster et al. 1998*), and was more predictive of necrotising enterocolitis than TNF (*Harris et al. 1994*). IL-6 combined with TNF gave 100% specificity in diagnosing early neonatal sepsis (*de Bont et al. 1994*). Combining IL-6 with CRP

yielded 98% sensitivity and 91% specificity in detecting early neonatal sepsis (*NG et al. 1997*). 100% sensitivity was reported for IL-6 in identifying infected newborns who had a negative CRP test (*Buck C, 1994*). However, earlier studies found no correlation between plasma levels of IL-6 and the febrile response in newborns suspected of sepsis (*de Bont et al. 1993*). Furthermore, non-septic babies with physiological jaundice were reported to have had significantly higher plasma IL-6 levels than adult controls (*Ozdemir et al. 1994*). Serum IL-6 levels were elevated in all children with bacterial sepsis, with significantly higher levels in non-survivors, whereas no IL-6 was detectable in control subjects (*Sullivan et al. 1992*). High IL-6 levels were also found in serum of patients with septic shock (*Jacobs et al. 1996*) and in CSF of children with acute bacterial meningitis (*Helfgott et al. 1989*). The median serum concentration of IL-6 was 1000 times higher in patients with meningococcal septic shock than in patients with bacteraemia, meningitis, or combined septic shock and meningitis, and levels correlated with mortality (*Waage et al. 1989*). Children with bacterial acute otitis media (AOM) had significantly higher IL-6 levels than those with non-bacterial AOM. Pneumococcal AOM was associated with higher levels than H. Influenzae or Moraxella AOM (*Tehro et al. 1995*). Urinary levels of IL-6 were found elevated in febrile children with UTI, and were reduced after initiation of antibiotic therapy (*Jantausch et al. 2000*). Serum IL-6 levels effectively predicted bacteraemia in patients presenting with sepsis in the A&E department (*Moscovitz et al. 1994*).

IL-8 became measurable by a fully automated random access assay in 1996. Cytokines generate an inflammatory response both systematically and locally, at the mucosal level. Like interleukin-6, urinary IL-8 concentrations are elevated in patients with febrile UTI, and the response appears to be related to the virulence of the invading bacteria (*Jantausch et al. 2000*). IL-8 has also been detected in CSF of patients with meningitis (*Marty et al. 1994*). IL-8 was present in the pulmonary oedema fluid of all patients with ARDS. The high concentrations of IL-8 in pulmonary oedema fluid, coupled with the relatively low concentrations of IL-8 in the plasma, suggested that the lung was the primary source of IL-8 in the patients with ARDS (*Miller et al. 1996*). IL-8 has been found a more sensitive (Sensitivity 100% and 95% respectively) marker for

histologic chorioamnionitis and preterm delivery than amniotic fluid culture and G stain *Cherouny et al.1993*). This supports the role of IL-8 in recruitment of neutrophils into chorionic membranes and placenta during intrauterine infection, and that IL-8 is released into the amniotic fluid during the early phase of ascending infection before bacteria or their products are detectable in amniotic fluid. Cord IL-8 concentrations measured in septic newborns were also significantly elevated compared with non-septic newborn infants (*Berner et al. 1998*). Other workers found elevated cord IL-6 levels in babies with many non-infectious neonatal complications while IL-8 was elevated only in early neonatal bacterial infection (*Santana et al. 1997*). In newborn infants evaluated for sepsis, IL-8 was positive at the first suspicion of bacterial infection (BI) in 100% of infants with culture proven BI and in 78% of infants with clinical BI. CRP was initially positive in 33% of culture proven BI and in 27% of infants with clinical BI and was raised in all, 12-60 hours after the initial evaluation. The combination of both tests increased the sensitivity from 91 to 100%. IL-8 rose earlier and tended to return to normal within 24 hours (*Franz et al. 1999*).

In septic patients, high amounts of circulating IL-8 concentrations correlated with fatal outcome, whereas only low plasma concentrations of IL-8 were present in patients with non-septic multiorgan failure. This suggests that the signals involved in the exacerbation of IL-8 production are different, depending on infectious or non-infectious aetiology (*Marty et al 1994*). Levels of cytokines (TNF, IL-1, IL-6, and IL-8) were higher, and persisted for longer, in plasma and broncho-alveolar lavage (BAL) from patients with ARDS associated with sepsis and more so in the non-survivors (*Headley et al. 1997*). In pertussis, inflammatory parameters, such as ESR, CRP and α_1 acid glycoprotein, were normal, while cytokines, especially IL-6 and TNF, were significantly elevated (*Ostermann et al. 1994*). All cytokines (IL-1, TNF, IL-8 and IL-6) were significantly higher in children with acute febrile illnesses (*Torre et al. 1993*). Increased levels of ICAM-1 were found in a number of pathologic conditions including malignancy, SLE, hepatitis, HIV, miliary TB and neonatal sepsis (*Sessler et al. 1995*). Newborns with early onset sepsis had high cord blood levels of sICAM-1, which did not correlate with maternal blood levels of this cytokine, indicating that the neonate's

response to sepsis is different from the mother (*Lehrnbecher et al. 1996*). A rise in the concentration of ICAM-1 has been described in adults with meningococcal sepsis or meningitis (*Baines PB, 1999*). Increased levels were also found in septic adults admitted to ICU and these levels correlated with severity of sepsis and shock and subsequent organ failure and eventual outcome (*Sessler et al. 1995*).

VIII. 4. Cytokine Assay

VIII. 4. a. Bioassay

All cytokines were initially described as biological factors before being purified and chemically characterized. Therefore, virtually all cytokines can be measured by bioassay. The major problem with bioassays is that they are not specific. The thymocyte proliferation assay for IL-1 can be influenced by other cytokines; IL-6 and TNF can replace IL-1, and IL-2 and IL-4 can synergise with IL-1. Modifications, such as neutralization with specific antibody or saturating the assay with IL-2, or IL-4, still do not solve the problems arising from yet unknown cytokines or other biologically active molecules like steroids and prostaglandins, which are known to alter cytokine responses. The measured biological activity in some cytokines has been less than that measured by immunoassay. Inhibitory factors, cytokine-binding molecules, or biologically inactive but immunoreactive cytokines can result in this discrepancy. Chloroform has been used to extract an agent interfering with IL-1 assay. α_2-Macroglobulin, an acute phase protein in some species, binds to IL-1 and IL-6. Furthermore, bioassays are labour intensive, expensive, and subject to considerable operator influence. They are not suitable for routine clinical chemistry. However, they are very sensitive, detecting orders of nanograms per litre. The materials required for assays are easily obtained, compared to the difficulty in obtaining good antibodies for immunoassay. Thus bioassays may identify which areas warrant investing in the development of immunoassays. IL-6-dependent mouse-to-mouse hybridoma cells 7TD1, is a bioassay that is specific to IL-6 with a limit of detection less than 1 pg of recombinant mouse IL-6 (*Calandra et al. 1991*). Bioassays for IL-6 using hybridoma cell lines detect IL-6 at concentrations of 5-

100 pg/ml. However, although not responding to other known cytokines, these assays could be influenced by combinations of cytokines and/or inhibitory substances present in human body fluids or produced by cultured cells. Moreover, some pharmacologic agents possibly present in samples from patients might also influence the bioassay. Since these agents are often protein-bound, as is IL-6 in human plasma, it is difficult to separate them from IL-6 (*Scindler et al. 1990*). It has proved difficult to detect the presence of biologically active IL-1 or TNF in the peripheral circulation during the acute phase (*Helfgot et al. 1989*).

VIII. 4. b. Immunoassay

Immunoassays are simpler to use and control. However, they are expensive and generally have been validated as research tools, often only for cell culture supernatants. They may lack the sensitivity for use with plasma, and some are subject to interference with rheumatoid factors or heterophilic antibodies. They are frequently imprecise and often do not demonstrate acceptable recovery profiles from plasma, CSF, or synovial fluid. It has been suggested that laboratories set up their own assays to ensure the validity of their results and encourage production of reagents such as cytokine-specific antibodies. The immunoradiometric assay (IRMA) needs incubation for 16-20 hours. Reported sensitivity of IRMA for TNF and IL-6 was 5pg/ml, and 4pg/ml for IL-1β. In normal subjects, neither IL-1, nor TNF or IL-6 could be detected by IRMA. Radio-immunoassay (RIA) correlated well with the hybridoma bioassay for IL-6 ($r=0.87$) (*Damas et al. 1992*).

VIII. 4. c. Standardization

The problem of standardization in cytokine assay is enormous. A single cytokine may have different biological activities, and numerous preparations (natural and recombinant) of a cytokine might be available. Such materials may differ in potency in bio- and immunoassay. Comparing data from different laboratories requires proper cytokine standards and carefully prescribed assay conditions such as the choice of serum supplements, type of sample, and sample preparation. Cytokine standards are becoming

available. Quality control will be important to address the problems of cytokine assays in clinical chemistry (*Wicher & Evans 1990*). The evaluation of the precise role of these lymphokines requires specific assays. Specific immunoassays satisfy this requirement, but the high sensitivity required poses quite a problem. Bioassays are often used to detect the small quantities of cytokines present in biological fluids. Purified natural IL-1 preparations were shown to be contaminated with IL-6, and some antisera to IL-1 contained anti-IL-6 activity. Both IL-1 and IL-6 are active in the Thymocyte assay. The detection limit of this assay for both is 100 pg/ml. The detection limit of the IL-6 assay on hybridoma cell line, which is highly specific to IL-6, is 0.5pg/ml. IL-1 can be specifically measured with D10 cells, the detection limit for which is around 1pg/ml (*Helle et al. 1988*).

Part Three

A Study of the Prevalence and Prediction of Serious Bacterial Infection in Febrile Young Children.

Chapter IX

Methods

Venue and subjects

IX. 1. Venue

The Edinburgh Sick Children's Hospital NHS Trust is one of only two Trusts in Scotland dedicated solely to the treatment of children, both in the acute and community setting. The hospital provides a fully combined child health service for the children of Lothian, South-East Scotland and beyond. Patients referred from the A&E and other departments are seen in an ambulatory/short-stay unit which has been opened only recently. In-patients are accommodated in 138 beds distributed among medical and surgical wards as shown in Table 12. There are 27 consultants in the surgical directorate, 23 paediatric medical consultants and nine consultants in community paediatrics. The hospital also employs a number of consultants in the support services and a few numbers of the staff from the University of Edinburgh also hold honorary posts at the hospital. The hospital operates a computerised information system designed to provide speedy access to information needed by clinical staff to help with their treatment of patients.

The A&E department is staffed with a consultant in accident and emergency medicine and two full time staff-grade doctors. A number of senior house-officers work in a partial-shift system and are supported by both medical and surgical middle grade staff (specialist registrars). A consultant paediatrician with special interest in ambulatory

paediatrics supports the A&E staff and also runs an ambulatory clinic on site. A number of nurses were specially trained and qualified as paediatric A&E nurse practitioners and they are actively involved in patient management and can provide independent advice and treatment for simple ailments. The department runs a telephone advice service for the parents, which is run by nurses under the supervision of the medical staff. The department is well equipped and operates a 24 hour stand-by service for resuscitation and advanced life-support. All admissions to the hospital, except those referred from other district hospitals, go through the A&E department.

About 35000 children are treated in the A&E Department each year. On average, 45000 patients are seen annually in the outpatient clinics, 72% of whom are review patients and the remainder are new referrals. Each year, around 3800 patients are seen in the ambulatory clinic attached to the A&E department. The average annual number of in-patients is 9500, over half of which is from the A&E department. From a total number of 20446 beds occupied in 1998/99, 13712 (67%) were for medical cases, excluding cardiac and neurology cases. During the last three years increasing numbers of children were being treated as day-cases. There was an increase of 16% (to 4476) in 1997/98, and 18% (to 5287) in the year 1998/99 in the number of day cases. This was mirrored by a 1% reduction in admissions in 1998/99. These achievements were largely due to the development of Ambulatory Paediatrics and changes in clinical practice emphasizing a short-stay philosophy. There was also an increase in the numbers of children being treated in their local community. Accompanying these changes were large improvements in waiting times for both outpatient appointments and day-case and in-patient treatments. The year 1998/99 witnessed an 18% reduction in the total number of patients waiting for admission.

Twenty percent of the 35000 patients attending the A&E department at Edinburgh Sick Children's Trust (ESCT) annually present with medical problems. Varying with the season, 30%-75% of these have infectious illnesses. In winter, an average of 35 cases of infectious diseases are seen daily with up to 26 admissions a day. Fever is a major symptom in the majority of these children. A pilot study was done to study the determinantnts and appropriateness of hospitalization of febrile children from

the emergency department. Data were collected from all children presenting with an infectious illness over nine weeks (10 December 1997 to 22 February 1998). A study proforma was used to collect clinical and laboratory data and on it the admitting physician indicated the reason for admission. The definitive study's aims were to to determine the probability (prevalence) of serious bacterial illness in highly febrile children and identify the clinical and laboratory determinants of SBI.

IX. 2. **Subjects**

The target population was all the children aged 0-5 years of age who lived in the council areas served by the Edinburgh Royal Hospital for Sick Children (*Table 13*). The study spanned over a whole calendar year, from 1 September 1998 to 31 August 1999. The study group consisted of children seen at the emergency department fulfilling the inclusion criteria (*Appendix 4*):

1. Age $\leq$5 years
2. Temperature $\geq$38.5°C
3. Informed written parental consent obtained

Patients referred for admission from district hospitals were not included. Patients were prospectively enrolled as they presented to the emergency department. The numbers of all patients attending the previous day were collected from the hand-written attendance list and were verified by cross-examining with the computer-generated emergency room register. Patients eligible for the study who were not enrolled were identified by examining the date of birth and temperature on the ER evaluation form completed by the nurse. Demographic data and relevant clinical information, including the time of arrival, source of referral, and recent antibiotic and antipyretic use, were recorded. The administrative outcome, i.e. admission to hospital or discharge from the emergency department, was noted and further clinical information was collected by review of the case notes. This included the initial diagnosis, route and duration of antibiotic use, admission to ITU, laboratory and radiographic investigation results and the discharge diagnosis. Blood culture results were cross-examined and verified with a list of all blood cultures done during the study period obtained from the microbiology

department. A written study protocol was distributed among the medical and nursing staff concerned with the management of medical patients at the emergency room. This was also prominently displayed at different sites of the department. Upon arrival to the department a triage nurse saw the patient and recorded the axillary temperature using Sure-Temp electronic thermometer (*WelchAllyen™, Henleys Medical Supplies Ltd Brownfields, Welwyn Garden City, Herts AL7 1AN*). This device takes about 15 seconds to record axillary temperature with an accuracy of ±0.1°C (0.2°F). The nurse then alerted the investigator or the attending doctor if the patient met the study criteria. The nurse attached a clinical assessment form to the clinical notes of the patient and delivered a study information sheet to the parent. The attending doctor obtained a written informed consent and completed the assessment form as he/she took the medical history and examined the patient. The emergency department policy indicated a CBC on all patients with similar age and temperature criteria. The majority of the patients were also investigated by a blood culture.

Other tests, such as urine culture and radiography, were done at the discretion of the attending physician. During the study period, September 1998 to August 1999, urine was collected by suprapubic aspiration from babies less than 6 months and a clean catch specimen was collected from older patients. Urine bags were only used for children not thought to have a high risk of infection. Spun urine was microscopically examined for pyuria and reported as the number of white blood cells seen per high power field. Significant results were expressed in absolute numbers. Urine was cultured using standard quantitative techniques in the hospital microbiology laboratory. Chest radiography was done at the discretion of the assessing physician and the radiologist's report was immediately available to guide management in the emergency room. The radiologists had access to clinical data on the radiography request form but were blinded to the study group or outcome.

An extra 1-2 ml of blood was collected for cytokine assay immediately on entry to the study. Sera for comparison were obtained from afebrile children who were not acutely ill and were attending various clinics at the outpatient department. The

investigator reviewed the case notes of all patients and collected informed written consent from the parents. Inclusion criteria were:

1. Age ≤5 years
2. Parental consent.

Exclusion criteria were:

1. Acute febrile illness
2. Current antibiotic treatment
3. Recent vaccination (in the last 48 hours)
4. Chronic or intermittent infections (e.g. cystic fibrosis)
5. Primary or secondary immunodeficiency
6. Neoplastic disease including haematological malignancies
7. Renal disease e.g. nephrotic syndrome and nephritis
8. Blood disorders e.g. active ITP, sickle cell disease
9. Significant recent trauma or burns (in the last 48 hours)
10. Autoimmune diseases e.g. SLE
11. Chronic inflammatory disease e.g. rheumatoid arthritis, Crohn's disease
12. Prolonged corticosteroid treatment
13. Prolonged non-steroidal anti-inflammatory drug use

The study protocol was approved by the Ethics Committee for the Lothian Health Authority.

IX. 3. The Clinical assessment form

The assessment was based on the Acute Infant Observation Scale (Yale Infant Observation Scale) developed by McCarthy et al, with a slight modification to make it easier to use in a busy emergency department (*Appendix* 5). The clinical assessment data were collated from history, observation and physical examination variables. Each variable consisted of three specific items and each item was scored on a three-point scale of impairment; 1 (no or mild impairment), 2 (moderate impairment) and 3 (severe impairment). An overall subjective impression of the severity of illness, and the probability of SBI were similarly scored (*Appendix 5*). On the form, serious bacterial

illness (SBI) was defined as bacteraemia or septicaemia, urinary tract infection, pneumonia, osteomyelitis or septic arthritis, meningitis or gastro-enteritis of bacterial aetiology. Physicians chose the options descriptive of the patient's condition rather than ticking the scale points. Physicians used their discretion in assigning the scores according to the age and developmental stage of the child. Historical and observational parameters could be used interchangeably.

IX. 4. Definition of outcome variables

The outcome result was the presence of serious bacterial infection (SBI) defined as bacteraemia / septicaemia, urinary tract infection, pneumonia, osteomyeltis, septic arthritis and soft tissue infection, and bacterial gastro-enteritis. Bacteraemia was defined on the basis of a single bacterial pathogen grown from blood by standard culture techniques. Bacterial organisms regarded as non-pathogenic in immuno-competent patients, such as staphylococcus epidermidis, were considered contaminants. Bacterial meningitis was considered present in the context of clinical signs and cytological evidence of meningeal inflammation with a pathogenic organism grown from CSF, blood or throat swab. Urinary tract infection was defined as a significant ($\geq 10^5$ cfu/ml) growth of a single urinary tract pathogen according to universal criteria. Other evidence of UTI, such as gram stain of urine for bacteria, nitrites or leucocyte esterase, was not routinely sought. A conservative definition of pneumonia was used in that only findings reported as consolidation were considered pneumonia. Other abnormalities described with less definitive terms, such as patchy infiltrates, peri-bronchial or peri-hilar infiltrates were not considered pneumonia. Readings other than consolidation, such as patchy infiltrates or peribronchial thickening, were not considered indicative of pneumonia. Bacterial gastro-enteritis included Clostridium difficile infections, either the organism grown on culture or its toxin detected in the stools. Osteomyelitis and cellulitis were considered bacterial infections on clinical grounds but other evidence of baterial aetiology was necessary to make a diagnosis of pyogenic arthritis.

IX. 5. Statistical analysis

Non-parametric data were analysed using the Mann-Whitney U-test for continuous variables. Normally distributed data were analysed using the two-tailed t-test. Categorical data were analysed by the χ^2 test. The clinical assessment score (The Acute Illness Observation Scale, modified) was analysed as continuous data. Backward step-wise logistic regression analysis was used to identify independent parameters predictive of SBI. Receiver-operator characteristic curves (ROC) were used to compare the differential performance of variables. Based on the ROC curves, cut-off values and corresponding optimum sensitivities and specificities for each variable were determined. The level of statistical significance was 0.05. Statistical analyses were performed using the SPSS statistical software package, version 10.0 for Windows (SPSS, Inc, Chicago, IL).

Table 12: Bed allocation at the Edinburgh Sick Children Hospital

Specialty	Number of wards	Number of beds
Medical	4	61
Surgical	3	56
Infectious diseases	1	6
High dependency	1	6
Intensive care	1	6
Neonatal unit	1	3

Table 13: Mid-year population estimates: Council areas by age group 0-5 years*.

Age (years)	1	2	3	4	5	*Total*
City of Edinburgh	4894	4935	4997	4870	4995	***24691***
Midlothian	948	936	911	946	1020	***4761***
TOTAL	5842	5871	5908	5816	6015	**29452**

*Source: Lothian & Edinburgh City Council.

Chapter X

Methods

Cytokine Assay

1-2 ml of blood was collected in tubes containing EDTA and aprotonin (trasylol) and labelled by the patient's addresograph. Blood was gently shaken and then centrifuged at 13000 rpm for three minutes using a microfuge kept in a side room at the A&E department. Plasma was removed, using disposable pipettes, and divided into three fresh plain tubes without anticoagulants. The tubes were similarly lablled by a sticker from the patient's case notes. The tubes containing plasma were placed in a refrigerator (4°C) in A&E for only a few hours according to the manufacturer's instructions. The tubes were then transferred to the adjacent University of Edinburgh laboratory and kept frozen at -20°C until the time of assay. The technician who performed the assays had no access to any clinical details concerning the patients. Cytokine ELISA kits were provided by the *Immunodiagnostic Systems Limited, Boldon Business Park, Boldon Tyne & Wear, NE35 9PD, United Kingdom*. The assays were done at the Department of Child Life & Health Laboratories, by Dr J Smith, PhD, University of Edinburgh. The kits were kindly funded by Mason Company.

X. 1. IL-6 assay

IL-6 kit:

The IL-6 kit is a solid phase sandwich Enzyme-Linked Immune-Sorbent Assay (ELISA). A monoclonal antibody specific for IL-6 has been coated onto the wells of the provided microtitre strips. Samples, including standards of known IL-6 concentrations, control specimens and unknowns are pipetted into these wells. During the first incubation, the IL-6 antigen and a biotinylated monoclonal antibody specific for IL-6 were simultaneously incubated. After washing, the enzyme (streptavidin-peroxylase) was added. After incubation and washing to remove all the unbound enzyme, a substrate solution acted upon by the bound enzyme was added to induce a coloured reaction product. The intensity of this coloured product was directly proportional to the concentration of IL-6 present in the samples.

Reagents Preparations:

Standard buffer diluent:

5 ml of standard diluent buffer was diluted in 50 ml of distilled water.

Standards and Controls:

Two standard diluents were provided by the manufacturer, one for culture supernatant and one for sera or plasma samples. The standard diluent for human serum was used in this assay. Standards were reconstituted with 1.1 ml of standard buffer diluent to give a concentration of 200 pg/ml IL-6. Controls were resuspended in 1 ml of standard buffer diluent to give a concentration of 64±13 pg/ml IL-6.

Dilution of biotinylated anti-IL-6:

The biotinylated anti-IL-6 was diluted with the biotinylated antibody diluent in a clean glass vial according to the number of wells to be used:

Number of Wells used	Biotinylated Antibody (µl)	Biotinylated Antibody Diluent (µl)
16	40	1060
24	60	1590
32	80	2120
48	120	3180
96	240	6360

Dilution of Streptavidin-HRP:

0.5 ml of HRP diluent were added to a 5 µl vial of Streptavidin-HRP. Further dilutions were made according to the number of wells required:

Number of Wells	Streptavidin-HRP (µl)	Strep-HRP Diluent (ml)
16	30	2
24	45	3
32	60	4
48	75	5
96	150	10

Dilution of Washing Buffer:

2 ml of washing buffer was diluted in 400 ml of distilled water.

IL-6 Assay Method:

A. Before use, all reagents were mixed thoroughly without making foam.

B. For each round of assay, the number of microwell strips required to test the number of samples available was determined and removed from the pouch. The appropriate number of wells needed for running blanks and standards was also prepared. Every sample, and every blank and standard and optional control sample was assayed in duplicate.

C. 100ul of appropriate standard diluent were added to standard wells B1, B2, C1, C2, E1, E2, F1, and F2. A standard vial was reconstituted with the appropriate volume as described above. 200ul of standard were pipetted into wells A1 and A2. 100ul from A1 and A2 were transferred to B1 and B2 wells, respectively. The contents were mixed by repeated aspirations and injections. This procedure was repeated from the wells B1, B2 to wells C1, C2 and from C1, C2 to D1, D2 and so on creating two parallel rows of IL-6 standard dilutions ranging from 200 to 6.25pg/ml. 100ul from the content of the last microwells used (F1, F2) were discarded.

D. 100ul of appropriate standard diluent were added to the blank wells (G1-G2).

E. 100ul of samples and 100ul of control were added to the sample wells and to the control wells (H1, H2), respectively.

F. Biotinylated anti-IL-6 was prepared as above.

G. 50ul of diluted biotinylated anti-IL-6 were added to all wells.

H. The wells were covered with a plate cover and incubated for 1 hour at room temperature.

I. The cover plate was removed and washed as follows:

   1. The liquid was aspirated from each well
   2. 0.3ml of washing solution was dispensed to each well
   3. The contents of each well were aspirated again.
   4. Steps 2 and 3 were repeated twice.

J. HRP solution was freshly prepared as above.

K. 100ul of HRP solution were dispensed into all wells, including the blank wells. The wells were covered with the cover plate.

L. The microwell strips were incubated at room temperature for 30 minutes.

M. The plate cover was removed and the wells emptied. The microwell strips were washed according to point (I) above.

N. 100ul of ready-to-use TMB substrate solution were pipetted into all wells, including the blank wells. The wells were then incubated in the dark, by wrapping the plate in aluminium foil, for 15 minutes at room temperature.

O. The Optical Density values of the plate were monitored and the substrate reaction stopped before positive wells were no longer properly readable (maximum time allowed was 20 minutes).

P. The enzyme-substrate reaction was stopped by pipetting 100ul of 1.8 N sulphuric acid into each well, including the blank wells, to completely and uniformly inactivate the enzyme. Results were then read immediately after the addition of sulphuric acid.

Q. The absorbance of each well was read on a spectrophotometer using 450nm as the primary wavelength and 620nm as the reference wavelength.

Plate Scheme Used:

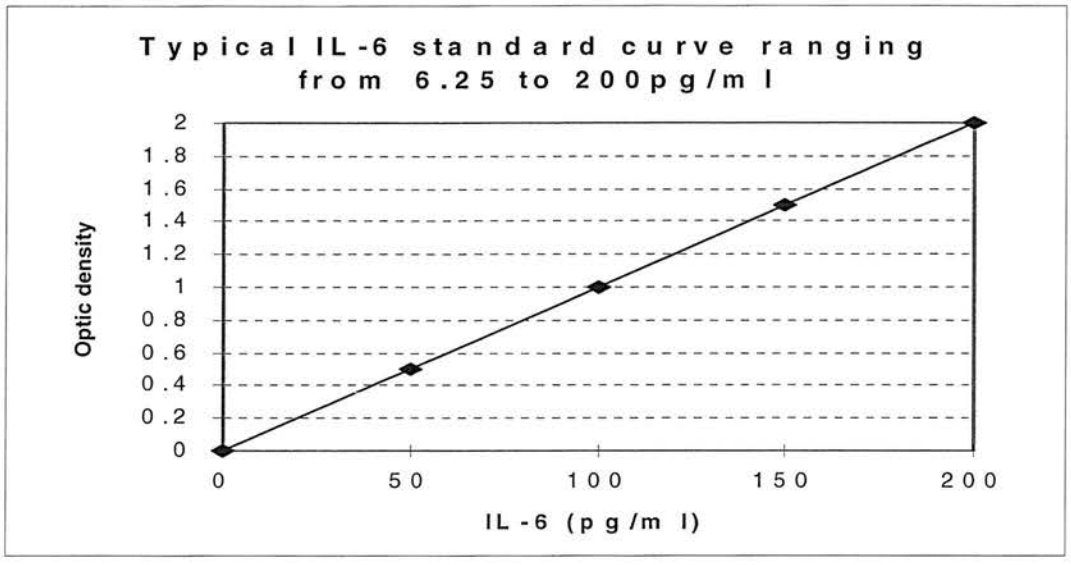
Sample wells

Standard Concentrations (pg/ml)

	1	2	3	4	5	6	7	8	9	10	11	12
A	200	200	65	65	86	86/50	120	120/50	132	132	147	147/50
B	100	100	70	70/50	87	87	122	122	135	135/50	150	150/50
C	50	50	73	73/50	88	88/50	124	124	136	136	151	151/50
D	25	25	74	74	89	89	125	125	138	138/50	152/50	152/50
E	12.5	12.5	75	-	90/50	-	126	126	141	141	158	158
F	6.25	6.25	79/50	79/25	91/50	-	127	127	143/50	143/25	159	159
G	Blank	Blank	81	81	92	92	130	130	144	144/50	161	161
H	Ctrl	Ctrl	83/50	-	93	93	131	131	145	145	163	163

Data Analysis:

A linear standard curve was generated by plotting the average absorbance on the vertical axis versus the corresponding IL-6 standard concentration on the horizontal axis. The amount of IL-6 in each sample was determined by extrapolating OD values to IL-6 concentrations using the standard curve.



Limitations of the Procedure:

The standard curve should not be extrapolated beyond the 200 pg/ml standard curve point. The dose-response is non-linear in this region and good accuracy is difficult to obtain. Therefore concentrated samples (>200pg/ml) had to be diluted with standard diluent and the results were multiplied by the dilution factor. The influence of various drugs, aberrant sera (haemolysed, hyperlipaemic, jaundiced, etc....) and the use of plasma instead of serum specimens has not been thoroughly investigated. The rate of degradation of native IL-6 in various matrices has not been investigated. The immunoassay literature contains frequent references to aberrant signals observed with some human sera and attributed to heterophilic (or anti-mouse) antibodies. The possibility of this occurrence cannot be excluded

Performances and Characteristics:

Sensitivity:

The minimum detectable dose of IL-6 is less than 2 pg/ml. This has been determined by adding 3 standard deviations to the mean optical density when the zero standard was assayed 32 times. (data provided by the manufacturer)

Precision:

	Intra-assay				Inter-assay			
Sample	n	Mean (pg/ml)	SD	CV%	Sample	n	Mean (pg/ml)	SD	CV%
A	8	198.7	1.667	0.83	A	29	198.9	3.772	1.89
B	8	28.69	1.118	3.86	B	29	25.68	1.505	5.84

Linearity of dilution:

A human serum pool containing 100pg/ml of measured IL-6 was serially diluted in standard buffer diluent over the range of assay. Linear regression of samples versus the expected concentration yielded a correlation of 0.99.

Recovery:

Recovery of IL-6 added to pooled normal serum was 96.2% (85.4% to 100%) for IL-6 concentration ranging from 200 to 6.25 pg/ml (data provided by the manufacturer).

X. 2. **IL-8 Assay**

IL-8 kit:

The IL-8 kit is a solid phase sandwich Enzyme-Linked Immune-Sorbent Assay (ELISA). A monoclonal antibody specific for IL-8 has been coated onto the wells of the provided microtitre strips. Samples, including standards of known IL-8 concentrations, control specimens and unknowns are pipetted into these wells.

During the first incubation, the IL-8 antigen and a biotinylated monoclonal antibody specific for IL-8 were simultaneously incubated. After washing, the enzyme (streptavidin-peroxylase) was added. After incubation and washing to remove all the unbound enzyme, a substrate solution which acted on the bound enzyme was added to induce a coloured reaction product. The intensity of this coloured product was directly proportional to the concentration of IL-8 present in the samples.

Reagents Preparations:

Standard buffer diluent:

5 ml of standard diluent buffer was diluted in 50 ml of distilled water.

Standards and Controls:

Two standard diluents were provided by the manufacturer, one for culture supernatant and one for sera or plasma samples. The standard diluent for human serum was used in this assay. Standards were reconstituted with 1.42 ml of standard buffer diluent to give a concentration of 2000 pg/ml IL-8. Controls were resuspended in 0.5 ml of standard buffer diluent.

Dilution of biotinylated anti-IL-8:

The biotinylated anti-IL-8 was diluted with the biotinylated antibody diluent in a clean glass vial according to the number of wells to be used:

Number of Wells used	Biotinylated Antibody (µl)	Biotinylated Antibody Diluent (µl)
16	40	1060
24	60	1590
32	80	2120
48	120	3180
96	240	6360

Dilution of Streptavidin-HRP:

0.5 ml of HRP diluent were added to a 5 µl vial of Streptavidin-HRP. Further dilutions were made according to the number of wells required:

Number of Wells	Streptavidin-HRP (µl)	Strep-HRO Diluent (ml)
16	30	2
24	45	3
32	60	4
48	75	5
96	150	10

Dilution of Washing Buffer:

2 ml of washing buffer was diluted in 400 ml of distilled water.

IL-8 Assay Method:

A. Before use, all reagents were mixed thoroughly without making foam.

B. For each round of assay, the number of microwell strips required to test the number of samples available was determined and removed from the pouch. The appropriate number of wells needed for running blanks and standards was also prepared. Every sample and every blank and standard and optional control sample was assayed in duplicate.

C. 100ul of appropriate standard diluent were added to standard wells B1, B2, C1, C2, E1, E2, F1, and F2. A standard vial was reconstituted with the appropriate volume as described above. 200ul of standard were pipetted into wells A1 and A2. 100ul from A1 and A2 were transferred to B1 and B2 wells, respectively. The contents were mixed by repeated aspirations and injections. This procedure was repeated from the wells B1, B2 to wells C1, C2 and from C1, C2 to D1, D2 and so on creating two parallel rows of IL-8 standard dilutions ranging from 2000 to 62.5pg/ml. 100ul from the content of the last microwells used (F1, F2) were discarded.

D. 100ul of appropriate standard diluent were added to the blank wells (G1-G2).

E. 100ul of samples and 100ul of control were added to the sample wells and to the control wells (H1, H2), respectively.

F. Biotinylated anti-IL-8 was prepared as above.

G. 50ul of diluted biotinylated anti-IL-8 were added to all wells.

H. The wells were covered with a plate cover and incubated for 1 hour at room temperature.

I. The cover plate was removed and washed as follows:
   1. The liquid was aspirated from each well
   2. 0.3ml of washing solution was dispensed to each well
   3. The contents of each well were aspirated again.
   4. Steps 2 and 3 were repeated twice.

J. HRP solution was freshly prepared as above.

K. 100ul of HRP solution were dispensed into all wells, including the blank wells. The wells were covered with the cover plate.

L. The microwell strips were incubated at room temperature for 30 minutes.

M. The plate cover was removed and the wells emptied. The microwell strips were washed according to point (I) above.

N. 100ul of ready-to-use TMB substrate solution were pipetted into all wells, including the blank wells. The wells were then incubated in the dark, by wrapping the plate in aluminium foil, for 12-15 minutes at room temperature.

O. The O.D. values of the plate were monitored and the substrate reaction stopped before positive wells were no longer properly readable (maximum 20 minutes). Incubation time of substrate solution is usually determined by the ELISA reader performances: many ELISA readers record absorbance only up to 2.0 O.D.

P. The enzyme-substrate reaction was stopped by pipetting 100ul of 1.8 N sulphuric acid into each well, including the blank wells, to completely and uniformly inactivate the enzyme. Results were then read immediately after the addition of sulphuric acid.

Q. The absorbance of each well was read on a spectrophotometer using 450nm as the primary wavelength and 620nm as the reference wavelength.

Plate Scheme Used:

Standard Concentrations (pg/ml) | sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	2000	2000	C5		C23		C38/75		C47		C57/75	
B	1000	1000	C6		C24		C29		C50		C58/75	
C	500	500	C7/75		C27		C40		C52		C59/75	
D	250	250	C8/75		C23		C41		C52		C66	
E	125	125	C5/75		C33		C41		C53/75		C67	
F	62.5	62.5	C20		C34/75		C43		C54/75		C68/75	
G	Blank	Blank	C21		C36		C44		C55		C74/75	
H	Ctrl	Ctrl	C22		C37		C45/75		C56		C80	

Data analysis:

A linear standard curve was generated as for IL-6 and the amount of IL-8 in each sample was determined by extrapolating OD values to IL-8 concentrations usng the standard curve.

Limitations of the Procedure:

These were the same as for IL-6 assay.

Performance characteristics

Sensitivity

The minimum detectable dose of IL-8 was less than 25 pg/ml. This has been determined by adding 3 standard deviations to the mean optical density obtained when zero standard was assayed 34 times (*data provided by the manufacturer*).

Precision

	Intra-assay				Inter-assay				
Sample	n	Mean (pg/ml)	SD	CV%	Sample	n	mean (pg/ml)	SD	CV%
A	8	1934	11.71	0.60	A	40	1977	39.21	1.96
B	8	267.3	5.35	2.0	B	40	249.9	15.34	6.13

Linearity of dilution

A human serum pool containing 1000 pg/ml of measured IL-8 was serially diluted in standard buffer diluent over the range of the assay. Linear regression of samples versus the expected concentration yielded a correlation coefficient of 0.99.

Normal serum values

The average concentration of IL-8 detected in 72 normal serum is 407±331 pg/ml, ranging from 0 to 1938 pg/ml (*data provided by the manufacturer*).

Recovery

Recovery of IL-8 added to normal serum was 100% (84 to 114%) for IL-8 concentration ranging from 2000 to 62.5 pg/ml (*data provided by the manufacturer*).

X. 3. **sICAM-1 Assay**

The principles of sICAM-1 assay were exactly the same as for IL-6 and IL-8.

Sensitivity

The minimum detectable dose of sICAM-1 for this assay was less than 0.1ng/ml. This has been determined by adding 3 standard deviations to the mean optical density obtained when the zero standard was assayed 32 times.

Precision:

Sample	n	Mean (ng/ml)	SD	CV%	Sample	n	Mean (ng/ml)	SD	CV%
A	8	7.82	0.081	1.03	A	22	8.05	0.317	3.93
B	8	1.17	0.033	2.82	B	22	1.03	0.084	8.15

Normal serum values

The average concentration of sICAM-1 detected in 77 normal human serum samples is 571±168 ng/ml ranging from 219 to 1042 ng/ml (*data provided by the manufacturer*).

Chapter XI

Results

Patient Characteristics and Clinical Outcome

XI. 1. Patient characteristics

The pilot study showed that a third of A&E attendants presented with an infectious illness, and 42% of these were febrile. 41% of patients with infectious illnesses were admitted to hospital and 56% of hospitalized children did not, in retrospect, require treatment that was not administrable at home (*Table 14*).The height of fever and uncertainty of diagnosis were prominent reasons for admission, whereas only 3% of the children were admitted for social reasons. Furthermore, clinical and demographic determinants of hospitalization were not the same as those dictating hospital-based treatment (data not shown).

From 1 September 1998 to 31 August 1999, a total of 9980 non-trauma cases were seen at the emergency department of the Edinburgh Royal Hospital for Sick Children. Of these, 6128 (60%) patients presented with an infectious illness. 618 patients fulfilled the study criteria (age $\leq$5 years and temperature $\geq$38.5°C) and clinical and laboratory data were prospectively collected from these patients (*Figure 6*). Of the 618 febrile patients (with axillary temperature $\geq$38.5°C), 342 (55.3%) were males and 276 (44.7%) were females, p=0.009. The median age was 19.3 months, range 0.3-71.8

months. The vast majority (77.3%) of the febrile patients were 36 months of age or younger, and there were only three infants less than a month old (*Table 15*). Nearly half (47.9%) were seen during the winter months (December to February) while less than 5% presented during the summer months (June-Aug), (*Figure 7*). 346 (57.7%) patients were seen out of working hours (between 17.00 and 09.00), and 254 (42.3%) came during working hours (9.00-17.00). Time of presentation to ER was not recorded for 18 patients. Two hundred and twenty two patients (35.7%) were self referred, 320 (51.9%) were referred by general practitioners, and 77 (12.5%) were brought in by ambulance. The source of referral was not recorded for one patient. The majority of the patients were initially attended by junior doctors; 433 (71%) were seen by a SHO, 101 (16.6%) by a registrar and 74 (12.1%) by a staff grade. A consultant and a senior registrar saw one each (0.2%). This information was not available for 8 patients (*Figure 8*). 289 patients (46.8%) had received an antipyretic before being assessed by a physician and 91 (14.7%) had received an antibiotic for the presenting illness. The median temperature recorded at the emergency room was 39.0°C, range 38.5°C- 40.5° C. Only 15 patients (2.4%) had temperatures higher than 40.0° C, and there was no case of hyperpyrexia (≥41.0°C).

XI. 2. Hospital admission

399 patients (64.6%) were admitted to the hospital and 219 patients (35.4%) were discharged from the emergency department. Sixty four (16.2%) of the admissions were to the high dependency or intensive care units. The median duration of hospital stay was 2 days, however, the majority of patients (44.9%) were admitted for only one day (*Figure 9*). 22 (5.6%) patients were discharged on the same day. The total patient-days spent in hospital over the one year period was 980. Almost all admitted patients (98%) had at least one laboratory, or radiographic, test done, compared to 82.2% of those who were discharged from the emergency department. The majority of admitted patients (85.2%) were investigated by a blood culture, while this test was done on an ambulatory basis in less than half (48.4%) of the patients. 113 (28.3%) of the admitted patients were treated with parenteral (usually intravenous) antibiotics, while this mode

of therapy was administered on an ambulatory basis on only two (0.9%) occasions. A third (33.3%) of hospitalized patients did not receive any antibiotics, while the remaining third (38.3%) were prescribed oral antibiotics.

Hospitalized patients were significantly younger than those who were discharged from the emergency department: median age 17.1 and 24.7 months, respectively (p=0.001). Equal proportions (64.3% vs 64.9%) of males and females were admitted (p=0.892). Patients were equally likely to be admitted when they came during normal working hours (66.5%) or out of hours (62.1%) (p=0.197). Patients were more likely to be admitted when referred by their GP (74.7%) than when self-referred (44.1%), (LR 52.0, p=0.0001). Admitted patients had statistically, but not clinically, significant higher temperatures than those who were discharged from the emergency room; median temperature 39.1°C vs 39.0°C respectively, p=0.0001. Moreover, the presence of a high fever (≥40.0°C) did not influence the decision to admit, p=0.103. Admitted patients were both subjectively and objectively more ill; they were more tachypnoeic (median respiratory rate 40 vs 35), more tachycardic (median heart rate 160 vs 150), and scored significantly higher (median score 7 vs 4) on the clinical assessment scale (p-values >0.0001 for all parameters). Junior (SHO) doctors admitted similar proportions of patients to middle grade doctors (registrars and staff-grades); 67.7% vs 59.3%, p=0.061 (χ^2 test with continuity correction).

XI. 3. **Prevalence of serious bacterial infection**

A total of 571 (92.4%) of febrile patients were investigated by one or more laboratory or radiographic tests (*Table 16*). Virological tests were done in 142 (23%) of the patients, 66 (46.5%) of these yielded a positive result (*Table 17*). 165 (26.7%) patients had a serious bacterial infection. Table 18 shows the incidence of illnesses in the study population. The incidence and types of serious bacterial infection are shown in Figure 10 and table 21 and are detailed in the following sections. Some patients had more than one illness classifiable as SBI therefore the cumulative proportions of SBI are more than one.

XI. 3. a. Bacteraemia

Of the 441 blood cultures taken, twelve (2.7%) grew pathogenic organisms (*Table 22*). All cases of bacteraemia occurred in hospitalized patients. Seven (58.3%) of the pathogenic isolates were streptococcus pneumoniae, two of these were identified as serotype 23 & type 2, and the rest were not typified. A four month old baby had pneumococcal meningitis and two older infants had uncomplicated pneumococcal bacteraemia. In more than half (57%) of pneumococcal bateraemia the organism was not detected at the time of discharge from the hospital. One had a potential source of fever (otitis media) and two had specific viral infection (RSV+ve bronchiolitis and chickenpox). The estimated attack rate of invasive pneumococcal disease was 28 per 100,000 for the under-five children in the population served by the Edinburgh Hospital for Sick children. Two girls with E.coli septicaemia also had the same organism isolated from urine. The patient with meningococcal septicaemia had classical clinical features of this illness, purpuric rash, hypotension and thrombocytopenia. The patient with streptococcus agalactae septicaemia did not have meningitis. The patient with blood culture positive for staph. aureus was highly febrile and with peri-oral ulcers and cervical adenopathy and was admitted as eczema herpeticum and received nine days of parenteral antibiotic therapy in hospital. Twenty four (5.4%) of blood culture isolates were of organisms considered contaminants in immuno-competent patients. There were 16 isolates of coagulase negative staphylococcus, two corynebacterium species, one each of streptococcus mitis, strep. salvarius, and strep. sanguius. There were two more isolates of unidentified streptococcus species.

XI. 3. b. Meningitis

Lumbar puncture and CSF examination and culture were done in 29 (4.7%) patients. Three patients had meningitis; an incidence of 0.5%. The clinical details of these patients are detailed in Table16. The five year old girl presented with clinical features of meningitis with a purpuric rash and thrombocytopenia. Lumber puncture was not done. The diagnosis was based on the clinical picture and she received 10 days of hospital treatment.

XI. 3. c. Urinary tract infection

Urine was collected from 343 (55.5%) of 618 patients fulfilling the age and temperature criteria. Children who had urine obtained had higher median temperature than those who did not have urine collected. This difference (39.1°C, vs 39.0°C) was statistically (p=0.026), but not clinically, significant. They were more likely to be admitted (p=0.0001), but did not look more ill, as judged by the median clinical score (p=0.46) and the subjective assessment of the severity of illness (p=0.663). There was no difference between those who did or did not have urine obtained for culture with regard to, sex (p=0.267), median age (p=0.150), and the presence of a localizing sign (p=0.234). Patients with a potential source of fever (otitis media, URTI, GE) were equally likely to have urine obtained for culture as those who did not have an identifiable cause of fever (p=0.249). A third of patients (13/42) with a specific viral syndrome (bronchiolitis, croup, stomatitis, chickenpox), had urine obtained for culture. It can therefore be concluded that the patients from whom urine was obtained are a reasonably representative sample of the studied population of febrile children.

Girls and boys in different age categories (<12 months, 1-2 years >2 years) were equally likely to be tested for UTI (*Table 19*). There were 39 positive urine culture results. All were caused by E. coli. The majority of positive urine cultures (72%) were associated with significant pyuria on microscopic examination. The vast majority (82.1%) of patients with positive cultures were admitted to hospital. The majority (74.3%) of these were treated with antibiotics before discharge, a third (34.5%) with parenteral antibiotics. Follow up data were not available for those who were discharged before culture results were available. Information about further isotopic confirmation of pyelonephritis or urological anomaly was not sought.

The overall prevalence of urinary tract infection was 11.4% (95% CI, 8%-15%), with higher rates in girls (18.1%) than in boys (5.5%). The highest prevalence (26.2%) was in girls below one year of age (*Table 19*). UTI was equally common in patients with or without a potential source of fever (13.6% vs 10.6%), p=0.426. Furthermore, patients with specific viral syndromes (bronchiolitis, croup, stomatitis and chickenpox) were equally likely to have UTI (7.7%) as those without a clinically identifiable viral illness

(10.6%), p=0.741. Two infants (5.1%), 3.7 and 12.5 month old girls, were bacteraemic. E. coli was grown from urine and blood of both patients. There was no significant difference between patients with and those without UTI with regards to age, temperature, and the degree of illness as judged by the clinical assessment score. Conventional laboratory tests (WBC, ANC, CRP and ESR) were significantly higher in patients with UTI, while there was no difference in cytokine levels between patients with and without UTI, (*Table 24*).

XI. 3. d. **Pneumonia**

268 patients (43.4%) had a chest radiograph done. Boys and girls were equally investigated by a chest radiograph, p=0.960. Patients who had chest radiography done were younger, more febrile and more tachypnoeic. They also looked more ill, and were more likely to be hospitalized. Patients were more likely to have CXR obtained when they did not have a potential source of fever (*Table 20).* The vast majority (95%) of patients with clinical signs suggestive of pneumonic consolidation had CXR obtained. On the other hand, 40.3% of those who had CXR done did not have physical signs of pneumonia. Clinical signs suggestive of pneumonic consolidation were; dullness to percussion, crepitations, bronchial breathing and localized reduced air entry. Other signs, such as aegophony and tactile fremitus, were not among the signs reported in the case notes. Respiratory rate above the upper limit for age was considered a sign of pneumonia. Wheezing was not considered indicative of pneumonia. Forty six (17.2%) chest radiographs were reported normal, 113 (42.2%) had abnormalities other than consolidation (infiltrates, peri-bronchial thickening, etc.) and 109 (40.7%) had pneumonic consolidation. Thirty eight patients without physical signs of pneumonia had radiographic evidence of pneumonia (focal consolidation). Only one of these tested positive for RSV antigen. The proportion of patients with occult pneumonia was therefore 14.2%. Table 25 shows the characteristics of patients with radiography-confirmed pneumonia. From the 268 patients who had a CXR done, 221 (82.5%) underwent blood culturing. Of the 109 patients with pneumonia, 94 (86.2%) had blood culture performed. Four of these blood cultures (4.3%) grew pathogenic organisms (2

streptococcus pneumoniae and one each of N. meningiditis and E. coli). The majority (78%) of the patients with pneumonia were hospitalized. Of all the patients, 35.8% were treated with intravenous antibiotics, 56% with oral antibiotics, and nine patients (8.2%) did not receive any antibiotics.

XI. 3. e. **Bacterial enteritis**

86 (13.9%) patients had stools taken for culture. Eleven (12.8%) yielded a positive test. Seven patients tested positive for Cl. difficile, two of these occurred in association with other SBI (meningitis and pneumonia). The latter two infections occurred during the winter months (November - January). The five others occurred in spring (March and April) and were not related to other infections. There were two infections with Campylobacter jejnei and one each of Salmonella group D and Shigella sonnei.

XI. 3. f. **Soft tissue infection**

There were two cases of clinically diagnosed soft tissue infection usually considered bacterial in origin; one with periorbital cellulitis, the other with cellulitis complicating chickenpox. Blood cultures from both patients were negative. There were no cases of septic arthritis or osteomyelitis.

XI. 4. **Non-SBI**

Patients who did not have microbiologic or radiographic evidence of any of the above infections were considered to have non-serious bacterial illness. The 453 (73.3%) such patients were a heterogeneous group, which included illnesses that were microbiologically defined, such as respiratory syncytial virus positive bronchiolitis, and rotavirus positive gastroenteritis. Others were defined by specific clinical presentation, such as varicella and herpes simplex stomatitis, while the diagnosis in the majority was based on clinical features and the failure to demonstrate a specific, viral or bacterial, pathogen. The latter group included otitis media, tonsillitis (with or without a positive throat swab culture), viral upper respiratory tract infection and non-specific viral illness.

The clinical diagnosis of otitis media was made without any indication of the causative organism because tympanocentesis was not in routine use. 52 (8.4%) patients were discharged with a diagnosis of otitis media, 22 of these had been hospitalised. Six patients discharged with a diagnosis of otitis media were reclassified as SBI according to investigation results, probably not available at the time of discharge. Two had pneumonic consolidation, two with UTI, and one with both pneumonia and UTI. The sixth patient, an 11 month old boy, had Streptococcus pneumoniae grown from blood culture. 222 (36%) of the patients had throat swabs taken for cultures. Seventeen of these cultures (7.7%) were positive for group A β haemolytic streptococcus. 54 (8.8%) of these patients were discharged with a diagnosis of tonsillitis. Although potentially serious, these infections were not included in the SBI group, in-keeping with the conventional definition of this type of infection. 138 (22.4%) were discharged with upper respiratory tract infection, bronchiolitis, or lower respiratory tract and chest infection as the diagnosis. 64 (10.4%) patients had nonspecific viral illness, with no clinically identifiable focus of infection. 18 (2.9%) patients had a specific clinically identifiable viral infection such as herpetic stomatitis, chickenpox or croup. A miscellaneous group of diagnoses comprised 26 (4.2%) patients. These included diseases such as allergy, arthralgia, Kawasaki disease, mesenteric adenitis and peticheal rash. 58 patients (9.4%) were discharged with a diagnosis of fever of unidentified origin. 17 patients (2.8%) were discharged without a diagnosis. 96 patients (15.5%) had a febrile convulsion during the course of their presenting illness. 19 (19.8%) of these had a SBI distributed as follows; 10 UTI, 7 pneumonia, and one bacterial gastroenteritis. A further 14.8 month old boy had pneumococcal bacteraemia and was discharged as febrile convulsion due to viral illness.

Table 14: Number of patients admitted with an infectious illness requiring hospital treatment*

Treatment	**Number of patients treated**	**Percent (%)**[δ]
Intravenous fluids	102	16.1
Oxygen	98	15
Suctioning	61	9.6
Naso-gastric tube feeding	42	6.6
Intravenous antibiotics	102	16.1
Any of the above	276	43.5
None of the above	359	56.5

*: Data from the nine week pilot study

δ: The cumulative proportion is >1 because some patients received more than one type of treatment.

Table 15: Demographic characteristics of the study population

Gender	**Number (%)**
Male	342 (55.3)
Female	276 (44.7)
Age (months)	
0-1	3 (0.5)
1.1-3	9 (1.5)
3.1-6	27 (4.4)
6.1-12	127 (20.6)
12.1-36	311 (50.3)
>36	141 (22.8)

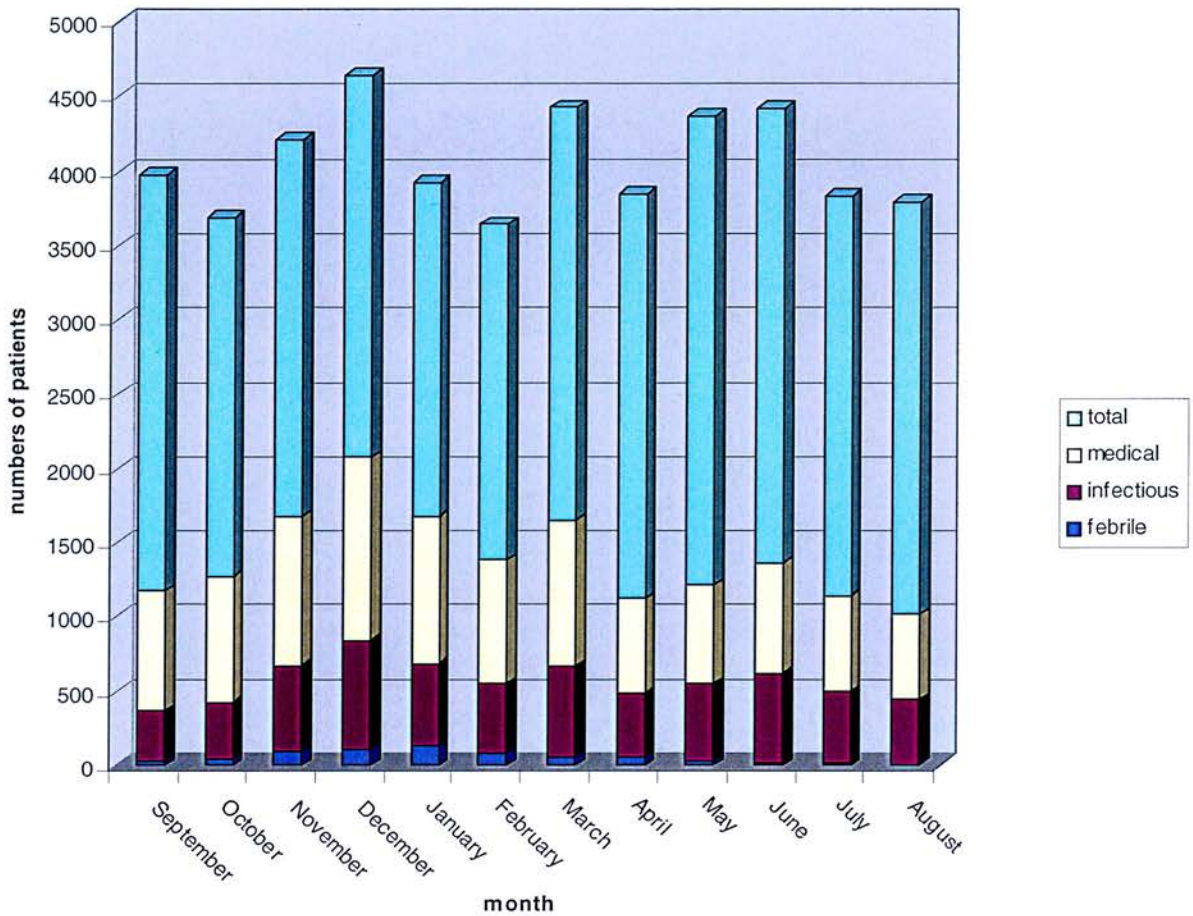


Figure 6: Types of illness seen at the emergency department during the study year

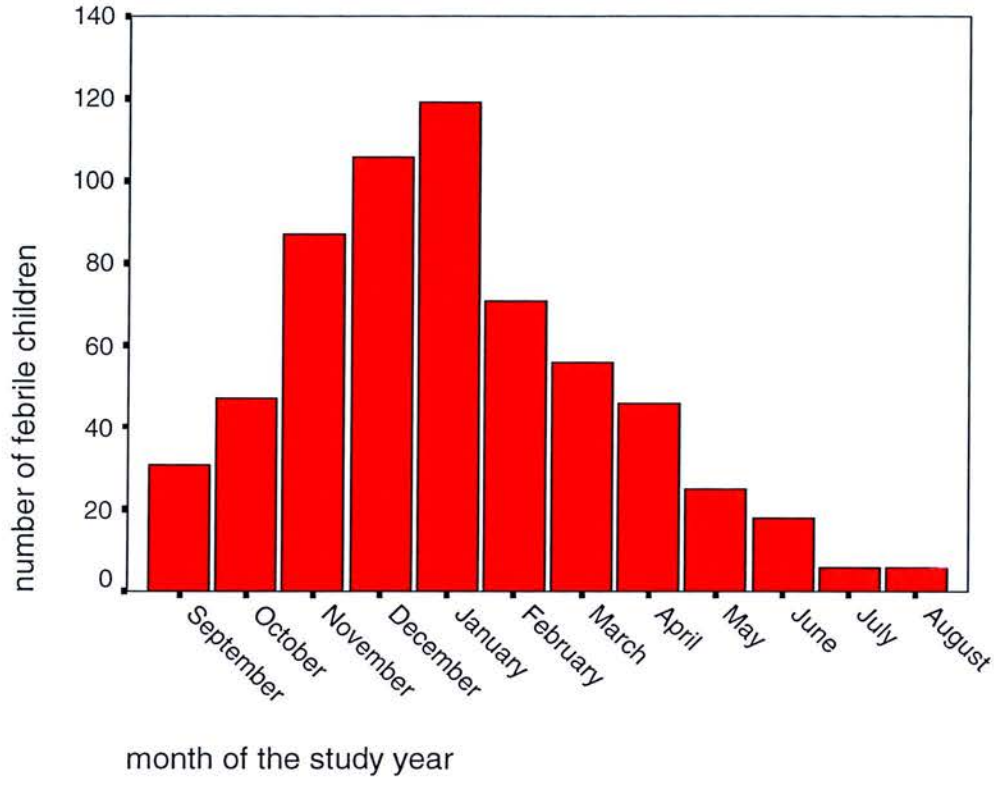
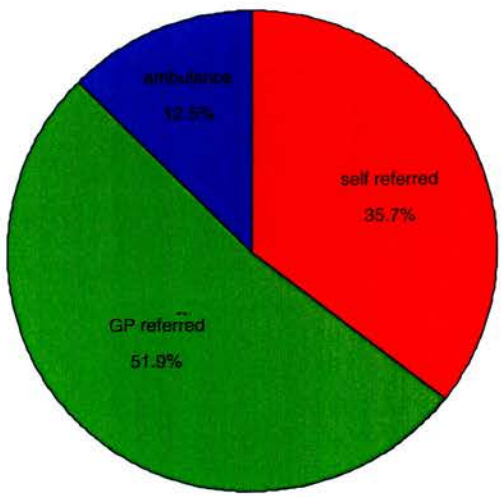


Figure 7: Seasonal distribution of febrile illness during the study year.



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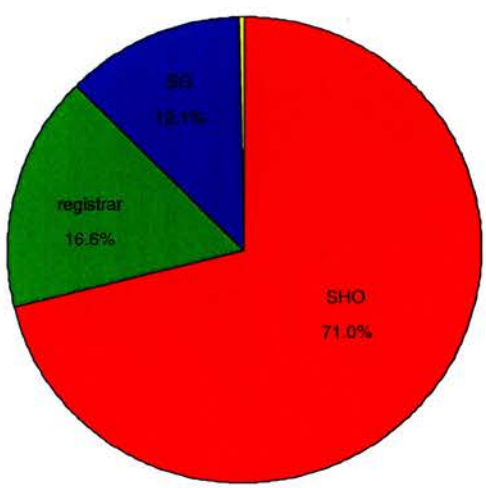

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Figure 8: a) source of referral and, b) grades of physicians first attending the patients at the emergency department.

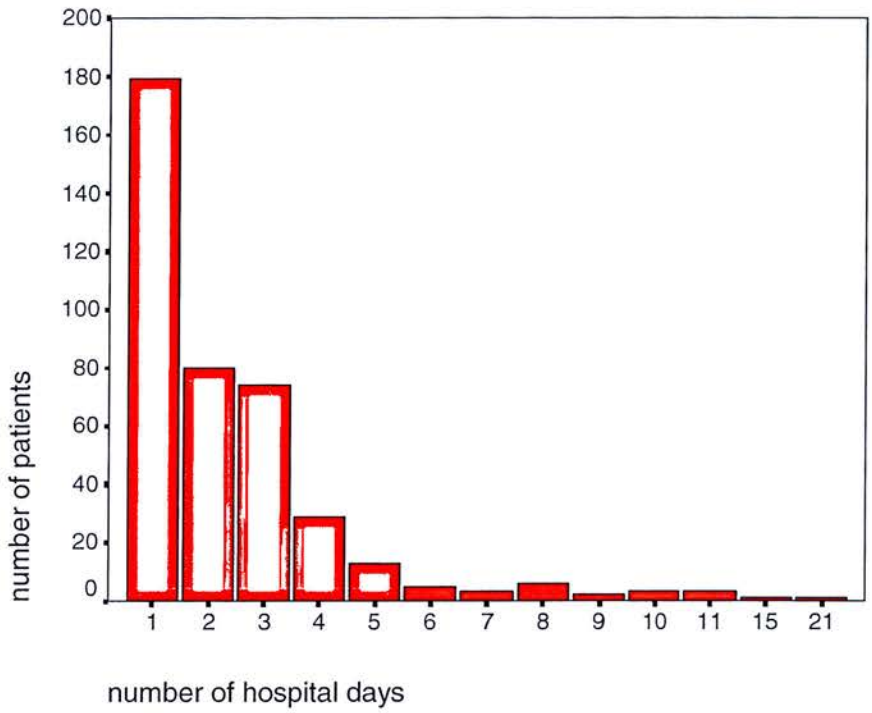


Figure 9: Length of hospital stay for hospitalized patients.

Table 16: Investigations for infectious illness performed on the study population

Test	Number (%)
Chest radiograph	268 (43.4)
Blood culture	441 (71.4)
Urine analysis/culture	343 (55.5)
Cerebrospinal fluid analysis/culture	29 (4.7%)
Stool analysis/culture	86 (13.9)
Throat swab culture	224 (36.2)
Viral titre/culture/antigen test	142 (23%)
Complete & differential blood count	501 (81.1)
CRP	234 (37.9)
ESR	100 (16.2)

Table 17: Sources of positive virological tests

Source	Number (%) positive
Nasopharyngeal aspirate	41 (62.1)
Mouth swab	1 (1.5)
Skin lesion	2 (3)
Stools	13 (19.8)
Stools & CSF	1 (1.5)
Stools & throat	5 (7.6)
Urine	1 (1.5)
Serological titre	2 (3)

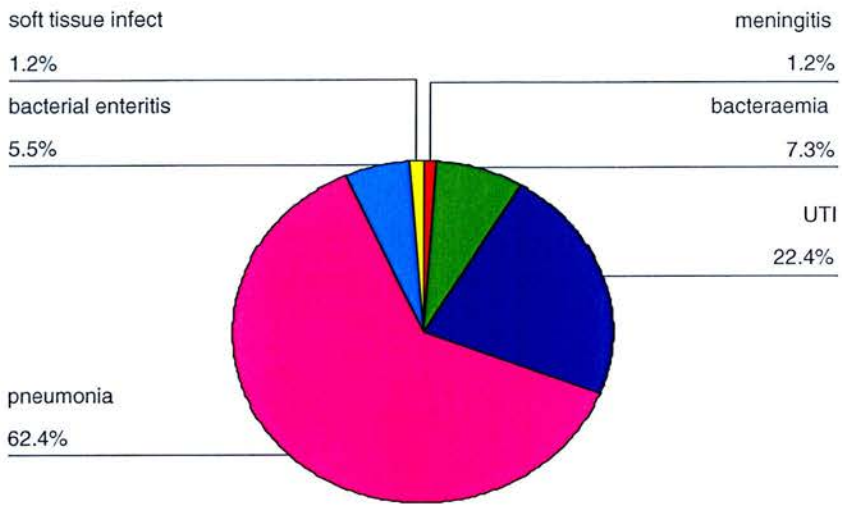
Table 18: Types of infectious illnesses in the study population

Illness	Number (%)
Bacteraemia	12 (1.9)
Bacterial meningitis	3 (0.5%)
Pneumonia	109 (17.6)
Urinary tract infection	39 (6.3)
Bacterial gastroenteritis	11 (1.8)
Bacterial soft tissue infection	2 (0.3)
Non-serious bacterial infection	453 (73.3)

Table 19: Incidence of UTI by age and sex

Age (years)	**<1**		**1-2**		**>2**	
Gender	Male	Female	Male	Female	Male	Female
Number of patients	96	70	108	88	138	118
Tested for UTI (%)	56.3	60	55.6	63.6	50	52.5
UTI present (% of tested)	9.3	26.2	3.3	17.9	4.3	12.9

a)



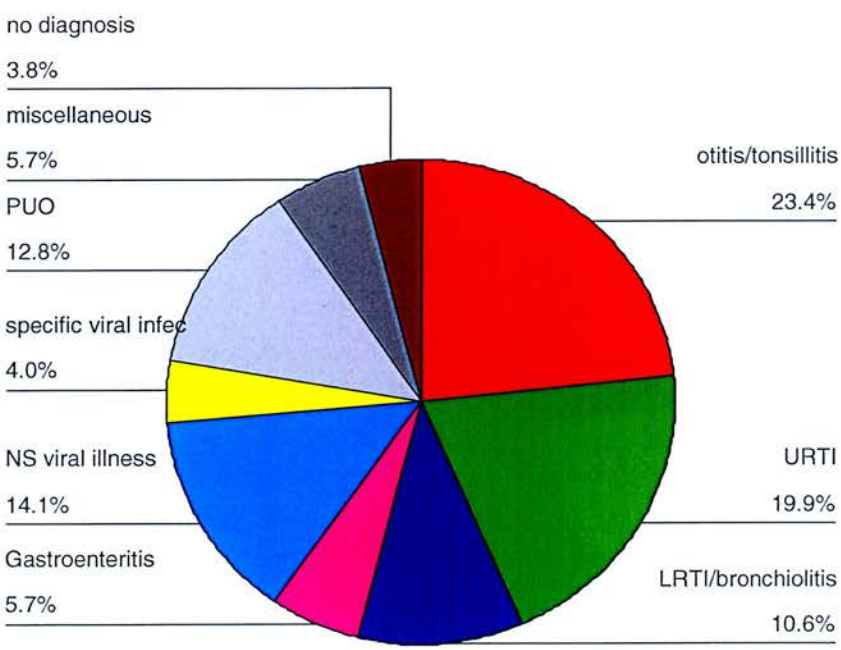


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Figure 10: Proportions of patients with a) SBI and, b) non-SBI.

Table 20: Clinical characteristics influencing the ordering of a chest radiograph

	CXR obtained	**CXR not obtained**	**p-value**
% male	55.2	55.4	0.960
Admission to hospital	77.2 %	54.5 %	0.0001
Presence of potential source of fever	30.2 %	43.1 %	0.001
Presence of signs of pneumonic consolidation §	35.8 %	1.4 %	0.0001
Presence of signs of pneumonia*	59.7	30	0.0001
Looking ill	84.2 %	52.9 %	0.0001
SBI likely	78.4 %	40.8 %	0.0001
Median age (mo)	17.4	20.7	0.008
Median clinical score	7	5	0.0001
Median temperature (°C)	39.1	39.0	0.009
Median respiratory rate (bpm)	40	34	0.0001

*: Including tachypnoea

§: See text

Table 21: Incidence of infectious illness across the age groups

	Number (%) of patients in each age group			
	0-1 mo	1-3 months	3-36 months	>36 months
Total number of patients	3	9	466	140
SBI	0	3 (33)	128 (27.5)	34 (24.3)
Meningitis	0	0	2 (0.4)	1 (0.7)
Bacteraemia	0	1 (11.1)	11 (2.4)	0
Pneumonia	0	2 (22.2)	84 (18)	23 (16.4)
Urinary tract infection	0	1 (11.1)	30 (6.4)	8 (5.7)
Bacterial enteritis	0	0	11 (2.4)	0
Soft tissue infection	0	0	0	2 (1.4)

Chapter XII

Results

Predictors of Serious Bacterial Infection

XII. 1. Clinical and conventional laboratory tests

There was no age or sex difference in the incidence of SBI. Patients referred by their general practitioners were more likely to have a SBI than those who were self-referred. Temperature, respiratory rate and heart rate and the clinical score were all significantly higher in patients with SBI (*Table 27*).The incidence of SBI steadily rose with the rise in temperature (*Figure 14*). 501(81.1%) of the patients were evaluated by a white blood cell count; and 151 of these had SBI. The total WBC was significantly higher in the patients with SBI (p=0.0001). Absolute neutrophil counts were significantly higher in patients with SBI compared to those with non-SBI (p=0.0001) (*Figure 15*). One hundred patients (16.2%) had ESR measured on presentation to the emergency room, and 20 of these patients had SBI. ESR was significantly higher in patients with SBI. CRP was done in 234 (38%) of the patients, and was significantly higher in those with SBI (*Table 32*). The performance of the conventional tests in predicting SBI are compared in T*able 29*.

XII. 2. Cytokines

XII. 2. a. Cytokines in febrile patients and afebrile controls

Eighty-nine patients were recruited as the control group. These were children who attended outpatient clinics during the study year. They were afebrile and did not have an acute illness at the time of blood collection (see exclusion criteria above). 50 (56.2%) were males and 39 (43.8%) were females. The median age was 23.7 months, range 0.1-57.9 months. From the 618 subjects, 342 (55.3%) were males, and their median age (range) was 19.3 (0.3-71.8) months. There was no significant difference in median age (p=0.247) or gender representation (p=0.882) between the controls and the subjects.

Serum IL-6 was measured in 60 (67.4%) of the controls and was detectable in four patients. IL-8 assay was done in 48 (54%) of the controls and was detectable in only one patient; an infant who had cerebral palsy resulting from group B, β-haemolytic streptococcal meningitis and was having increasing seizure frequency, but was clinically well at the time of blood collection (*Table 26)*. Soluble ICAM-1 was detectable in the sera of all the 67 (75%) controls from whom enough serum was available to measure this cytokine. The cytokine was normally distributed (p=0.200, Kologrov-Smirnov test) with a mean (SD) serum value of 895.68 (304.52) ng/ml (*Figure 11*). The minimum and maximum values were 108.00 ng/ml and 1600.00 ng/ml, respectively.

The 618 febrile patients who fulfilled the study criteria of age and temperature are hereby referred to as subjects. 312 (50.5%) of these patients had serum IL-6 measured. Interleukin-6 was detectable in the majority (95.2%) of febrile patients. The median serum IL-6 in these patients was 33.50 pg/l, range 00.00-5867.00 pg/ml, and interquartile range 15.00-76.37 pg/ml. Serum IL-6 was not detectable in 15 (4.8%) of the febrile patients. There was a significant difference in serum IL-6 between the afebrile controls and the highly febrile patients, mean ranks 40.92 and 214.50, respectively (p=0.0001). Serum IL-8 was measured in 264 (43.0%) febrile patients and was detectable in 71 (26.7%). The median value in the study group was below detectable level, with interquartile range 0.00-2.70 pg/l. The difference in serum levels between the

febrile patients and the afebrile controls was significant (p= 0.0001). Serum sICAM-1 was detected in all of the 295 febrile patients tested (48% of all the patients). The median value was 993.90 ng/ml, the minimum and maximum values, respectively, were 172.40 & 3949.90 ng/ml, with interquartile range 767.50 – 1232.80 ng/ml. Serum ICAM-1 in febrile patients had a normal distribution, with a few outliers (*Figure 12*). The difference in the serum sICAM-1 levels between controls and subjects was significantly different, mean ranks 148.22 vs 189.06, respectively, p=0.004 (*Figure 13*).

XII. 2. b. **SBI vs non-SBI**

Serum IL-6 was measured in 312 patients and of these 115 had serious bacterial infection. There was no significant difference in serum IL-6 between patients with and without SBI, p=0.362. Serum from 264 patients was assayable for IL-8; eighty-six patients had SBI. There was no significant difference in serum IL-8 between the two groups (p=0.884). Of 295 patients whose serum sICAM-1 was measured, 95 had SBI. Serum sICAM-1 was significantly higher in patients with SBI; p=0.005 (*Table 30*). Standing alone, none of the three cytokines was useful in discriminating SBI from non-SBI in a febrile child (*Table 31*).

Considering the twelve patients with a positive blood culture, there was no significant difference in serum IL-6 between bacteraemic and non-bacteraemic patients; median serum IL-6 (range) was 44.54 (0.00-5867.00 pg/ml) and 33.77 (0.00-1305.09 pg/ml) respectively, p=0.258. This was due to the wide overlap in the cytokine levels between the two groups. Nevertheless, IL-6 was detected in all bacteraemic patients who were tested for the cytokine, except the 19 months boy with meningococcal septicaemia who had extremely elevated serum IL-8 and sICAM-1 levels (Table 22). IL-6 was also highly elevated in all tested patients meningitis (Table 23). There was also no difference in serum sICAM-1 between bacteraemic and non-bacteraemic patients; median levels (range) being 1170.00 (662.20-3949.90 ng/ml) and 986.50 (189.40-3447.20 ng/ml) respectively (p=0.150). However, bacteraemic patients had significantly higher serum IL-8 values than non-bacteraemic patients; median (range) levels 101.70 (0.00-1652.00 pg/ml) vs. 0.00 (0.00-526.00 pg/ml) (p=0.0001). All the seven patients with either

bacteraemia or meningitis, who had IL-8 measured, had detectable serum IL-8. A serum IL-8 value of 20 pg/ml was 85.7% sensitive and 91.1% specific in detecting either meningitis or bacteraemia, with a negative predictive value of 99.6%. Six of the seven patients with either bacteraemia or meningitis who were tested for serum IL-8, had levels above 20 pg/l, while only one of the 235 patients who did not have either of these conditions had a serum level above this value.

XII. 3. Predictive model for serious bacterial infections among febrile young children

Variables significantly associated with SBI in univariate analysis (*Tables 32&33*) were sequentially entered in a logistic regression analysis to construct a model predictive of serious bacterial infection. Respiratory rate, serum CRP and serum sICAM-1 were identified as independent predictors of SBI. Hosmer-Lemeshow goodness-of-fit test results were not significant (χ^2, 3.53, p=0.897), which statistically supports the robustness of this prediction model;

$\{L = (\log (p/1-p^*) = \{(0.04\times RR) + (0.08\times CRP) + (0.001\times sICAM)-4.0\}$.

P*: Probability of SBI.

The model was derived from 122 patients from whom all test parameters were available. Thirty seven (30%) of these had SBI, and 26 (71%) were correctly identified by the model. The model was 52% sensitive and 84.7% specific for SBI. The respective positive and negative predictive values were 70.3% and 71.8%.

XII. 4. Correlations between infection parameters

Non-parametric (ρ) correlations between the infection markers are shown in table 29. There was little or no correlation between the three cytokines (*Table 34*).

Table 22: Details of bacteraemic patients

Organism	**Age** mo	**Sex**	**WBC** 10^3/dl	**ANC** 10^3/dl	**CRP** mg/dl	**IL-6** pg/ml	**IL-8** pg/ml	**sICAM** ng/ml	**Discharge diagnosis**
S. Pneumoniae (type 23)	4.1	M	16.8	12.8	14.6	5867.00	-	-	Pneumococcal meningitis
S. Pneumoniae	17.1	M	26.3	15.5	1.5	36.55	38.79	872.55	PUO, shivering
S. Pneumoniae	11.3	M	20.9	15.9	1.5	3005.40	102.22	2611.80	Otitis media
S. Pneumoniae (type 2)	14.8	M	16.5	13.5	-	-	-	-	Febrile convulsion, viral illness
S. Pneumoniae	16.4	F	27.1	16.8	2.6	-	-	-	Pneumococcal bacteraemia
S. Pneumoniae	24.5	M	38.2	32.1	18.0	-	-	-	Pneumococcal septicaemia
S. Pneumoniae	6.5	F	12.9	7.0	1.8	38.95	101.17	1170.20	Chickenpox
N. Meningiditis (type B)	19.3	M	21.8	16.4	1.5	0.00	1652.10	3949.90	Meningococcal septicaemia
E. coli	3.7	F	56.3	50.7	-	50.14	4.18	1801.40	Pneumonia, coliform sepsis
E. coli	12.5	F	41.4	37.4	14.5	-	-	-	UTI
Gr. B βHS	1.7	M	12.6	10.6	1.5	896.40	829.52	662.20	Gr. B βHS. septicaemia
Staph. aureus	13.9	M	14.3	10.2	-	11.3	-	992.70	Infected eczema

Table 23: Details of patients with meningitis

Organism	Strep. pneumoniae (type 23)	Neisseria meningidits (gr. A,typ1, subtypep1-14)	No organism isolated
Source	Blood & CSF	Throat swab	Clinical meningococcal meningitis
Age (mo)	4-1	17	66.5
Sex	M	F	F
CSF cells (/μl)	6720	1500	-
WBC (/mm^3)	16.8	12.6	26.8
ANC (/mm^3)	12.9	9.2	25.2
CRP (mg/dl)	14.6	-	-
IL-6 (pg/ml)	5867.00	737.99	1305.90
IL-8 (pg/ml)	-	84.52	-
sICAM-1 (ng/ml)	-	1227.10	3164.00

Table 24: Clinical and laboratory characteristics of patients with UTI

Parameter (median)	UTI	No UTI	p-value
Age (mo)	14.8	17.4	0.106
Temperature (°C)	39.0	39.1	0.333
Clinical score	5	6	0.406
WBC ($\times 10^3$/l)	18.7	12.6	0.0001
ANC ($\times 10^3$/l)	12.5	8.6	0.0001
ESR (mm/h)	52.5	23	0.041
CRP (mg/dl)	11.9	1.5	0.001
IL-6 (pg/ml)	27.87	34.84	0.840
IL-8 (pg/ml)	0.00	0.00	0.092
sICAM-1 (ng/ml)	1130.85	980.4	.071

Table 25: Clinical and laboratory* characteristics of patients with and without pneumonia

Parameter	Patients with pneumonia	Without pneumonia	p-value
% males	56.7	55	0.721
Median age (m)	20.3	19.1	0.658
Temperature (°C)	39.1	39.0	0.129
Clinical score	7	5	0.0001
Respiratory rate (bpm)	46	36	0.0001
WBC ($10^3/mm^3$)	14.7	13.2	0.112
ANC ($10^3/mm^3$)	8.9	8.7	0.423
ESR (mm/h)	50	23	0.008
CRP (mg/dl)	3.4	1.5	0.032
IL-6 (pg/ml)	36.17	32.8	0.963
IL-8 (pglm/l)	0.00	0.00	0.925
sICAM-1 (ng/ml)	1028.70	972.50	0.118

*: Median values

Table 26: Cytokine levels in controls

Sex	**Age** (months)	**IL-6** (pg/ml)	**IL-8** (pg/ml)	**Clinical details**
Male	0.1	0.42	-	Sibling of galactosaemia
Female	53.4	4.49	-	Listless, duplex kidneys, no UTI
Female	14.1	5.89	-	Galactosaemia
Female	22.9	25.41	-	Diarrhoea, milk fat intolerance
Female	18.5	-	215.72	Increasing seizure frequency, CP from GBβHS meningitis

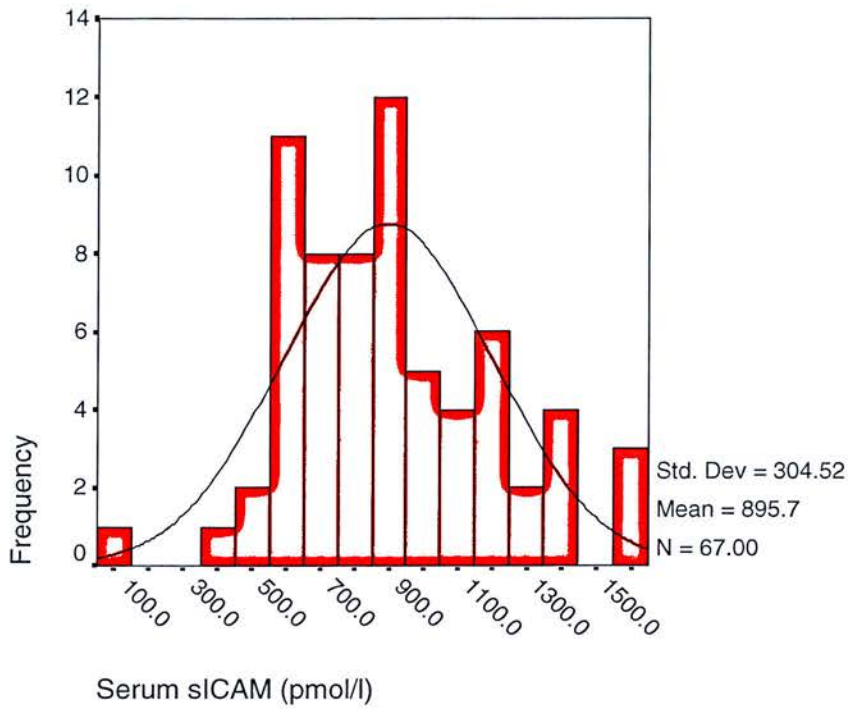


Figure 11: Distribution of serum sICAM-1 in controls

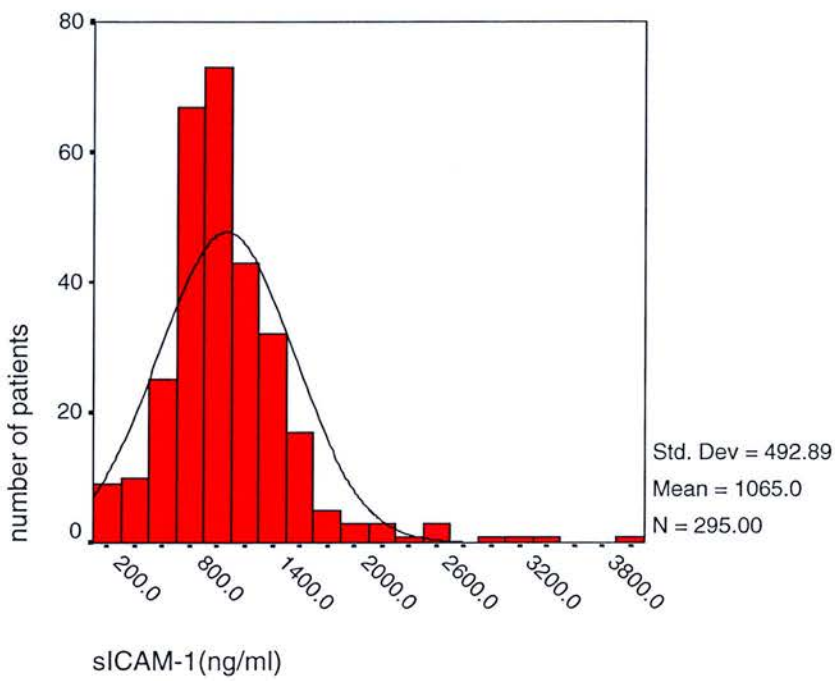


Figure 12: Distribution of serum sICAM-1 in febrile young children.

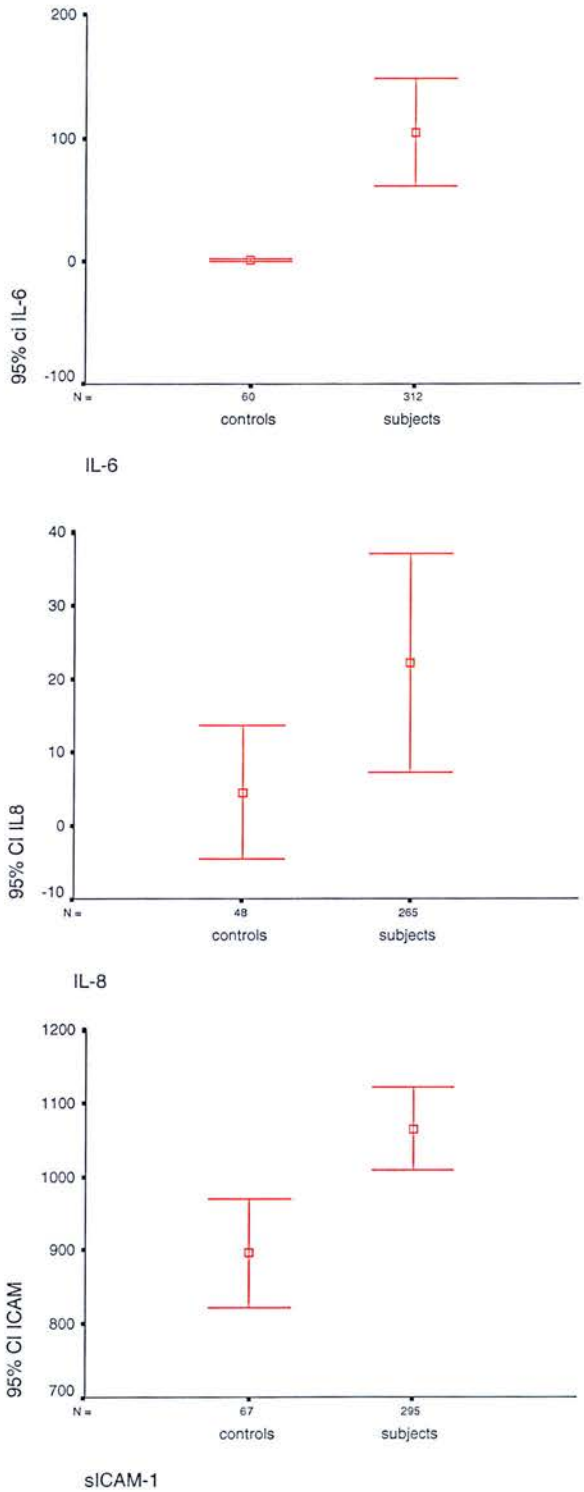


Figure 13: Mean (95% CI) serum cytokines in febrile subjects and afebrile controls.

Table 27: Demographic and clinical characteristics* of patients with and without SBI

	Number	Age (mo)	Boys %	GP referral %	Temp. (°C)	RR (bpm)	HR (bpm)	Clinical score
SBI	165	18.3	51.5	75.2	39.1	43	165	7
Non-SBI	453	19.6	56.5	60.2	39.0	36	156	5
p-value	-	0.395	0.248	0.001	0.017	0.0001	0.034	0.0001

*Median values for continuous variables.

Table 28: Performance of selected range of clinical score in predicting SBI

Score	Sensitivity	Specificity	PPV	NPV	LR (95% CI)	Post-test probability of SBI
>4	85	30	33	83	1.2 (0.4-3.1)	33
>7	55	65	39	78	1.6 (0.8-1.9)	40
>10	17	92	40	73	2.1 (0.6-3.5)	46
>13	7	96	40	72	1.6 (0.6-2.3)	40

Pre-test probability = 27%.

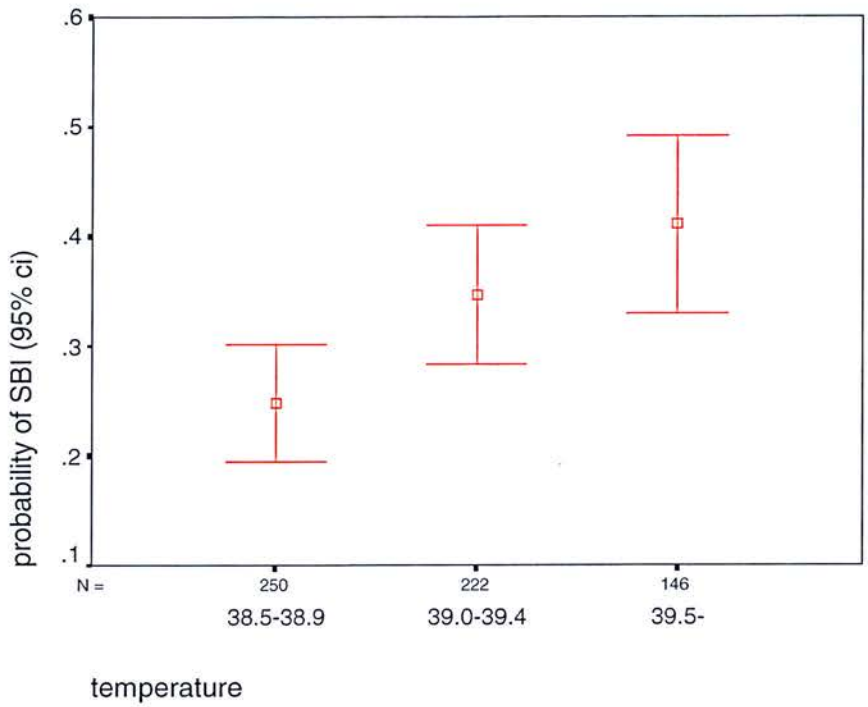
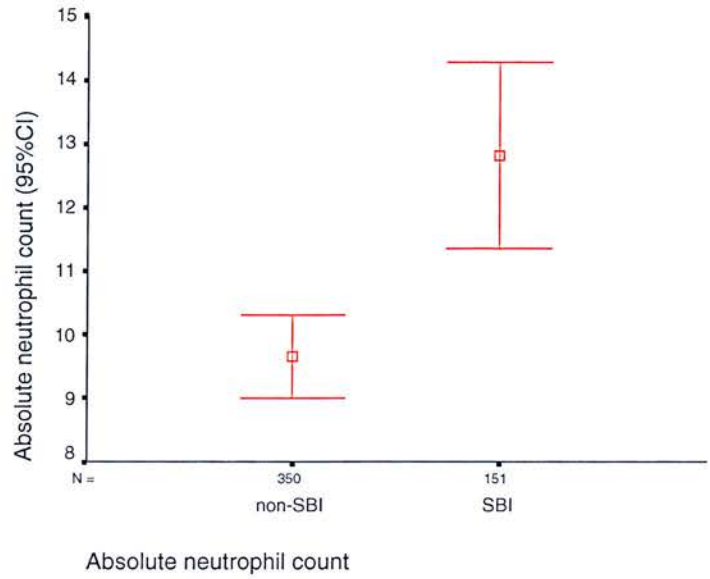


Figure 14: Increase in SBI with rise in temperature.



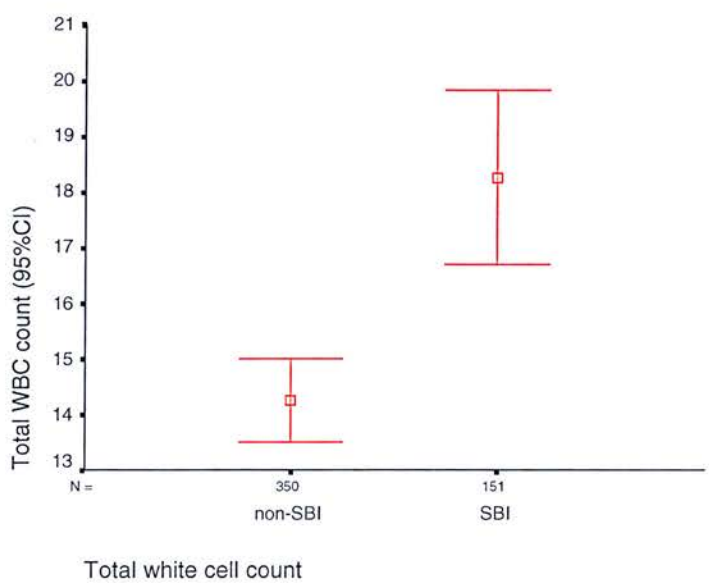


Figure 15: Mean (95%CI) WBC and ANC in patients with and without serious bacterial infection.

Table 29: Performance of selected ranges of values of conventional tests in predicting SBI.

	Sensitivity	**Specificity**	**PPV**	**NPV**	**LR** (95% ci)	**Post-test probability**
WBC (10^3/ml)						
5-15	-	-	-	78	1.6 (0.3-4.8)	41
>15	56	66	42	78	1.7 (0.3-5.1)	42
>20	36	83	47	75	2.1 (0.4-5)	48
ANC (10^3/ml)						
1.5-10	-	-	-	76	1.5 (0.3-4.8)	39
>10	53	64	39	76	1.5 (0.3-4.7)	39
>15	30	84	44	74	1.9 (0.4-4.6)	45
CRP (mg/dl)						
>5	43	75	45	73	1.7 (0.4-3.8)	45
>10	29	84	47	71	1.8 (0.5-3.3)	46
>15	12	96	56	69	2.7 (0.9-2.7)	56
ESR (mm/h)						
>20	75	49	27	89	1.5 (0.7-1.9)	27
>30	65	66	33	88	1.9 (0.8-2.3)	32
>40	60	75	38	88	2.4 (0.8-2.5)	38

Pre-test probability =27%.

Table 30: Median (range) serum cytokine levels in patients with and without SBI

	IL-6 (pg/ml)		**IL-8** (pg/ml)		**SICAM-1** (ng/ml)	
	SBI	Non-SBI	SBI	Non-SBI	SBI	Non-SBI
Number	115	197	86	178	95	200
Median	36.52	31.62	0.00	0.00	1056.60	952.65
Range	0 -5867	0-671.64	0 -1652	0 -526	323.90-3949.90	172.40-3062.80
p-value	0.362		0.884		0.005	

Table 31: Performance of selected cut-off values of the cytokines in predicting SBI

Test	**Sensitivity %**	**Specificity %**	**PPV %**	**NPV %**	**LR+ (95%CI)**	**Post-test probability %**
IL-6 (pg/ml)						
>5	91	10	37	66	1.0 (0.5-2.1)	33
>15	75	25	37	63	0.9 (0.6-1.8)	31
>35	53	55	41	67	1.2 (0.3-3.8)	35
>75	30	76	42	65	1.2 (.4-3.4)	35
>200	13	90	44	64	1.3 (0.5-2.6)	49
IL-8 (pg/ml)						
>5	22	79	34	68	1.0 (0.4-2.7)	27
>10	16	83	32	67	1.0 (0.4-2.5)	27
>20	12	89	34	68	1.1 (0.5-2.2)	29
>40	10	91	36	68	1.1 (0.5-2.1)	29
>100	6	97	50	68	2.0 (1.0-2.0)	44
sICAM-1 (ng/ml)						
>600	97	13	35	90	1.1 (0.7-1.6)	34
>750	83	24	34	75	1.1 (0.4-2.7)	34
>1000	59	58	40	75	1.4 (0.3-3.7)	40
>1200	36	74	39	71	1.4 (0.4-3.7)	40
>1600	13	91	40	62	1.4 (0.8-1.7)	40

Pre-test probability = 27%

Table 32: Clinical features and laboratory results of patients with and without SBI

Characteristic	SBI		Non-SBI		p-value
	n*		n*		
Median age (mo)	165	18.3	453	19.6	0.395
Sex (%male)	165	51.5	453	56.1	0.248
Referral (%GP-referred)	165	75.2	452	60.2	0.001
Temperature (%>39°C)	165	54.5	453	45.3	0.041
Median respiratory rate (bpm)	150	43	391	36	0.0001
Median heart rate (bpm)	160	165	427	156	0.011
Median clinical score	113	7	276	5	0.0001
Median total WBC (10^{10} /dl)	151	16.1	350	12.5	0.0001
Median absolute neutrophil count (10^{10} /dl)	151	10.6	350	7.9	0.0001
Median ESR (mm/hr)	20	47	80	22.5	0.007
Median CRP (mg/dl)	76	3.7	158	1.5	0.001
Median serum IL-6 (pg/ml)	115	36.52	197	31.62	0.362
Median serum IL-8 (pg/ml)	86	0.00	178	0.00	0.884
Median serum sICAM-1 (ng/ml)	95	1056.60	200	952.65	0.005

*: number of patients for whom the test parameter was available.

Table 33: Performance characteristics of clinical and laboratory tests in predicting SBI*

	Sensitivity (%)	**Specificity** (%)	**PPV** (%)	**NPV** (%)	**LR** (95% CI)	**ROC, AUC** $^{\Phi}$ (95% CI)	**PTP**§
Clinical score (>10)	17	92	40	73	2.1(0.6-3.5)	0.63 (0.57-0.69)	46
WBC ($>20\times10^3$/ml)	36	83	47	75	2.1(0.4-2.2)	0.63 (0.58-0.69)	48
ANC ($>15\times10^3$/ml)	30	84	44	74	1.9 (0.4-4.6)	0.61 (0.55-0.66)	45
CRP (≥15 mg/l)	12	96	56	69	2.7 (0.9-2.7)	0.62 (0.55-0.70)	56
ESR (≥40 mm/hr)	60	75	38	88	2.4 (0.8-2.5)	0.70 (0.54-0.85)	38
IL-6 (>200 pg/ml)	13	90	44	64	1.3 (0.5-2.6)	0.53 (0.46-0.60)	43
IL-8 (>100 pg/ml)	6	97	50	68	2.0 (0.95-2.0)	0.47 (0.40-0.55)	49
sICAM-1 (>1200 ng/ml)	36	74	39	71	1.4 (0.8-1.7)	0.61 (0.53-0.67)	40

*: Pre-test probability = 27%

Φ: Reciever operator curve, area under the curve

§: Post-test probability

Table 34: Correlation matrix (p-value) between tested parameters of infection

	WBC	ANC	CRP	ESR	IL-6	IL-8	sICAM-1
Temperature	0.17 (0.0001)	0.18 (0.0001)	0.22 (0.001)	0.04 (0.688)	0.21 (0.0001)	-0.05 (0.383)	0.04 (0.466)
WBC	-	0.91 (0.0001)	0.47 (0.0001)	0.30 (0.003)	0.43 (0.0001)	-0.02 (0.808)	0.25 (0.0001)
ANC	-	-	0.47 (0.0001)	0.22 (0.027)	0.47 (0.0001)	-0.02 (0.765)	0.25 (0.0001)
CRP	-	-	-	0.66 (0.0001)	0.47 (0.0001)	-0.07 (0.433)	0.26 (0.002)
ESR	-	-	-	-	0.19 (0.146)	-0.10 (0.478)	0.42 (0.001)
IL-6	-	-	-	-	-	0.07 (0.285)	0.13 (0.035)
IL-8	-	-	-	-	-	-	0.11 (0.082)

Chapter XIII

Discussion

XIII. 1. Prevalence of SBI

As there is no standard case definition of serious bacterial infection, a definition in common use was chosen as the primary outcome of the study. Most studies have considered bacteraemia, meningitis, urinary tract infection, pneumonia, bacterial gastroenteritis and osteomyelitis and soft tissue infection as SBI (see 1.2 chapter 1; definition of SBI). However, the inclusion of illnesses with variable potential aetiologies, such as pneumonia, could have affected the performance characteristics of markers of bacterial infection. In studies such as this, classification bias is almost inevitable because even in the face of positive culture results there is not enough evidence to discriminate infection from colonization. Nevertheless, as a main objective of the study was to find means of reducing uncertainty in evaluating a febrile child, and where in real life scenarios a febrile young child with radiographic pneumonia would be assumed to have a bacterial infection and treated as such, it was reasonable to include pneumonia in the definition of SBI. Furthermore, the use of a-priori-defined protocol for SBI diagnosis in a prospective study should have reduced the inherent ascertainment bias that rises from varying definitions of illness. Based on these considerations, patients with bacteraemia, bacterial meningitis, urinary tract infection, bacterial gastro-enteritis, and pneumonia and soft tissue infection (regardless of the bacteriologic proof of the later two groups of infections) were included in the SBI group.

Although mortality from infection has now substantially declined, infectious illnesses consume a major proportion of resources allocated for health care, both in hospital and primary care. Infection is the third commonest cause of mortality

considering all age groups and ranks tenth as a cause for hospitalization (*Wilson D & Bhopal R, 1998*). In general practice, infectious diseases account for 40% of new illness episodes, with the highest rates occurring in young children (*Fleming et al, 2002*). Following the introduction of Hib vaccine, streptococcus pneumoniae emerged as the commonest cause of SBI in children, although occasional cases of invasive Hib infection still occur due to failed or incomplete vaccination. The recently introduced heptavalent pneumococcal vaccine is expected to further reduce SBI in young children. However, a recent study in Scotland has shown that invasive pneumococcal disease is caused by 33 different serotypes, and only 48.4% of the isolates were serotypes included in the currently licensed PCV-7 vaccine, which is expected to provide protection in 71.8% of children below 5 years against serotypes in the vaccine *(McChlery S, et al. 2005)*. Furthermore, non-vaccine serogroups are recovered more frequently from children with meningitis than those with other invasive pneumococcal disease (*Ispahani P, et al. 2004*). The quest for more protective measures and more accurate diagnostic and surveillance methods therefore continues.

This study shows that a significant proportion of the young childhood population is first seen at or referred to the only paediatric hospital in the region with a febrile illness. Over 6% of non-trauma patients seen in the emergency department were febrile young children. These patients accounted for two percent of the total 5-year-child-population residing in the Lothian region served by the hospital. Lee and Harper estimate that 8% of total paediatric emergency department visits are for young children (≤ 36 months) with high (≥39.0°C) temperatures (*Lee & Harper, 1998*). More than three quarters (77.3%) of the current study patients were aged ≤ 36 months. Half of the febrile patients were between 12 and 36 months old, reflecting the higher incidence of acute respiratory infections in this age group. This is partly due to the waning of maternally acquired immunity and the increasing exposure of these children when they depart from their protective homes to attend day care centres and nurseries. This was further supported by the seasonal distribution of infectious illnesses whereby the great majority of cases were seen during the cold months of the year. Half the patients were seen between December and February, while less than 5% of the patients were seen in the

summer months. The study spanned a complete calendar year to allow for the expected seasonal variation in the incidence of different infectious illnesses. Neonates were usually followed up and admitted to the regional neonatal unit situated not far from the children's hospital. There was no SBI in the few neonates seen at the emergency department. The prevalence of febrile illness in the studied population was underestimated because historic fever (high temperature recorded at home) was not taken into account. Extremes of fever (>40C) were rare, as has been observed in earlier studies *(McCarthy et al. 1976, McCarthy et al. 1977)*.

During the period of study, the majority (65%) of febrile patients were hospitalised. Although a large proportion of the patients were admitted for only one day, these patients collectively consumed a significant proportion of hospital days. Moreover, patients hospitalized for acute illness were more likely to have more detailed investigations. Almost all hospitalized patients were investigated for an infectious cause or a marker of infection, while only a third of admitted children had parenteral antibiotics. The findings from the pilot study agree with other studies which suggest that a substantial proportion of child hospitalization may be unnecessary (*Kemper et al.1988, Perrin et al.1989, Gloor et al. 1993, McConnochie et al. 1997*). This over-admission is due to the uncertainty surrounding undifferentiated illness in children compounded by the lack of consistency in the evaluative process of febrile children. Therefore, the decision to admit may be made before a choice to treat or observe is made. Management guidelines based on objective criteria and known *priori* probability of serious illness in febrile children might alleviate this uncertainty and help implement a more rational and cost-effective way of dealing with this problem. An encouraging sign, nonetheless, was that nearly half of the patients who were discharged from the emergency room were investigated with blood culture. Discharging patients in the face of suspected bacteraemic illness demonstrates the growing awareness and utilization of the ambulatory service, which has recently been established within the premises of the emergency department. About half of the patients had already been seen by their GPs, and these patients were more likely to be admitted and had a higher incidence of serious bacterial infection. This is in-keeping with the findings of Duffy et al who studied

hospitalization of adult patients in the same region (*Duffy et al. 1998*). These findings further support the role of community-based management of young children considered to be at low risk of SBI. This can be further facilitated by the knowledge of the prevalence of infectious illnesses and the likelihood of serious bacterial infection in young patients presenting with a high fever.

The incidence of serious bacterial infection in the studied population (27.6%) was within the range of prevalence figures quoted by other authors. Nademi et al found one out of three children referred with fever to have a SBI, with 42% of microbiologically proven meningitis and/or sepsis caused by meningococci (*Nademi Z, et al, 2001*). Using a definition of SBI similar to that used in our study, Bleeker et al reported a 25% incidence in a group of 1-36 month old children (*Bleeker et al, 2001*). Pulliam et al found 18% of their patients (1-36 months old) had a serious bacterial infection. Bacterial gastroenteritis was not counted as a SBI and only patients without clinically detectable infections were studied, accounting for the lower incidence (*Pulliam et al. 2001*). Galetto-Lacour et al reported a 23% incidence of SBI in children aged 7 days to 36 months. The commonest (68%) was UTI (*Galetto-Lacour et al. 2001*). In a more recent study, the same authors studied children 7 days to 36 months old who did not have a localising cause for their fever. Twenty-nine (29.3%) of 99 patients were diagnosed with SBI (*Galetto-Lacour et al. 2003*). Gendrel et al studied 1500 children aged 1 month to 15 years of age who were admitted with a fever ≥38.5°C. The responsible infectious agent was identified in 360 patients, and 114 (31.7%) of these had a serious bacterial infection. There were 5 cases of meningitis caused by H. influenzae type b, in a population supposedly covered by the Hib vaccine. Among the other bacterial infections were septicaemia caused by gram-negative organisms, such as E. coli and Salmonella species (*Gendrel et al. 1999*). Lopez and colleagues identified 150 patients with invasive bacterial disease from a total of 445 (33.7%) febrile young children (*Lopez et al. 2003*). Invasive bacterial illnesses included septicaemic enteritis, acute pyelonephritis, sepsis and meningitis, arthritis and soft tissue infection, and osteomyelitis. These children had higher probability of SBI as they were all hospitalized and all underwent blood analysis to rule out the possibility of bacterial infection. Similar

to our findings, they identified a heterogeneous group of organisms causing infections, such as E. coli septicaemia, salmonella enteritis, meningococcal meningitis, and staphylococcal arthritis, among others. These differences in the reported incidences of bacterial illness are explained by differences in the study populations, physician's criteria (temperature and age) for ordering cultures and other tests in febrile children, interpretation of radiographic and laboratory test results and the coverage rates of Hib and pneumococcal vaccines.

The majority (80%) of SBI in the current study occurred in children ≤36 months of age, the group of patients most widely studied. All cases of bacteraemic illness and bacterial gastroenteritis, and two of the three cases of meningitis, were in these younger patients. The three febrile neonates (<1 months) did not have a SBI, but the only child in the 1-2 months range had a bacteraemic SBI. This underscores the importance of careful assessment and extensive evaluation of these young infants. Beyond this age the incidence of SBI was similar across all age groups. An age related variation, albeit differently, in the incidence of SBI was also noted by other workers. In the study by Galetto-Lacour et al, infants <3 months had a 26% incidence compared to 20% in those 3-12 months old and 23% in 12-36 months old (*Galetteo-Lacour et a. 2001*). Bonsu and Harper retrospectively studied consecutive febrile young infants (0-89 days old) and reported a 1% rate of bacteraemia (*Bonsu & Harper, 2004*). Baker and Bell found that the incidence (12.6%) and spectrum of causative organisms of SBI in febrile neonates was similar to that observed in febrile infants 29 to 60 days old. However, the authors demonstrated that infants younger than one month were different in that SBI was unpredictable and concluded that protocols based on risk criteria are not applicable to these young patients (*Baker et al. 1999*). In a commentary, De Angelis noted that febrile infants younger than one month were different and suggested no changes should be made to the current practice of extensive evaluation under broad spectrum antibiotic cover (*Baker et al. 1999*).

XIII. 1 .a. Bacteraemia

In the studied population, all positive blood cultures were in children younger than three years of age. The incidence of bacteraemia appeared to be constant across the

two year period (1998 and 1999) represented by the pilot study (2.5%) and definitive study (2.7%), respectively. The prevalence of bacteraemia and the types of causative organisms were comparable to that reported in other studies of febrile children. Isaacman et al retrospectively studied young children (3-36 months) seen in a paediatric emergency unit who had blood culture obtained for evaluation of fever. Two percent of the cultures grew a pathogenic organism (*Isaacman et al. 2000*). In a post-Hib vaccine study, Lee and Harper reported a 1.6% incidence of bacteraemia in unselected 3-36 months old children with a high (>39.0°C) fever. The risk of bacteraemia was significantly lower in the 3-6 months age group than in older age groups. Beyond six months, there was no significant difference in the odds of bacteraemia between various age categories (*Lee and Harper, 1998*). Our findings are different in that patients younger than 6 months had a higher incidence of bacteraemia (7.1%) than older patients (1.1%). There were more cases of gram negative bacteraemia, which tend to affect younger age groups, in our patients. We witnessed no bacteraemic illness beyond 36 months of age.

Similar to most studies from Western countries, the absence of Haemophilus influenzae type b was evident in our study. In the study by Lee and Harper ninety-two percent of the isolates were pneumococci, and the others were Salmonella (5%), N meningiditis (1%), and group A streptococcus (1%). Haemophilus influenzae type b was not isolated from any of the children (*Lee and Harper, 1998*). Bandyopadhyay et al found 37 (3%) of 1202 young children (2-36 months) with fever without source to have a positive blood culture. Streptococcus pneumoniae was the most prevalent (84%) organism, and only two patients (0.08%) developed serious bacterial infection (*Bandyopadhyay et al. 2002*).

In our population, the rate of positive culture in patients with no focus of infection (occult bacteraemia) was 1.4%. The majority (71.4%) of isolated organisms were streptococcus pneumoniae. The prevalence of occult bacteraemia has been further reduced in populations covered with the conjugate pneumococcal vaccine (PCV7). Stoll and Rubin studied young children (2-36 months) who had blood culture taken for evaluation of fever and who were discharged from the emergency room. Three (0.91%)

out of 329 cultures were positive, all streptococcus pneumoniae. The studied population had received the heptavalent pneumococcal vaccine (PCV-7), so the authors suggested that routine practice of obtaining blood cultures may no longer be indicated in previously healthy, well-appearing, highly febrile young children who have received at least 1 dose of PCV7 (*Stoll et al. 2004, Rubin et al. 2004*). Although in the majority of children occult pneumococcal bacteraemia resolves without therapy (*Baraff, 2000*), untreated pneumococcal bacteraemia can result in complications, including persistent bacteraemia and fever, pneumonia, cellulitis and meningitis (*Jaffe et al. 1987*). There is an estimated 2.7% risk of meningitis from untreated pneumoccal bacteraemia (*Rothrock et al. 1997*). Non-vaccine serotypes of streptococcus pneumoniae have been isolated with increasing frequency in the years following the introduction of the heptavalent vaccine. Furthermore, antibiotic resistance of these serotypes has increased (*Farrell DJ, 2007*). Other workers have reported a decrease in the carriage rate of penicillin resistant pneumococci after the introduction of the 7-valent vaccine (*Cohen R, 2006*).

In our study population, the causative organisms of bacteraemic illness were more heterogeneous. E. coli was isolated in two infants, both with concurrent urinary tract infection. The baby with group B streptococcal septicaemia was less than two months, an age that was not commonly included in other studies. A highly febrile infant with infected eczema had Staph. aureus isolated from both skin swab and blood culture. These organisms usually cause specific focal infections, and cause a more severe illness when they invade the blood stream. They were therefore expected to be detected more frequently in our study population, which included hospitalized children. In a population of febrile infants (<1 year) at an African teaching hospital, Ayoola et al reported a 38.2% incidence of bacteraemia, with E. coli and S. aureus each accounting for over a third of cases (*Ayoola et al. 2002*). Meningococcal infections were particularly difficult to ascertain. This organism was not recovered from the blood in patients with clinically diagnosed meningococcaemia, and from those with clinical meningitis who had CSF pleocytosis and meningococci isolated from throat swabs. This could be due to the administration of antibiotics before referral, as is usually the practice upon clinical suspicion of meningococcaemia. Many patients treated for meningococcaemia do not

have this infection (*Jaskiewicz et al. 1994*), while it can pass undetected, clinically, in a significant proportion of patients (*Dashefsky et al. 1983*, *Kuppermann et al. 1999*). Although occult meningococcal bacteraemia is rare, observed in only 0.06% of children with fever without source in two multicentre studies (*Jaffe et al. 1987, Bass et al. 1993*), the risk of serious sequelae is greater and includes purpura fulminans and meningitis. Meningitis is said to occur in half of the patients with meningococcal bacteraemia who are not treated with antibiotics (*Kuppermann et al. 1999, Barraf. 2000*). It is evident, therefore, that the need for obtaining blood culture and rigorous evaluation of febrile young children will continue regardless of the immunization status of the patient or vaccination coverage in the population.

We have demonstrated that a significant proportion of serious bacterial infection, including UTI and bacteraemia, occurred in children discharged with apparently less serious diagnoses, such as otitis media or URTI. These patients were at risk of having their underlying disease (UTI) going unchecked or developing more serious illness, such as meningitis, from undetected bavteraemia. Other workers also have emphasised the frequent occurrence of SBI in febrile children with seemingly benign illness. Isaacman et al found that the proportion of patients with bacteraemia with a focal bacterial infection was not dissimilar to that in patients without a focal bacterial infection (*Isaacman et al. 2000*). Schutzman et al noted a 3% incidence of bacteraemia in children with otitis media, similar to those seen in children with no source of infection (*Schutzman et al. 1991*). In the study by Lee and Harper, 31% and 12.8% of positive blood culture occurred, respectively, in patients discharged with a diagnosis of otitis media and viral illness. The incidence of occult bacteraemia was the same in patients with and without otitis media (*Lee & Harper, 1998*). Levine et al have shown that the risk of SBI, particularly that of UTI, is appreciable in infants with RSV positive bronchiolitis. Seven percent of RSV +ve patients, compared to 12.5% of RSV-ve patients, had a SBI (*Levine et al. 2004*). There was a 1.1% rate of positive blood culture in RSV+ve patients, compared to a 2.3% rate of bacteraemia in RSV-ve patients. The occurrence of SBI was 10.1% in RSV+ve children ≤28 days and 5.5% (all UTI) in children >28 days. The authors stated that a septic workup in infants ≤28 days is

indicated even if an RSV infection is documented and that the presence of RSV does not obviate the need for urine culture in infants >28 days (*Levine et al. 2004*). Titus and Wright retrospectively analysed RSV+ve febrile infants and compared them with matched infants who tested negative for RSV. Only 2 (1.2%) of the 174 RSV+ve patients had SBI (both UTI), compared to 22 (12.6%) of the control group. Nonetheless, the authors recommended that urine cultures be obtained in all febrile infants with RSV infection (*Titus and Wright. 2003*).

On the other hand, patients with specific viral syndromes (croup, stomatitis and varicella) are unlikely to have an underlying bacteraemia. Greens et al retrospectively studied young children (3-36 months) with a recognizable viral syndrome (RVS) who had a blood culture done. Two of 876 (0.2%) cultures were positive for pathogenic bacteria. The authors concluded that highly febrile young children with uncomplicated RVS have a very low rate of bacteraemia and need not have blood drawn for culture (*Greenes et al. 1999*). It is perhaps prudent to consider and search for SBI in febrile infants even when a diagnosis of respiratory infection was clinically made.

XIII. 1. b. **Meningitis**

The incidence of meningitis in the study group was 0.5%. Streptococcus pneumoniae was isolated from the blood of a 4 month old baby with typical clinical and cytologic appearances of bacterial meningitis, and was thus considered pneumococcal meningitis (*Shackley F, 2000*). The two other patients had clinical features of meningococcal septicaemia and meningitis, but this organism was not isolated from CSF. Around 15% of febrile patients with purpuric rash will have meningococcal disease and, in the presence of meningeal signs, such patients are considered to have meningococcal meningitis even if the organism is not isolated from CSF (*Wells et al. 2001)*. However, viruses, such as enteroviruses and adenovirus, and bacteria other than meningococci, also can cause a non-blanching rash (*Neilsen et al. 2001*). Likewise, meningococci isolated from the throat of a patient showing clinical signs of meningitis and CSF pleocytosis would be considered the causative agent of this disease in the absence of other pathogenic bacteria grown from CSF. This low incidence of meningitis

might allow for a less aggressive approach in evaluating young children showing no signs of meningitis in whom the invasive lumber puncture can be omitted.

XIII. 1. c. Urinary tract infection

During the study period results of urinalysis were provided in terms of white cell numbers per high power field. Significant pyuria was quoted as wbc >40, although counts higher than 100 were given in absolute numbers. Colony counts of $\geq 10^5$/ml of a single known urinary pathogen only were considered indicative of UTI, irrespective of the result of urinalysis. This is a more conservative definition than those used in many other studies. In spite of this, the overall prevalence of UTI in our population (11.4%) was high, with exceptionally high rates in female infants. However, not all febrile children were tested for UTI and therefore the high rates of UTI could be due to selective testing of patients thought likely to have the illness. Furthermore, the high rates in boys below one year (9.3%) could be explained by the almost non-existence of circumcision in the studied population. Estimates of the prevalence of UTI in febrile children vary among studies. This is due to the variation in the definition of UTI, method of urine collection, and eligibility criteria with inherent selection bias. Roberts et al reported an incidence of 4.1% from bag urine in children 0-2 years old with unexplained fever. All infections were in girls, who had a 7.4% incidence. The lower reported rate can be explained by the more stringent criteria used in testing for and defining UTI; Infants with symptoms or signs attributable to other organ systems were excluded, a colony count of $>10^5$/ml of a single organism was not considered UTI unless it was grown from two successive urine specimens, and isolated pyuria was not considered a urinary infection (*Roberts et al. 1983*). Crain et al. reported a 7.5% incidence of UTI in febrile infants younger than 8 weeks of age, and estimated that more than half of UTI would have been missed if normal urinalysis (<5 WBC phpf) results were used to forgo obtaining a urine culture (*Crain et al. 1990*). Buys et al studied 545 febrile children of all ages with urine collected by suprapubic aspiration and found 44 (8%) children with bacteruria. Two thirds of the bacteruric children had abnormalities of the urinary tract, while radiological abnormalities were found in a third of children, mainly neonates, with

sterile pyuria (*Buys et al. 1994*). Shaw et al obtained catheter urine culture from all infants younger than 12 months and girls younger than 2 years with temperature ≥38.5C° without a definite source for fever. The reported overall prevalence of UTI was 3.3% with higher rates in white girls (16.1%), uncircumcised boys (8%), and those with a history of UTI (9.3%), malodorous urine (8.6%), and abdominal tenderness (13.2%) (*Shaw et al, 1998*). Bauchner et al evaluated 664 children younger than 5 years of age presenting with an acute febrile illness and no symptoms indicating UTI. Only 11 (1.7%) had significant bacteruria. The authors concluded that searching for silent UTI is unwarranted in the presence of another explanation for fever (*Bauchner et al. 1987*). Febrile children with viral syndromes, such as bronchioilitis, and other identifiable sources of fever outside the urinary tract may be at low risk for UTI (*Kuppermann et al. 1998, Hoberman et al. 1993*). However, the presence of less well-defined potential sources of fever, such as upper respiratory tract infection, otitis media and gastro-enteritis, does not exclude urinary tract infection (*Shaw et al. 1998*).

The incidence of bacteraemic UTI (5.1%) was comparable to the 6% incidence of bacteraemic UTI reported by Crain. None of the infants studied by Krober et al had bacteraemia associated with UTI. These results are different from those reported by McCracken and Ginsburg who found a 21% incidence of bacteraemia in infants 1-2 months old with UTI (*Ginsburg et al. 1982*). The patients in the latter study were sick young infants many of whom were referred by other health facilities. In a more recent study, Pitetti and Choi reported a 22.7% incidence of bacteraemia in infants younger than two months and 3% in those aged 2-36 months of age with UTI (*Pitetti & Choi, 2002*). In febrile children, bacteraemic UTI is not clinically distinguishable from, and has comparable outcome to non-bacteraemic UTI (*Honkinen et al. 2000*). The goals of treatment of acute UTI- eliminating the acute infection, preventing sepsis, and reducing the likelihood of renal damage- are all achievable by outpatient treatment. However, since bacteraemia in itself is an indication for hospital treatment and observation, hospitalization is necessary if the patient has clinical sepsis or is likely to have bacteraemia based on clinical or laboratory evaluation.

XIII. 1. d. **Pneumonia**

Because not all patients were investigated by chest radiography, the true prevalence of pneumonia in the study population of febrile young children cannot be determined with certainty. It would seem that patients considered at lower risk of pneumonia did not have a chest radiograph ordered. Patients who had a chest radiograph done were significantly younger, looked more ill, had a higher median temperature and respiratory rate, and were more likely to be admitted. On the other hand, while a chest radiograph was obtained in almost all patients who had clinical signs of pneumonia, a third of those who did not have such signs were also investigated by chest radiography. Furthermore, pneumonia was defined more stringently than in most of other studies in that any radiographic findings short of focal consolidation in absolute terms, were not considered pneumonia. The prevalence of pneumonia may have been underestimated by considering all the patients with equivocal pneumonias, e.g. patchy infiltrates, and those who did not have a radiograph as "no pneumonia" patients- the latter would have specifically underestimated the occurrence of occult pneumonia. Assuming that those who did not have CXR done did not have pneumonia, the incidence of pneumonia in this population is 17.6%. This is comparable to the 19% incidence reported by Leventhal in febrile children of all ages (*Leventhal et al. 1982*). Zukin et al reported a 14% incidence of pneumonia in children up to the age of 17 years who were evaluated with a chest radiograph (*Zukin et al. 1986*). Kramer et al studied younger (3-24 months) febrile children with chest radiographs taken at their initial visit to the emergency department. Twenty seven percent had radiographically diagnosed pneumonia (*Kramer et al, 1992*). Patterson examined febrile young children (≤2 years) with and without symptoms and signs of LRTI by chest radiography and found parenchymal opacification in 14% of the patients (*Patterson. et al. 1990*).

The incidence of bacteraemic pneumonia in this study was 2.8%. This was similar to the findings of Hickey et al who reported a 2.7% yield of pathogenic bacteria in paediatric patients with radiographic evidence of pneumonia (*Hickey et al 1996*). Ramsay et al reported a 2% rate of positive blood culture in children, of all ages, with acute lower respiratory tract infection (*Ramsey et al. 1986*). Streptococcus pneumoniae

was the causative organism of bacteraemic pneumonia in most of the previous studies. In our study, pneumococci were responsible for only half of bacteraemia associated with pneumonia. E.coli was grown from blood and urine of a 3.7 month girl who had chest crepitations and 20 white cells/mm^3 in the CSF. She was discharged with a diagnosis of pneumonia and coliform sepsis. Gram negative bacteraemic pneumonia is a highly specific subgroup of pneumonia that occurs mainly in infants and debilitated elderly patients and has a high mortality. The most important organism is Klebsiella, which causes Friedlander's pneumonia. Less commonly, E. coli, pseudomonas, and other species are implicated. A nineteen month old boy was admitted with a purpuric rash and had physical and radiographic evidence of pneumonia. N. meningiditis was grown from blood obtained upon admission. Primary meningococcal pneumonia is a recognized clinical entity that is associated with pleural effusion or empyema in 15% of cases (*Eastbrook et al. 1996*). Blood culture is rarely positive in patients with radiographic pneumonia and in clinical practice its use should be individualized to those who appear ill.

XIII. 2. **Predictors of SBI**

XIII. 2. a. **Clinical assessment**

Patients with SBI scored higher (median 7) on the clinical assessment scale than those without SBI (median 5). However, the score was not clinically helpful in identifying patients with SBI: only 17% of such patients scored more than 10, compared with 8% of those without SBI. Thus, a score higher than 10 was 17% sensitive and 92% specific in identifying SBI. A score of >10 increased the likelihood of SBI by only 19% over that of the pre-assessment probability. The physician's subjective assessment was equally unhelpful. The performance of the scale in our study mirrored that reported in recent studies. Strait et al recorded the acute illness observation score for febrile children (0-36 months old) without an apparent focus of infection. Patients with an occult bacteraemia had a mean score of 9 (95% CI 8-11) compared to a mean of 8 (95% CI 7-8) for children without bacteraemia. The difference, though statistically significant

(p=0.03), was not of clinical use in distinguishing patients with occult bacteraemia (*Strait et al. 1999*). Several recent studies have demonstrated the inaccuracy of the observation scale in identifying patients with serious bacterial infection. Kuppermann et al studied 100 febrile children aged 2 years or younger and found no difference in the YOS between children with documented bacterial infection and those with laboratory-documented respiratory viral infections (*Kuppermann et al. 1999*). Pulliam et al prospectively studied febrile children 1 to 36 months of age with clinically undetectable SBI and found them indistinguishable from those without SBI in YOS (*Pulliam et al. 2001*). Similarly, young children (7 days – 36 months) with and without SBI had comparable YOS, as reported by Galetto-Lacour et al (*Galetto-Lacour et al. 2001*). In a more recent study, the same authors reported a 23% sensitivity (95% CI 5-54%) and 82% specificity (95% CI 67-92%) for a YOS >10 in predicting SBI (*Galetto-Lacour et al. 2003*). In our study, the clinical score has been recorded by different physicians with varying levels of expertise. The resulting inter-observer variability is unquantifiable and is likely to reduce the reliability of this measurement. Furthermore, the case mix in the emergency department resulted in inclusion of patients with severe viral illness who looked equally sick on the parameters measured on the scale. The antipyretic given to a large proportion (47%) of patients before being evaluated also could have altered the physical appearance of the child and, therefore, influenced the clinical assessment.

XIII. 2. c. Traditional laboratory aids

Algorithms for evaluating febrile children have commonly included WBC although studies have shown that it is not a reliable indicator of SBI (*Barraf, 2000, Bachur et al. 2001*). In our population of febrile young children the white cell and absolute neutrophil counts, although significantly higher in patients with SBI, were not helpful in identifying these patients. Using the commonly used cut-off value of $15\times10^3/mm^3$, WBC was only 56% sensitive and 66% specific in detecting SBI. Theses values are comparable to those reported in the more recent studies evaluating blood indices. Pulliam et al reported a sensitivity and specificity of 64% and 67%, respectively, for a WBC at $\geq15\times10^3/mm^3$, for predicting SBI in a 1-36 months child

population (*Pulliam et al. 2001*). Ayoola et al studied one-year-old African children with a high incidence (38.2%) of bacteraemia and concluded that a WBC at a cutoff of $15\times10^3/mm^3$ would have missed 79.5% of bacteraemic infants (*Ayoola et al. 2002*). Studying children with fever and a rash, Carrol et al found that WBC was higher in children without meningococcal disease (MCD) than those with meningococcal disease (MCD) (*Carrol et al. 2002*). More recently, Galletto-Lacour also demonstrated a poor sensitivity of 52% and only fair specificity of 74% for a WBC at a cutoff of $15\times10^3/mm^3$ (*Galletto-Lacour et al. 2003*).

We tried, as did other workers, using different cut-off values and combinations of indices to work out clinically useful sensitivity and specificity trade-offs, but this was not rewarding. Bonsu et al reported a sensitivity and specificity of 45% and 78%, respectively, for WBC at a cutoff of $15\times10^3/mm^3$ in detecting bacteraemia in febrile young infants (<90 days). Even at a much lower cutoff of $5\times10^3/mm^3$, WBC would have missed 21% of patients with bacteraemia (*Bonsu et al. 2003*). At a cut-off of $17.1\times10^3/mm^3$, WBC had a 69% sensitivity and 80% specificity for occult bacterial infection (OBI) in the study of Isaacman. The addition of CRP to the blood cell count added little diagnostic utility (*Isaacman et al. 2002*). Kuppermann et al found an ANC value of $\geq10\times10^3/mm^3$ to be a better discriminator of pneumococcal bacteraemia than a WBC $\geq15\times10^3/mm^3$ (*Kuppermann et al. 1998*). Lee and Harper, assessing the risk of pneumococcal bacteraemia in the post-Hib immunization era, concluded that WBC at a cutoff of $\geq18\times10^3/mm^3$ could lead to a safe reduction in antibiotic use (*Lee & Harper, 1998*). In a cost-effectiveness analysis, Lee et al estimated that, at a 1.5% rate of bacteraemia, taking blood culture and administering antibiotics selectively at a WBC cut-off of $15\times10^3/mm^3$ prevents 48 cases of meningitis and saves 86 life-years per 100000 patients. Lowering the WBC cutoff to $10\times10^3/mm^3$ would incur significant extra costs per life-year saved. However, if the rate of bacteraemia declined to 0.5%, then the strategy of CBC testing loses its cost-effectiveness (*Lee et al. 2001*). Bonsu and Harper found that the odds of bacteraemia were not decreased or substantially increased at WBC values outside published norms. The authors concluded that WBC count is an

inaccurate screen for bacteraemia in young children, and decisions to obtain blood culture should not rely on this test (*Bonsu et al. 2003*).

CRP and ESR are widely used markers of infection and inflammation; ESR is more commonly used in the USA, while CRP is favoured in Europe. In our study, only a few patients were tested by CRP (38%) and/or ESR (16.2%) and therefore selection bias was more likely with these tests. Two commonly quoted cut-off values of CRP; 5 mg/dl and 15 mg/dl were compared. At a cut-off value of 5 mg/dl, CRP was only 43% sensitive for SBI, with a negative predictive value of 73%. Increasing the threshold to 15 mg/dl dramatically reduced the sensitivity to 12%, with marginal gains in the positive predictive value (from 45 to 56%), and increased the likelihood of SBI by 29%. The poor performance of CRP in early detection of bacterial infection has been attributed to the lag in the production of CRP following injury. The increase in CRP in response to bacterial infection occurs about 12-36 hours after the onset of clinical signs. Therefore, a negative CRP at the initial evaluation does not rule out serious infection and cannot be relied upon in decision making (*Mathers et al. 1987, Pourcyrous et al. 1993*). The low sensitivity of CRP found in our study was therefore not surprising, because only CRP values obtained at the initial presentation were considered. Had serial or second day measurements been taken, we could have seen more favourable performance of this time-honoured test. ESR across all the conventional ranges of values was more sensitive but less specific than CRP and did not contribute much in reducing the uncertainty about the presence of SBI. Studies vary widely in their findings regarding the usefulness of CRP as a marker of bacterial infection. Fernandez-Lopez reported a sensitivity and specificity of 63.4% and 84.2%, respectively, of CRP at an optimum cut-off value of 27.5 mg/l in identifying invasive bacterial disease in febrile children 1-36 months old (*Fernandez-Lopez et al. 2003*). Pulliam et al found that CRP, at a cutoff of 7 mg/dl, was more powerful than WBC in predicting SBI, with a sensitivity and specificity of 79% and 91%, respectively (*Pulliam et al. 2001*). Their study population was a convenience sample of 1-36 month old febrile children, 18% of whom had SBI. Galletto-Lacour et al studied febrile children aged 7 days to 36 months with no localizing sign of infection and reported a 29% incidence of SBI. Multilevel likelihood ratio revealed that CRP

performed better than IL-6 and WBC indices in predicting SBI. CRP values of <40 mg/L and >100 mg/L generated posttest probabilities for SBI of 9.7% and 86.5%, respectively. The WBC indices only modestly changed SBI probabilities from pretest probabilities (*Galletto-Lacour et al. 2003*). Gendrel et al reported a sensitivity of 98% for CRP at a cutoff of 10 mg/l to discriminate bacterial from viral infection. Because of the very low cutoff, well below the mean for viral infections (19.5 mg/l), the reported specificity was only 50%. IL-6 was more specific than CRP for bacterial infection (*Gendrel et al. 1999*). Isaacman et al studied the utility of CRP for detection of occult bacterial infection in febrile young (3-36 months) children in whom no focal abnormality was evident on physical examination. 11.3% of 256 children had occult BI (6.6% pneumonia, 3.5% UTI, and 1.2% with occult bacteraemia). The overall prevalence of bacteraemia was 2.1%. CRP, at an optimal cutoff of 4.4 mg/dl had a sensitivity of 63% and a specificity of 81% for detection of occult bacteraemia. No significant difference was detected in the distribution of CRP levels between patients presenting within 12 hours of illness onset compared with later presentation (Isaacman *et al. 2002*). Fowlie and Schmidt systematically reviewed diagnostic tests (mainly haematological indices and CRP) for bacterial infection. The authors found enormous variation in the reported accuracy of the tests and concluded that the current diagnostic tests, either singly or in combination, were of limited value (*Fowlie and Schmidt, 1998*).

XIII. 2. d. **The cytokines**

Among the many cytokines tested, IL-6 and IL-8 were the most intensively studied as markers of bacterial infection. IL-6 is widely acknowledged as an initiator of the acute phase response, components of which (CRP and ESR) have long been used as markers of bacterial infection. IL-8 is the most important chemoattractant to neutrophils which were for long considered the wholemark of bacterial infection. These cytokines rise at the onset of infection while CRP reaches the maximum concentration with a noticeable delay. Patients suffering from SBI present to hospital after early inflammatory events. The more proximal pro-inflammatory cytokines (TNF and IL-1) were found elevated in septic patients mainly upon admission to the ICU, while IL-6 and

IL-8 concentrations were elevated throughout the inpatient period of recovery. It is therefore not surprising that the latter were the most consistently detected cytokines in septic patients. Furthermore, IL-6 assay is among the most sensitive cytokine assays, with massive incremental changes observed in patients with proven bacterial infection. It was therefore anticipated that these cytokines will more sensitively detect SBI at its early stages, when it non-specifically presents as acute fever of unknown origin. ICAM-1 plays a key role in trafficking neutrophils invited by invading bacteria and is therefore expected to be elevated in bacterial infections. Soluble ICAM-1 has been shown to be a good predictor of sepsis in neonates but its role in febrile children has not been proven. ICAM-1 belongs to a different group of molecules involved in inflammation, is known to have kinetics different from the pro-inflammatory cytokines and is a more distant player in the cytokine cascade. Thus, a wider net is cast to capture markers of infection by measuring it simultaneously with the pro-inflammatory cytokines. Conflicting findings have been reported regarding the utility of these cytokines as markers of sepsis, largely in severely ill patients admitted to intensive care units or in selected groups of children with no identifiable source of an infection. Based on these considerations, IL-6, IL-8 ans sICAM-1 were analysed in this study as predictors of SBI in an unselsected group of young febrile children.

To the limits of assay detection, IL-6 was absent in the serum of almost all afebrile control patients. From the four controls with detectable IL-6, three probably had some on-going inflammation, either from milk intolerance, tissue damage from galactosaemia, or low grade kidney inflammation in the young girl with duplicated kidneys. The presence of IL-6 in the sibling of a galactosaemic patient could not be explained; this was possibly due to a subclinical infection. IL-6 was undetectable in the serum of 15 (4.8%) of the febrile patients probably due to late presentation. Compared to CRP, IL-6 is a rapidly responsive protein with a short half-life and may not remain detectable long after the onset of infection (*Panero et al. 1997, Harris et al. 1994*). The duration of fever could not be reliably ascertained in these patients. Serum IL-6 was not useful in differentiating all SBI from more benign febrile infection and did not significantly increase the projected probability of SBI, in general, among the studied

population. There was no difference in serum IL-8 and IL-6 values between patients with and without SBI. Compared to IL-6 and IL-8, serum sICAM-1 was significantly raised in patients with SBI and independently predicted SBI in the multiple regression model. On the other hand, both IL-6 and IL-8 were elevated in all patients with bacteraemia and/or meningitis. It is possible that levels of IL-6 and IL-8 (in contrast to ICAM-1, which is a more distal player in the inflammatory cytokine cascade) had waned by the time blood was collected and persisted only in patients with pathogens circulating in blood or localizing in the meninges after a period of bactraemia. It has been shown that the majority of infected patients will have undetectable IL-6 concentrations 24 hours later, and its sensitivity is therefore reduced 24 hours after the onset of infection (*Ng et al. 1997, Buck et al. 1994*). Persistent elevation of ICAM-1 over a week has been demonstrated in patients with sepsis (*Sessler et al. 1995*). This could be explained, in part, by the slower kinetics and delayed shedding of the soluble adhesion molecule.

IL-6 was elevated in seven out of eight patients with bacteraemia who had the test performed, and was highly elevated in all patients with meningitis. IL-8 was also highly elevated in the only patient with meningitis who had the test done. IL-8, IL-6, or both were highly elevated in all patients with bacteraemia and/or meningitis. Although the numbers involved were too small to draw solid conclusions, it would seem that IL-6 and IL-8 are highly sensitive indicators for the more invasive forms (bacteraemia and meningitis) of serious bacterial infections. Interestingly, these cytokines would have detected the three bacteraemic patients discharged from the emergency room with minor diagnoses, such as PUO with shivering, otitis media, and chickenpox. These patients looked ill enough to have blood obtained for culture and other tests. Staph. aureus bacteraemia in the child with infected eczema would also have been detected, with implications for the immediate management of his condition. Obviously, the inclusion of less well-defined illnesses, such as pneumonia, led to the overall poor performance of these cytokines in singling out serious bacterial infections. Combining IL-8 with IL-6 could enhance the specifity of the tests for bacteraemia and meningitis. Since other bacterial infections, such as UTI or pneumonia can be easily diagnosed by non-invasive and easily available methods, future studies may usefully focus on the utility of these

cytokines in the detection of bacteraemia and meningitis. By excluding equivocal illnesses, the performance characteristics of the cytokine may be expected to improve substantially.

Previous studies varied in the reported usefulness of these cytokines in various situations. Among the cytokines, IL-6 is the most intensively studied new diagnostic marker of infection, probably because of its acknowledged importance as an alarm cytokine (*Malik et al. 2003*). Strait et al compared IL-6 with traditional clinical and laboratory indicators of occult bacteraemia (2.5% incidence) in febrile non-toxic appearing young children (0-36 months). At a cutoff of 65 pg/ml (determined from ROC) the respective values of sensitivity, specificity, positive and negative predictive values for IL-6 were, 88%, 70%, 7% and 99.6%. IL-6 was equivalent to ANC and superior to WBC in predicting bacteraemia. However, there was a wide range and overlap in IL-6 values between cases and controls evidenced by the wide confidence interval (4.8-71.6) of the odds ratio (16.7) (*Strait et al. 1999*). In the study by Saladino et al, none of 50 patients without signs of sepsis or occult bacteraemia had detectable IL-6, whereas 10 of 11 patients with bacteriologically documented infections had detectable IL-6, giving a sensitivity and specificity of 91% and 98% for invasive bacterial disease (*Saladino et al. 1992*). Galetto-Lacour et al found IL-6 to be inferior to CRP in predicting SBI in young febrile children without focal signs. The sensitivity and specificity of IL-6 at a cut-off of 50 ng/L were 79% and 66%, compared to respective values for CRP (at a cut-off of 40 mg/l) of 89% and 75% (*Galetto-Lacour et al. 2001*). In a more recent study, the same authors reported a 36% sensitivity and 80% specificity for IL-6 >100 pg/ml in predicting SBI in a larger cohort of similar patients. The reported sensitivity and specificity of CRP>40 mg/L were both 79% (*Galetto-Lacour et al. 2003*). Gendrel et al reported 51% sensitivity and 85% specifcity for IL-6 at a cutoff of100 pg/ml in differentiating bacterial from viral infections. The respective values for CRP at a cutoff of 20 mg/L were 83% and 71%. Although serum IL-6 levels were generally lower in viral infections, some of the highest levels were in children with documented viral infections (*Gendrel et al. 1999*). Other workers have found much higher levels of serum IL-6 in certain viral infections (such as adenovirus) compared with others, such as

RSV and influenza (*Kawasaki et al. 2002*). Franz et al demonstrated that IL-8 reduced unnecessary antibiotic use in newborn infants suspected of early sepsis by 40% (*Franz et al. 2001*). In the study by Galetto-Lacour et al, serum IL-8 values were comparable between patients with and without SBI. IL-8 values were below detectable levels (40 pg/ml) in 52% of patients with benign infection and in 25% of those with SBI (*Galetto-Lacour et al. 2001*). Soluble ICAM-1 has been claimed to be a good marker for sepsis in the neonate (*Kuster et al. 1993*). However, Berner et al measured sICAM-1 in cord blood and in plasma from babies with early onset infection and did not find any difference from those found in healthy term babies. The poor sensitivity of sICAM-1 was explained by slower kinetics and delayed shedding of the soluble adhesion molecule. Furthermore, plasma levels of sICAM-1 appear to be dependent on the maturation of the immune system, because they are significantly lower in premature infants (*Berner et al. 1998*).

Franz et al compared conventional and new markers of bacterial infections in newborn infants. Different combinations of tests yielded only modest sensitivities (91%) and specificities (73%) in predicting bacterial infection (*Franz et al. 1999*). Mehr et al reviewed studies of cytokines as markers of bacterial sepsis in newborns and found that cytokines needed to combine with CRP to offset the effects of short half-life of cytokines and increase their accuracy. The authors concluded that data were too limited to establish a clinical role for cytokines in neonates (*Mehr et al. 2000*). Hsiao and Baker reviewed recent studies of markers of SBI in febrile children and concluded that available data did not support the use of interleukin titres over other available indicators of SBI (*Hsiao et al. 2005*). There are many factors that could affect the performance of the cytokines and the discrepancies among studies reporting their use in predicting invasive bacterial infection. The time course of changes among the cytokines is very different and variable. The timing of presentation and blood collection in relation to the onset of fever will therefore affect the serum levels of the cytokine. Antibiotics, which are commonly prescribed and administered at the beginning of suspected infections, have been shown to increase endotoxin and subsequently increase concentrations of cytokines (*Prins et al. 1995*). The method of blood sampling itself can affect the serum

cytokine concentrations (*Mehr et al. 2000*). Studies evaluating the use of cytokines as markers of infection used a wide range of cut-off values which were unique to each study. Furthermore, there was a wide variation in the limits of detection between assay kits used by different studies. For instance, some studies used sensitive kits detecting down to 0.35 pg/ml IL-6, while the limit of detection for other kits was over 50 pg/ml. The range of reported test sensitivity (57-100%) and specificity (43-100%) was therefore wide, with LRs ranging between 1.5 to ∞ (*Table 35*). The volume of blood required to perform a cytokine assay (0.1-0.4 ml) does not pose a significant problem, but manual immunoassay kits, with 2-4 hour turnover times, have so far been used thus hindering the wide clinical application of the assays. Automated assays with fast turnaround times are being introduced and this will greatly enhance the applicability of cytokine assays in clinical practice. The rapidly evolving understanding of the molecular pathophysiology of sepsis and the role of cytokines in this process coupled with technical advances in biochemical testing, present the promise in future of more rapid and accurate diagnosis.

XIII. 2. e. **The Predictive models**

Currently, no single test fulfills the criteria of an ideal diagnostic investigation. Therefore, constellations of tests are commonly used to identify bacterial infection. A battery of laboratory tests can be ordered at the initial evaluation, either together or in a step-wise manner. Combinations of test results are then used to make a judgment for further management. Another approach is to construct and analyse models, derived from clinical and laboratory data that can add to the diagnostic accuracy. Our model correctly classified 70% of the patients to have either SBI or non-SBI illness. This leaves a third of febrile children requiring other methods to decide upon their management. Bachur et al constructed a model consisting of 4 clinical parameters; positive urinalysis, WBC$\geq 20\times10^3/mm^3$ or $\leq 4.1\times10^3/mm^3$, temperature ≥39.6°C, and age <13 days (*Bachur et al, 2001*). The model had 82% sensitivity and 98.3% negative predictive value in predicting SBI in young infants. Data were retrospectively collected (with all the inherent bias) and sequentially entered into a decision-tree analysis. Furthermore, SBI was strictly defined by positive culture of urine, blood, or CSF. Still, 18% of patients

with SBI, including meningitis, were misclassified into the low risk group. Using logistic regression on retrospectively collected data, Isaacman et al constructed a model based on temperature, female gender, and ANC predictive of bacteraemia in febrile children. The model marginally increased the sensitivity (by 4%) and specificity (by 15%) over those developed by the practice guidelines developed by Baraff et al. However, the authors claim that the improvement in sensitivity and specificity represent huge medical and economic gains when applied to the thousands of febrile children evaluated yearly (*Isaacman et al. 2000*). In a more recent study, Bleeker et al found that a prediction rule based on clinical data was insufficient in identifying SBI, and that adding laboratory findings (WBC, CRP and urinalysis) only modestly improved the risk estimate (*Bleeker et al. 2007*).

XIII. 2. f. **Correlation between the parameters of SBI:**

There was an insignificant negative correlation between serum IL-8 and all the conventional indicators of bacterial infection, including temperature. This observation has not been previously reported and has no plausible explanation. IL-6 had a moderate positive correlation with CRP, the total WBC, and the absolute neutrophil count, and poorly correlated with ESR. These parameters are part of the acute phase response, in the induction of which IL-6 plays a major part. On the other hand; serum ICAM-1 had a stronger correlation with ESR while poorly correlating with the other tests. This is possibly due to the delayed rise of plasma fibrinogen which peaks in 24-48 hours and its slow decline with a half line of 4-6 days, kinetics simialr to those of sICAM-1 explained above.

XIII. 3. Strengths and limitations of the study

Not every eligible patient was enrolled. Therefore the study cannot be considered a consecutive series for the purpose of studying the true prevalence and markers of bacterial infection. Sampling bias may have occurred because well-appearing children may have been discharged without performing any tests on them, while more ill-appearing patients (who are presumably more likely to have SBI) were more likely to be

investigated. It is not practical, or ethical, to subject all febrile children to the various tests necessary to identify every possible cause of infection. Although illness outcome was not part of the study, attendance at the only paediatric A&E in the region was continuously monitored throughout the study period. Only one child, with three episodes of pneuomococcal septicaemia and meningitis, was readmitted for a febrile illness (only the first episode was included in the analysis). It was therefore unlikely that any serious infection was not accounted for. Moreover, all patients fulfilling the study criteria were traced and data available at the time of discharge collected, so the prevalence of febrile illness is a true picture of the prevalence in the population. Factors that could potentially affect infection rates in young children (perinatal history, underlying conditions, and infectious exposures) were not considered in this study. These factors are usually extracted from history and allowance made for the inherent higher pre-test probability of SBI in evaluating these patients. There was a small number of patients with each type of SBI, especially bateraemia and meningitis, lowering the power of the test and limiting the ability to determine the utility of the cytokines in each type of infection. A positive culture of normally sterile body fluids is the standard for diagnosing bacterial infection. Obviously this is impractical to apply for every case, especially those with clinical pneumonia. Culture can also be falsely negative either due to prior antibiotic use or because of the low yield caused by insufficient sample volumes, especially blood. The resultant misclassification of infected patients would markedly reduce the tests' predictive values. It was attempted to obviate this problem by a definition of SBI that encompassed both objective evidence of an infecting organism grown from pathological specimens and radiographic and clinical evidence of bacterial infection. The current study also used more strict criteria for the diagnosis of pneumonia, interpreted as consolidation in clear terms, and UTI, which required pure growth of a pathogenic organism. Otitis media and tonsillitis were not considered to be SBI.

In contrast to earlier studies, this study included all febrile young children regardless of the presence or absence of a focus of infection. It has been shown that patients with focal infection have similar incidence of SBI as those without a focus (*Isaacman et al. 2000*). The study also determined the prevalence of all SBI, not limiting to occult bacteraemia.

Furthermore, a wider age range, with different prevalence and special diagnostic considerations and problems, was studied. The study population is therefore more representative of real life scenarios and typical daily problems in a paediatric emergency room. This study is the first of its kind that has determined the prevalence of SBI in a defined population of young febrile children served by the only paediatric hospital in a geographical region.

Table 35: Accuracy of Cytokines in detecting SBI in neonates and young infants*

Source	Cutoff point	Sensitivity % (95% CI)	Specificity % (95% CI)	Likelihood Ratio (95% CI)	
				Positive	Negative
IL-6 level					
Kallman et al, 1996	135 pg/ml	93 (70-99)	86 (69-94)	6.5 (2.9-16.5)	0.08 (0.01-0.35)
Messer et al, 1996	100 pg/ml	82 (52-95)	46 (39-52)	1.5 (0.95-1.86)	0.4 (0.11-1.06)
Bhatiya et al, 2000	133 pg/ml	81 (57-93)	96 (87-99)	21.5 (6.2-79.2)	0.19 (0.07-0.4)
Panero et al, 1997	15 pg/ml	100	100	∞	∞
Kuster et al, 1998	25 pg/ml	86	57	2	0.24
Silveira and Procianoy, 1999	32 pg/ml	90	43	1.6	0.23
Kashlan et al, 2000	100 pg/ml	80	90	8	0.22
IL-8 level					
Nupponen et al, 2001	> 50 pg/ml	100 (57-100)	100 (96-100)	∞	∞
Franz et al, 1999	>70 ng/ml	69/80	91/87	7.7/6.2	0.34/0.23
	>53 ng/ml	84/91	85/53	5.6/1.9	0.19/0.17
Franz et al, 1999	>70 ng/ml	83	76	3.5	0.22
sICAM					
Kuster et al, 1998	425 ng/ml	82	79	3.9	0.23

*Adapted from, *Malik et al. 2003.*

Chapter XIV

Conclusion

The evaluation of febrile young children is an ever-lasting diagnostic challenge for the clinician in a paediatric emergency room, with a great deal of uncertainty and controversy surrounding the subsequent management of these same children. This study has detailed the epidemiology of serious bacterial infection in a defined population. A high rate of urinary tract infection in infants and young children has been demonstrated. Some of these patients, and some with bacteraemia, were considered to have minor illness at the time of discharge. Urine testing should be part of the assessment of febrile children under two years irrespective of their clinical diagnosis. Children with clinical diagnosis of upper respiratory tract infection, including otitis media, should be carefully evaluated and followed up for evidence of more serious illness. Pathogenic organisms not covered by currently available vaccines cause a significant proportion of SBI, including bacteraemia. These should always be considered when evaluating a young febrile child. A high hospitalization rate for young febrile children was demonstrated, with the potential for many unnecessary admissions. There is a requirement for more accurate markers of serious infection to direct the cost-effective management of these numerous patients. IL-6 and IL-8 appear to be sensitive markers for meningitis and the potentially serious bacteraemia. High serum IL-8 in particular is closely associated with bacteraemia and meningitis and its absence may be considered a negative predictor. The specificity for these two illnesses may be enhanced by the simultaneous measurement of IL-6 & IL-8. Future larger studies may usefully focus on the utility of these cytokines in the detection of these more serious illnesses.

Appendices

Appendix 1: The Acute Infant Illness Scale (Yale Observation Scale).

Observation item	**1** (no/mild impairment)	**3** (moderate impairment)	**5** (severe impairment)
Quality of cry	Strong with normal tone **OR** Content and not crying	Whimpering **OR** Whimpering **OR** Sobbing	Severe Impairment **OR** Weak **OR** Moaning
Reaction to parent stimulation	Cries briefly then stops **OR** Content and not crying	Cries on and off	Continual cry **OR** Hardly responds
Sate variation	If awake→stays awake **OR** If asleep and stimulated→awakes up quickly	Eyes closes briefly→ awake **OR** Awakes with prolonged stimulation	Falls to sleep **OR** Will not rouse
Colour	Pink	Pale extremities **OR** Acrocyanosis	Pale **OR** Cyanotic **OR** Mottled **OR** Ashen
Hydration	Skin normal, eyes normal **And** Mucous membranes moist	Skin, eyes-normal **AND** Mouth slightly dry	Skin doughy or tented **AND** Dry mucous membranes AND/OR Sunken eyes
Response (talk, smile) to social overtures	Smiles **OR** Alerts (≤2 mo)	Brief smile **OR** Alerts briefly	No smile Face anxious, dull, expressionless, **OR** no alerting

Appendix 2: Waskerwitz bacteraemia scoring system- classification of illness.

Score	Severity of illness
1	Child critically ill with localized illness
2	Child critically ill without localized illness
3	Child is sick - if sepsis work up is negative the patient can be discharged
4	Child is ill - may or may not have a localised infection and bacteraemia is suspected
5	Child is febrile - may or may not have a localised infection, but looks well and does not have bacteraemia

Appendix 3: Toxicity scoring in febrile children used by Schwartz et al.

Toxicity score	Clinical presentation
4+	Child looks seriously ill: severe lethargy, inactivity, inattentiveness, anorexia, apathy, weak cry.
3+	Child looks moderately ill during entire period of observation: moderate lethargy or irritability, poor eye-to-eye contact, diminished activity and playfulness, decreased appetite, attenuated cry, diminished resistance to performance of painful parts of physical examination.
2+	Child looks somewhat ill: some discernible and persistent lethargy and irritability, diminished activity and playfulness, decreased appetite.
1+	Child looks mildly ill at times but has intermittent periods of alertness, playfulness, interest in other people, and enough strength to resist otoscopic and intraoral examination.
0	Child generally alert, active, playful, interested in other people, good appetite at least for liquids, actively resists otoscopic and intraoral examination.

Appendix 4: The study protocol

Prediction of Serious Bacterial Infection Study: Protocol.

Subjects:

Children aged 5 years or younger who present to the accident and emergency department with a temperature of 38.5°C or more will be included in the study. The patients will be managed in the usual way according to the discretion of the physician attending the patient. Informed written consent will be collected from the parents.

The following data will be collected on specially designed forms:

- Date of birth and sex
- Date and time of attendance
- Source of referral
- Presenting complaint and other symptoms
- Clinical assessment score (*on a special form*)
- History of chronic illness (including perinatal complications)
- Current medication (e.g. steroids or non-steroidal antiinflammatory drugs)
- Recent antibiotic treatment (within the last 48 hours)
- Recent immunization (within the last 48 hours)
- Temperature (axillary) and vital signs
- Physical examination findings
- Provisional (working) diagnosis
- Investigations requested and their results
- Type of infection (bacterial/non-bacterial)
- Antibiotic treatment administered
- Disposition and follow up arranged

The following investigations will be performed, at the discretion of the physician or as per existing A&E policy, on all eligible consenting patients:

- Full blood count with differential WBC
- C-reactive protein

- Blood culture
- An extra ml of blood will be collected to be processed for later determination of serum cytokines levels.

Control group:

This will consist of the children attending the out-patient department from whom blood is being drawn for any reasons. Any child (but see attached exclusion criteria) can be included and informed written consent should be obtained from the parents. 1-2 ml of blood will be collected and processed as above.

The following investigations will be done:

- Interlekin-6 (IL-6)
- Interlekin-8 (IL-8)
- Soluble Intercellular Adhesion Molecule-1 (sICAM-1).

The following data will be gathered from this group:

- Date of birth
- Date of attendance
- Sex
- Reason for blood drawing (investigation performed)
- Medical condition (diagnosis)

Appendix 5: The Clinical Assessment Form

Prediction of Serious Bacterial Infection Study

Clinical assessment form.

"Please fill in this form before you do systemic examination of the child"

Patient's name:..... RC Date / Time .

	Score (degree of impairment)		
	Normal	**Moderate**	**Severe**
History			
Appetite	Normal	Not his/her usual	Hardly eating or drinking
Activity	Normal	Decreased	Inactive/refuses to play
Easiness to console	Normal	Responds intermittently	Inconsolable
Observation			
Quality of cry/vocalisation	Strong/content & not crying	Weak	Moaning/high pitched
Irritability	Not irritable	Irritable on handling	Irritable
Social response/eye contact/smile	Smiles/alerts	Brief smile or response	No smile/dull, anxious face
Physical examination			
Colour/peripheral perfusion	Pink	Pale extremities	Pale/mottled/cyanosed
Breathing pattern	Normal	Some distress	Laboured/grunting
Hydration	Moist mucous membranes	Dry mouth	Dry/doughy skin/sunken eyes
Overall assessment			
Severity of illness	Mild	Moderate	Severe
Possibility of **SBI**	Unlikely	Equivocal	Strong

* Feel free to use your discretion in assigning scores according to the age and developmental stage of the child.
* Please also note that some historical and observational parameters are inter-changeable.
* Serious Bacterial Infection (SBI) is bacteraemia or septicaemia, UTI, pneumonia, osteomyelitis or arthritis, meningitis or gastro-enteritis of bacterial aetiology.

Designation of the examining physician: SHO (1) Reg (2) Staff Grade (3)
Senior Reg (4) Consultant (5)

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Declaration

I hereby declare that this thesis has been wholly composed by me (Dr. OG Osman) and I own all work it contains. The thesis is written solely for fulfulment of MD from the University of Edinburgh and has not been submitted for any other degree or professional qualification.