The Role and Optimal Timing of Flexible Bronchoscopy and Broncho-alveolar Lavage Chemokine Measurement in Severely Immunocompromised Febrile Neutropenic Patients

Chien-Li Liew: 1042406

Thesis submission
for Master of Clinical Science,
Discipline of Medicine, Adelaide University

Supervisors: Professor L B To, A/Professor P N Reynolds

Submission date: 8th August 2008

The Role and Optimal Timing of Flexible Bronchoscopy and Broncho-alveolar Lavage Chemokine Measurement in Severely Immunocompromised Febrile Neutropenic Patients

Chien-Li Liew

TABLE of CONTENTS

        - 2.3h The diagnosis of invasive pulmonary aspergillosis
            - i Role of flexible bronchoscopy
            - ii Role of CT chest and other methods of detecting invasive fungal disease

    - 2.4 Chemokines in pulmonary disease

3. Aims

4. Methodology

- 4.1 Clinical trial design
- 4.2 Bronchoscopy technique
- 4.3 Analysis of specimens – microbiological
- 4.4 Analysis of specimens – chemokine measurement

5. Results

- 5.1 Patient characteristics
- 5.2 Bronchoscopic parameters
- 5.3 Safety of bronchoscopy
- 5.4 Yield
- 5.5 Antibiotic Use
- 5.6 Chemokine analysis
- 5.7 Other clinical outcome measures
- 5.8 Survival

6. Discussion

7. Development of a protocol for the management of severely immunocompromised febrile neutropenic patients

8. References

Statement of Originality

I, Chien-Li Liew, declare the research work described in this thesis to be original. This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution, and, to the best of my knowledge and beliefe, contains no material previously published or written by another person, except where due reference has been made within the text.

I give consent for a copy of my thesis to be made available to the university library for loan and photocopying, subject to the provisions of the Copyright Act 1968. I acknowledge that the copyright of published works contained within this thesis, as listed as references, resides with the copyright holders of those works

Signed_____

Name _____Date_____

Witnessed_____ Name_____Date_____

Acknowledgements

Professor L B To[1]: for supervision of research development and thesis
Professor P N Reynolds[2, 3]: for supervision of research development and thesis
Dr G Hodge[3]: for supervision of cytokine analysis
Professor M D Holmes[2, 3]: for assistance with methodology and thesis
Professor H P A Jersmann[2]: for assistance with methodology
Dr P C Robinson[2] for assistance with methodology
Dr M M Chia[2]: for assistance with methodology
Dr N Horvath[1]: for assistance with methodology and thesis
Professor A R Glanville[4]: for assistance with data analysis

1. Royal Adelaide Hospital and Institute of Medical and Veterinary Science, Department of Haematology
2. Royal Adelaide Hospital Department of Thoracic Medicine
3. Royal Adelaide Hospital and Institue of Medical and Veterinary Science, Lung Research Laboratory
4. St Vincent"s Hospital Department of Thoracic Medicine

1. ABSTRACT:

Respiratory infection remains a leading cause of morbidity and death in severely immunocompromised febrile neutropenic haematology patients, despite the introduction of numerous prophylactic strategies and advances in diagnosis and treatment. Prognosis is improved if an organism can be isolated and specific therapy commenced as soon as possible. Current practice in this population group is to commence empirical antibiotics and perform flexible bronchoscopy (FB) if temperature does not settle or *after* patients develop clinical or radiological features suggesting a respiratory source. This delay may result in a lower procedural diagnostic yield due to prior or prolonged anti-microbial treatment, and increased risk of respiratory compromise and procedural complications due to advanced respiratory infections. We hypothesised that proceeding to FB as early as possible after developing febrile neutropenia would improve treatment outcomes. With this randomised, prospective trial, we aim to further define the role of FB with reference to optimal timing of the procedure and its impact on diagnostic yield, future management and complication rate. We also aim to analyse the impact of proven infection on the cytokine profile of immunocompromised patients.

Methods: Patients with acute leukaemia, allogeneic bone marrow transplantation or chronic lymphocytic leukaemia (CLL) being treated with Fludarabine/ Mabthera without an obvious non-respiratory source of infection were prospectively randomised into early bronchoscopy or conventional management groups at onset of febrile neutropenia. Bronchoalveolar lavage (BAL) fluid chemokine levels (IP-10, RANTES, MIG, IL-8, MCP-1) were measured using a human Chemokine cytometric bead array (CBA) kit.

Results: Thirty-one episodes of febrile neutropenia in 29 patients were analysed; 17 conventional and 14 early. There was an increased yield in fungal growth in the early bronchoscopy group, which was not predicted by prior clinical or radiological changes. However, this had no impact on clinical management in the short-term due to the delayed growth. Overall diagnostic yield was not significantly different between the two groups. Procedural complication rate was negligible overall and there was no difference associated with either group. IP-10 and MIG were significantly lower in those patients who had a fungal pathogen isolated, compared with those study patients who did not (175.17 vs 1157.8, p=0.03, 30.33 vs 247.8, p=0.03 respectively). IP-10 levels were higher in the conventional than early group (1253.0 vs 261.14, $p = 0.035$) and the study population had higher MCP-1 (734 vs 2.83, p=0.006) and IL-8 levels (606.9 vs 14.25, p=0.00655) than normal controls. Those cases with fungal infection had higher mean MCP-1, RANTES and IL-8 levels than in normal controls (844.0 vs 2.83, p=0.007; 17.5 vs 2.1, p=0.03; 156.0 vs 14.25, p=0.004).

Conclusions: Early bronchoscopy as a component of the septic screen in febrile neutropenic patients was feasible and safe. A significant difference in fungal yield was seen in the early bronchoscopy group compared to conventional methods, with a negligible complication rate, but this did not result in a change in immediate clinical management or outcomes.

2. INTRODUCTION

- 2.1 Febrile neutropenia
  - 2.1a Use of empirical antibiotics in febrile neutropenia
  - 2.1b Pulmonary infection in severely immunocompromised patients
- 2.2 Pulmonary complications in acute leukaemia and allogeneic bone marrow (stem cell) transplantation
  - 2.2a Update on current issues
  - 2.2b Current consensus on diagnosis, prophylaxis and treatment
- 2.3 The role of flexible bronchoscopy in diagnosis of pulmonary infections
  - 2.3a Flexible Bronchoscopy in non-immunocompromised population
  - 2.3b Flexible Bronchoscopy in severely immunocompromised patients
  - 2.3c Yield of flexible bronchoscopy
    - i. Overview of literature on bronchoscopic yield compared to other techniques
    - ii Factors aiding yield
    - iii. Yield of individual techniques: bronchial washings/ bronchoalveolar lavage, protected specimen brush, transbronchial biopsy
  - 2.3d Safety of flexible bronchoscopy
  - 2.3e Timing of bronchoscopy
  - 2.3f Impact of bronchoscopic results on clinical management and outcome measures
  - 2.3g Open lung biopsy
  - 2.3h The diagnosis of invasive pulmonary aspergillosis
    - i Role of flexible bronchoscopy
    - ii Role of CT chest and other methods of detecting invasive fungal disease
- 2.4 Chemokines in pulmonary disease

2.1 **Febrile neutropenia**

Febrile neutropenia (FN) is recognised as a commonly encountered haematological emergency and is predominantly caused by infectious pathogens[175]. In patients treated with myelosuppressive chemotherapy or following bone marrow transplantation, the frequency of febrile neutropenia varies between 2% and 82%, depending on the nature of the underlying malignancy and chemotherapeutic regimes used[178]. The majority of febrile neutropenic episodes occur without focal symptoms, signs or radiological changes at the outset, and frequently, routine septic screen cultures are negative.

Pulmonary infections are a common cause of disease in febrile neutropenic patients, with bacterial pneumonia alone occurring in 5-30% of HSCT recipients. Clinical symptoms, signs and radiological changes are often absent, non-specific or delayed in this population, leading to delay in diagnosis[43, 50]. Studies have shown that respiratory symptoms or signs and chest xray (CXR) changes do not predict the likelihood of a respiratory source of infection or yield from bronchoscopy and bronchoalveolar lavage (BAL)[182] . Optimal outcome depends on commencing specific therapy as soon as possible, and prompt and targeted antibiotic therapy is essential for a favorable outcome. Even with aggressive empirical antimicrobial therapy, the mortality rate for febrile neutropenic immunocompromised patients *once pulmonary infiltrates develop* has been reported as 40%[179], with rates of more than 95% in those patients who develop respiratory failure and require mechanical ventilation [78, 180].

It has previously been reported that 75% of microbiologically documented pneumonia in neutropenic patients with haematological malignancy were caused by Gram-negative bacilli, *Staphylococcus* or *Streptococcus* species or *Aspergillus* species, compared with only 24% in patients with haematological malignancy but without neutropenia[44, 187, 193]. The incidence of polymicrobial infection is also higher in those who are more profoundly immunosuppressed. These major differences in microbiological profile justifies the separation of immunocompromised hosts into those with and those without neutropenia.

2.1a Use of empirical antibiotics in febrile neutropenia

Before the introduction of empirical broad-spectrum antibiotic therapy 30 years ago, febrile neutropenia accounted for most chemotherapy-associated deaths[176]. Since the routine use of empirical, broad-spectrum antibiotics to control bacterial infections was first introduced 30 years ago,[27, 59] mortality has significantly reduced. However, febrile neutropenia still continues to have severe consequences, especially in acute leukaemia and bone marrow transplantation recipients, when neutropenia is prolonged and other immune responses such as usual mucosal barriers are simultaneously impaired.

Since the earliest studies in febrile neutropenia[26, 27], further studies have shown the efficacy of an anti-pseudomonal *b*-lactam antibiotic in combination with an aminoglycoside[29-31] and this combination has become the backbone of published guidelines[32, 33]. Standard practice in neutropenic patients who become febrile is to perform a basic septic screen, consisting of cultures of blood, urine and sputum if possible, and then commence broad-spectrum empirical antibiotics covering bacterial organisms as described above. All guidelines recommend that

empirical treatment with antibiotics should be started immediately in cases of fever, with or without respiratory symptoms, regardless of the result of chest radiography[42, 45-6]. Reliable diagnostic procedures such as transtracheal aspiration or BAL may be performed, but this should never delay the administration of antibiotics. Further anti-microbials including anti-fungal agents are added until patients become afebrile, neutrophil recovery occurs, a specific organism is isolated or further microbiological information is obtained.
Controversy remains as to whether making a definitive diagnosis in these patients has an impact on overall outcome[70]. In addition, current guidelines state that „if a causative microbe is identified, the antibiotic regimen *may* be changed, if necessary, to provide optimal treatment with minimal adverse effects and lowest cost, but broad-spectrum coverage should be maintained to prevent breakthrough bacteraemia" [32]. There are no published randomised-controlled studies which compare pathogen-specific therapy alone with continuation of broad-spectrum antibiotics after a pathogen has been isolated, but multiple researchers have demonstrated that initially inappropriate antibiotic selections adversely affect outcomes[103]. Therefore, it is widely believed that early diagnosis is preferable, and in the absence of this, empirical therapy with broad-spectrum antibiotics is recommended[176].

Recently reported in the Internal Medicine Journal of Australia[57], was a prospective audit of 81 high-risk patients with 116 febrile neutropenic episodes, designed to measure the yield and the influence of positive culture results on the management of such patients – specifically examining the outcomes in patients whose antibiotic therapy was changed to be pathogen specific and who did not continue to receive broad-spectrum antibiotic therapy following isolation of a pathogen from blood cultures. Twenty-seven percent of blood cultures were positive, but in 8 of 40 episodes of bacteraemia, organisms were only isolated from blood cultures collected more than 5 days after broad-spectrum antibiotics had been started. Two were due to organisms susceptible to the empirical antibiotic therapy. Two more episodes had organisms isolated from blood cultures collected 1 and 2 days after starting antibiotics, but the same organisms had been isolated from blood cultures collected before starting antibiotics. Blood culture results were positive in 53 cases and led to a change in treatment in 25 of 116 (22%) episodes of febrile neutropenia. Nineteen of these changes were in response to a positive culture. Changes were made due to antibiotic resistance in nine episodes. One further patient had therapy already altered in response to clinical deterioration, and resistance to the previous antibiotic regime was later confirmed on blood cultures. In 12 episodes of bacteraemia, antibiotic therapy was optimized to pathogen-specific therapy and the empirical antibiotics were stopped, despite ongoing fevers. None of these patients required a further change in antibiotic therapy, and no adverse events occurred. In the remaining 28 positive blood culture results, no antibiotic changes were made in response. The decision to remove a central venous catheter was made in seven episodes of bacteraemic febrile neutropenia – 6 of these were following the positive blood culture.

In this study, urine cultures had a yield of almost zero in the absence of suggestive symptoms, and other papers have shown that serology is almost always non-diagnostic[174]. Interestingly, this paper suggested that routine chest radiographs are not required in the absence of signs or symptoms of pulmonary disease[58]. It is unclear whether this is due to low yield found, but this recommendation has not been echoed in most other guidelines.

2.1b Pulmonary infections in severely immunocompromised patients

Pulmonary complications occur in 40-60% of SCT recipients. In one of the largest studies to date, Rano and colleagues[102] noted that 3 variables independently predicted mortality in immunocompromised patients with pulmonary infiltrates:

- delay in diagnosis > 5 days (identification of cause of pulmonary infiltrates)
- increasing severity of illness
- need for mechanical ventilation

In the 1980s the diagnostic and therapeutic approach to patients with suspected pulmonary infection was limited to empirical treatment or open lung biopsy[41]. Today, a combination of multiple diagnostic techniques are utilised, including: non-invasive procedures such as computed tomographic (CT) scanning, analysis of expectorated or induced sputum, collection of extrapulmonary specimens such as blood or urine samples or nasopharyngeal washings or swabs, therapeutic testing with antibiotics in cases of probable bacterial pneumonia; and more invasive procedures such as FB with BAL, protected specimen brushing (PSB) or transbronchial biopsy (TBB), percutaneous needle aspiration or biopsy and open lung biopsy (OLB) videothoracoscopically or by open thoracotomy.

Similarly, the microbiologist may still use classical techniques, but there are also multiple new techniques emerging, for which clear roles are still being established.

In most institutions, Bronchoscopy is usually only requested if patients develop specific respiratory symptoms, signs or persistent radiological changes such as focal or diffuse infiltrates[88]. This usually occurs at least several days after the development of febrile neutropenia, when patients have received significant doses of antibiotics, and often at a time of worsening respiratory function, making the decision to bronchoscope difficult, due to the risk of worsening the patient"s respiratory state, or causing severe complications. Treatment with antibiotics prior to bronchoscopy has been shown to decrease the bronchoscopic yield in the HIV population and non-immunocompromised populations and probably in the immunocompromised haematology population [171, 181]. Patients with positive bronchoscopic results for histologically proven fungal disease have also been found to have had lower cumulative doses of the anti-fungal Amphoterecin B prior to bronchoscopy than those with negative bronchoscopies[17]. As such, it is highly likely that antimicrobial treatment prior to bronchoscopy impairs yield.

Current literature is controversial regarding the utility, optimal timing and specific tests which should be performed during bronchoscopy. There are inconsistencies in interpretation due to reporting of multiple variables, different populations included as "immunocompromised" and varying end-points or denominators. For this reason, bronchoscopic yield has been reported between 5 – 93%, but is generally accepted as low. This may be due to the use of anti-microbials prior to obtaining specimens for diagnosis, lack of sensitive tests available, or sampling errors. In those studies which obtained higher yield, this has never translated into improvements in clinical outcomes such as mortality. Only a few studies have focused on patients with febrile neutropenia alone[24,180,182]. Most studies to date involve immunocompromised patients who have already developed pulmonary infiltrates. Minimal literature exists regarding the timing of bronchoscopy in severely immunocompromised

patients, including those with acute leukaemia receiving chemotherapy, and recipients of allogeneic haematopoeitic cell transplantation. Previous studies have shown that FB is safe in neutropenic, thrombocytopenic patients without respiratory disease[50]. The gold standard in investigating for respiratory infection in this population is not straightforward. It is generally a trade-off between sensitivity of organism detection and specificity for disease syndromes related to those organisms.

In view of the above, our study therefore aims to answer clinical questions regarding the role of bronchoscopy as a diagnostic tool in severely immunocompromised febrile neutropenic patients, with specific reference to the safety, optimal timing, yield and impact on clinical management, of bronchoscopy. We hypothesized that proceeding to bronchoscopy as early as possible, as a component of the routine septic screen when immunocompromised patients develop febrile neutropenia, would lead to better outcomes due to increased yield and lower procedural risk, as patients would be less likely to have developed respiratory failure prior to bronchoscopy. As it is commonly difficult to differentiate between infective and non-infective causes of pulmonary disease in this population, we also aim to measure a series of cytokines in BAL fluid, to assess whether the profile can give further guidance as to the cause of disease, and assist in later developing less invasive methods of identifying a pulmonary source of infection, or predicting infection type. Due to the variation in incidence and prognosis of febrile neutropenia between subgroups of immunocompromised patients, we have focused our clinical trial on the more severe end of the spectrum of immunocompromised patients - those with acute leukaemia receiving chemotherapy, and recipients of allogeneic stem cell transplantation, in order to derive the most clinical benefit in this high-risk group. As it has been clearly shown that mechanical ventilation is associated with a worse prognosis, we aim to develop a protocol to diagnose and treat patients prior to this.

2.2 Pulmonary complications in acute leukaemia and allogeneic bone marrow (stem cell) transplantation

2.2a Update on current issues

Acute Leukemia: Overview and Infective Complications During Primary Disease or Chemotherapy

The approach to treatment of adults aged 18–60 years with Acute Myeloid Leukemia (AML) classically involves separate treatment phases. The first consists of induction chemotherapy in which the goal of myelosuppressive chemotherapy is to "empty" the bone marrow of all haemopoietic elements (both benign and malignant) and to allow repopulation of the marrow with normal cells, thereby yielding remission (< 5% marrow blasts). Once remission is achieved, additional consolidation therapy is given to reduce the undetectable burden of leukaemic cells to a level low enough that long-term disease-free survival (ie cure) might be possible.

The increased understanding of the pathophysiology of AML has led to the development of new targeted therapies. Whether any of these will prove to alter the natural history of AML and its complications when used either alone or in combination with each other or with standard chemotherapy remains to be determined, and further discussion is beyond the scope of this paper.

Acute Lymphoblastic Leukaemia (ALL) represents only 20% of adult acute leukemias, but an unexplained small increase in incidence has been recently observed. Treatment programs incorporate multiple drugs, with goals of rapid restoration of normal haemopoiesis, prevention of emergence of resistant subclones, adequate prophylaxis of sanctuary sites and elimination of recurrence. Therefore, similarly to AML, therapy is divided into several phases: induction, consolidation and intensification, and maintenance. CNS prophylaxis is essential in ALL and is usually delivered during induction and consolidation. The outcomes of salvage therapy either by chemotherapy or bone marrow transplantation remain poor, with complete remission rates ranging from 10-50%, but low disease-free survival[73]. The complication profile is similar to acute myeloid leukaemia.

Patients with both types of acute leukaemia are prone to bacterial and fungal infections as a result of prolonged neutropenia secondary to marrow infiltration and/or the effects of either induction or consolidation chemotherapy. Early commencement of empiric broad-spectrum antibacterial therapy is considered essential for febrile neutropenic patients, regardless of the cause of neutropenia. Currently, the standard regime differs between units, but there is generally a standardized protocol *within* units, after consultation with microbiologists and infectious disease physicians, with consideration of local organisms and antibiotic susceptibility patterns. More recently, fungal infection has emerged as a significant concern, as the risk of infection increases with severity and duration of neutropenia. Symptoms are often non-specific. Established fungal infections carry a high mortality, and empiric anti-fungal therapy is generally indicated in patients who have persistent pyrexia despite appropriate empirical antibacterial therapy. It is currently recommended that chest xrays (CXR) be routinely performed in all febrile neutropenic patients, and a low threshold for performing high resolution CT scans of the chest is maintained, as this may aid early detection of respiratory fungal infections. If a specific diagnosis of invasive pulmonary aspergillosis (IPA) is made in a patient undergoing chemotherapy, this is likely to have important implications on their antimicrobial regimen in subsequent cycles, and the conditioning regime prior to transplantation.

In case of pulmonary disease, once respiratory failure occurs, prognosis worsens significantly, and there is evidence that AML patients who require ventilatory support for acute respiratory failure rarely survive their ICU admission[201].

The use of prophylactic antibiotics in this population remains contentious and there is no evidence that their use improves survival.

Allogeneic Bone Marrow Transplantation: Overview and Infective Complications

The term "haemopoietic stem cell transplantation" has replaced the previously used "bone marrow transplantation", to incorporate the now broader range of donor stem cell sources including bone marrow, fetal cord blood, and growth factor–stimulated peripheral blood.

The ability to successfully transplant haemopoietic stem cells represents one of the landmark medical achievements of the twentieth century. First emerging in the 1960s as a novel therapeutic approach, hematopoietic stem cell transplantation (SCT) has since become an important treatment option for patients with a wide spectrum of non-malignant and malignant haematological disorders, genetic disorders, and some solid tumors requiring high-dose chemotherapy. Stem cell transplantation enables treatment of such malignancies with higher doses of chemotherapy, with replacement of malfunctioning or non-functioning bone marrow.

The most effective current anti-leukaemic approach to treatment is widely accepted to be allogeneic stem cell transplantation[62].

The past decade has witnessed a continued proliferation of transplant centers, a steady increase in the number of procedures performed, and a progressive shift in the care of recipients from the university hospitals to the community setting. In acute myeloid leukaemia, SCT outcomes have been particularly favorable, and discussions regarding transplantation are recommended to occur at diagnosis in many of these patients, unless obviously contraindicated. Traditionally, the main indication for SCT has been in the treatment of haematological malignancies and certain solid tumors, for which the technique serves as a "rescue" therapy to restore marrow function after lethal, marrow-ablating doses of radiation and chemotherapy employed to eradicate malignant cells. As use increases rapidly and SCT is indicated more for a variety of different disease entities, the incidence and spectrum of complications is likely to increase. It is therefore important that any protocols involving the use of potentially invasive diagnostic tools are based on clinical evidence if possible.

The procedure for allogeneic stem cell transplantation involves administration of high doses of chemotherapy, with or without total body irradiation, to ablate the existing bone marrow, maximize tumor cell kill and induce immunosuppression to prevent rejection of the donor stem cells. After infusion of donor stem cells, life-long immunosuppression is required.

Although advances in surgical techniques, immunosuppressive management, and prophylaxis and treatment of infectious diseases have made long-term survival an achievable goal, transplant recipients remain at high risk for developing a myriad of serious and often life-threatening complications, and significant morbidity. Paramount among these are pulmonary complications, which arise as a consequence of the immunosuppressed status of the recipient, the chemotherapy and radiation conditioning regimens as described above, and alloimmune mechanisms mediating host-versus-graft and graft-versus-host responses[62]. The single most consistent risk factor for such complications is type of graft-vs-host disease (GvHD) prophylaxis (T-cell depletion methods are associated with lower risk) and pre-transplant reduction in pulmonary function, as measured by FEV1[65].

Severe pulmonary complications are therefore far more common with allogeneic stem cell transplantation than autologous[74], due mostly to the higher immunosuppression requirements and GvHD prophylaxis in allogeneic transplantation. GVHD itself also causes an immunodeficient state by involving mucosal surfaces, the reticuloendothelial system, and bone marrow[106]. In one study, allogeneic marrow recipients had pulmonary complications requiring FB 3.37 times more often than did the autologous transplant patients[78]. The shift from bone marrow to stem cell harvest has improved the incidence and severity of infective complications, as the 4- to 5-fold higher number of $CD34^+$ cells in a peripheral blood stem cell product facilitates more rapid engraftment in comparison with a marrow product (median of 17 days versus 24 days for granulocytes and median of 28 days versus 47 days for platelets, respectively), which decreases the risk for bacterial infections in the early post-transplant period.[62] However, infections are still a major problem, and infective pulmonary complications are far more common in allogeneic SCT, with one study finding 81% of invasive pulmonary Aspergillosis in allogeneic SCT recipients, as opposed to 19% in autologous SCT[40]. Non-infectious acute lung injury syndromes (e.g., idiopathic pneumonia syndrome, diffuse alveolar hemorrhage) occur with similar frequency after both allogeneic and autologous transplantation[107]. As we are more focused on infectious pulmonary complications, this review will therefore concentrate primarily on allogeneic SCT.

It is estimated that up to 74% of SCT deaths are directly related to pulmonary disease[74, 78]. Despite the introduction of numerous prophylactic strategies and advances in diagnosis and treatment, pneumonia remains the leading infectious cause of death after SCT and pulmonary complications overall, the majority not diagnosed antemortem, are the most common cause of death. As a result of underdiagnosis, SCT recipients may not receive appropriate therapy for potentially treatable pulmonary complications. Infection is the most common of these, and in many cases is likely to be potentially reversible and treatable, if correct anti-microbials are commenced early. Pulmonary complications, both infectious and non-infectious, occur in up to 60% of HSC transplant recipients and nearly one-third of such recipients require intensive care after transplantation[108, 109]. Outcomes have been consistently shown to be worse in patients who require intubation and ventilation. In a review of more than 1,400 consecutive patients who underwent SCT at the Fred Hutchinson Cancer Research Center between 1986 and 1990, Crawford and Petersen documented the need for mechanical ventilation in 23% of patients[110]. Only 4% of ventilated patients in this series survived to discharge, a figure similar to that cited in other studies from this era[78, 109, 111-2]. More recent reports have provided a more favorable perspective, documenting survival rates of 16–26% in mechanically ventilated patients[92, 113-5]. Improvement in survival has also been documented with early institution of non-invasive mechanical ventilatory support[121], but these patients should be carefully selected.

Three significant independent risk factors for respiratory failure have been identified:

- receipt of HLA non-identical donor marrow
- active phase of malignancy
- age greater than 21 years[116-7].

The incidence of respiratory failure increases from approximately 10% with none of these risk factors, to more than 50% when all three are present.

A recent series reviewed the findings at autopsy of 71 SCT recipients, including 39 allogeneic transplants. Death occured a median of 1.3 months post-transplant in this group. Eighty-nine percent had pulmonary complications (63/71 pts), of which 28% were infectious. Only 28% of

complications were diagnosed antemortem, but diagnosis was more likely if the cause was infection (48% vs 20%). Six of the 13 patients with bronchopneumonia (46%) and 5 of the 11 patients with invasive pulmonary aspergillosis (45%) at autopsy were not receiving treatment for these conditions at the time of death. Importantly, 10 patients being treated for suspected invasive pulmonary aspergillosis, 7 patients treated for suspected pulmonary cytomegalovirus infection, 22 patients treated for suspected bacterial pneumonia, 2 patients treated for suspected Pneumocystis jiroveci pneumonia, and 12 patients treated for diffuse alveolar haemorrhage at the time of death had no evidence of these conditions at autopsy. This highlights the fact that even with current clinical acumen and diagnostic techniques, early detection and correct treatment remains difficult.

Dunagan et al[78] found that the mortality of SCT patients who did not develop pulmonary complications requiring bronchoscopy was 33%, compared with 61% in those undergoing bronchoscopy for suspected pulmonary complications. This was similar to that reported by Milburn et al[48] (67%) and Heurlin et al[82] (69%).

In SCT recipients, specific complications tend to occur within well defined time periods[108]. The timing and intensity of cyto-reductive therapies, the pattern of immune reconstitution and the institution-dependent use of infection prophylaxis influence duration and intervals[118]. There is currently no consensus on the routine use of prophylactic antibiotics in afebrile, asymptomatic neutropenic patients[119].

2.2b Current consensus on infection: diagnosis, prophylaxis and treatment

Bacterial pneumonia. Bacterial pneumonia is particularly prevalent during profound neutropenia. Approximately 20% of cases are fatal. Gram-negative pathogens, especially *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, predominate in the first 100 days whereas gram-positive organisms such as *Streptococcus pneumoniae* are responsible for later infections [120]. *Legionella* species have been reported to be an important cause of nosocomial pneumonia in some centers[122, 123].

Bacterial pneumonia is commonly heralded by fever, but respiratory symptoms and signs may be absent in the neutropenic host, and CXR abnormalities may be subtle, non-specific or absent in neutropenic patients, who may have a poor inflammatory response. High-resolution CT (HRCT) scanning is more sensitive.

Empirical antibiotics are usually commenced prior to invasive diagnostic sampling, and standard regimes include two synergistic broad-spectrum anti-pseudomonal agents, with the addition of broad-spectrum gram-positive cover.

Aspergillosis. Neutropenia has been repeatedly identified is an indicator of poor prognosis in patients with fungal infections. Mortality rates secondary to invasive infection with Candida Albicans have decreased due to the wide-spread use of prophylaxis. Invasive aspergillosis is now the most common of the fungal pneumonias, one of the most devastating complications of allogeneic SCT, and represents the leading cause of infectious deaths in this group[22], with mortality of at least 65%, and up to 92% in one series[67-8, 94, 153-5]. The emergence of resistance

in some sub-types[55] is of ongoing concern. IPA is confined to the lungs in most cases, but has been known to affect sinuses and the central nervous system. In contrast to other common pathogens, the incidence of invasive aspergillosis is increasing among severely immunocompromised patients, and currently approximates 10–15% in HSCT recipients[22, 95, 142]. Initially, neutropenia is the prevailing risk factor. A second, post-engraftment period of vulnerability coincident with the development of chronic GVHD and the attendant need to administer stronger immunosuppressive agents then occurs. The incidence of invasive aspergillosis has risen mainly in the latter group, as newer strategies for reducing the initial period of neutropenia has led to a decrease in pre-engraftment infections.

Importantly, there is a wide spectrum of illness; some patients initially have no symptoms and fever may be absent in up to two-thirds of patients [96, 143]. Cough and dyspnea are the most common presenting symptoms. Pleuritic chest pain may occur, reflecting the tendency of the organism to invade blood vessels and cause pulmonary infarction. Massive haemoptysis has been reported in up to 15% at the time of resolving neutropenia[144]. Seizures and focal neurological signs are rare but ominous. The plain chest radiograph may be normal in up to 10% cases[6], or may show single or multiple nodules, cavities, and subsegmental or segmental consolidation. In the later stages of infection, a sequestrum of necrotic lung tissue may separate from the surrounding parenchyma, resulting in the air crescent sign - a central nodule partially or circumferentially surrounded by air[145]. A highly characteristic CT finding is the "halo sign", a rim of low attenuation representing oedema or hemorrhage which surrounds a pulmonary nodule. Controversy exists surrounding the utility of this sign: Caillot and colleagues reported that the halo sign was present in more than 90% of neutropenic patients with invasive pulmonary aspergillosis when CT scans were performed at the onset of fever[146]. However, several other series have suggested that more often, CT findings are non-specific, and the halo sign itself appears late or is non-specific – other moulds and non-infectious pathology can cause a similar pattern. It is clear that even with the use of CT scan, difficulties in diagnosis exist. At present, establishing a definitive diagnosis of invasive pulmonary aspergillosis remains difficult and only approximately 30% of cases are recognized antemortem[147]. The recovery of *Aspergillus* species from respiratory tract cultures is highly suggestive of invasive infection in a severely immunocompromised patient, with a positive predictive value of 82%[148]. Unfortunately, the sensitivity of bronchoalveolar lavage and TBB (using current standard fungal assays) is only 35–57%[17, 97-9, 143, 149], due to sampling error. Trans-thoracic fine needle aspiration of accessible focal lesions has a reported yield in the range of 50–67%[143, 150, 151]. Another emerging diagnostic test for Invasive Aspergillosis is detection of Galactomannan, a water-soluble fungal cell wall polysaccharide component which is released during fungal replication, indicating active invasive disease. One prospective study demonstrated that ELISA detection of Galactomannan in serum had a sensitivity and specificity of 89.7 and 98.1%, respectively[152] The test became positive before development of respiratory clinical features or new radiographic findings in 68% of cases, preceding the definitive diagnosis of invasive aspergillosis using other means by a median of 17 days. This was thought to be highly significant and clinically useful, and Galactomannan is accepted as a diagnostic test for Invasive Aspergillosis, with serial sampling recommended. However, debate still exists as to the impact of prior anti-fungal therapy, and the cut-off points required for diagnosis.

There is currently no consensus or firm data regarding recommendations for prophylaxis of *Aspergillus* infections following chemotherapy or SCT. Low-dose intravenous Amphotericin B, aerosolized Amphotericin, and Itraconazole have all been employed, but none has consistently

been shown to decrease the incidence or clinical impact of invasive aspergillosis in this population. Measures to minimize environmental exposure to *Aspergillus* spores, including use of high-efficiency particulate air filters and laminar airflow in patient rooms and avoidance of areas of hospital construction or renovation, are currently emphasized.

Intravenous amphotericin B has long been considered to be standard treatment for invasive aspergillosis, but response to therapy is suboptimal and until recently, reported mortality has remained extremely high. This poor outcome is thought at least partially due to delay in establishing the diagnosis and commencing specific treatment. Nephrotoxicity has also been a significant complication, limiting empirical, long-term and indiscriminate use of Amphotericin B. Newer liposomal formulations are associated with less nephrotoxicity, but prolonged treatment can still be expensive and problematic. More recently, there has been a significant decrease in mortality in patients with a diagnosis of IPA, coinciding with multiple changes in transplantation practices, including use of non-myeloablative conditioning regimens, receipt of peripheral blood stem cells, more prompt diagnosis of IPA, and use of voriconazole[51].

Voriconazole has been shown to have superior efficacy and less toxicity than amphotericin B in a large randomized trial involving immunocompromised patients. Caspofungin, a parenteral echinocandin antifungal, has also now been approved for salvage therapy of invasive aspergillosis[156]. More recently, it has also been found to be as effective as liposomal Amphotericin as empiric therapy in patients with persistent fever and neutropenia. Importantly, Caspofungin has demonstrated an excellent safety profile with few serious drug-related adverse events and few therapy discontinuations resulting from drug-related toxicity. This is in dramatic contrast to Amphotericin B[53]. With respect to surgery for IPA, the results of two recent series[146, 149] suggest that wedge resection or lobectomy of localized disease may be useful if a lesion close to a pulmonary vessel has a high risk of causing massive haemoptysis, or a unique residual lesion exists in a patient for whom another course of chemotherapy would be needed with a high risk of recurrence of invasive pulmonary aspergillosis.

Currently, guidelines recommend systemic anti-fungal therapy as empirical treatment in all neutropenic patients who remain febrile after 4 days of broad-spectrum anti-bacterial therapy, when no alternative cause for fever has been isolated. It is also recommended not to withhold antifungal therapy if there is a high index of suspicion for aspergillus and bronchoscopy is non-diagnostic[91].

However, in these situations, ideal duration of treatment is difficult to determine, particularly if further courses of chemotherapy or ongoing escalation of immunosuppression is required, as commencing empirical anti-fungal treatment prior to obtaining diagnostic specimens can result in further attempts at obtaining respiratory specimens being negative. It is evident that prompt and accurate diagnosis is fundamental in this population.

Other fungal infections. The zygomycetes, including *Mucor* and *Rhizopus*, are an uncommon cause of invasive fungal infection after SCT, with a reported prevalence of less than 2%[157-8]. However, when infection occurs, pneumonia is most common and outcome is poor. Similar to *Aspergillus*, the zygomycetes are angio-invasive, leading to thrombosis, pulmonary infarction, and extensive hemorrhage and accounting for cavitation and halo sign. Amphotericin B, often in conjunction with surgical resection of necrotic lung tissue, is the mainstay of therapy, but mortality rates remain in the range of 60–80%[157-8].

Other emerging fungal pathogens which occasionally cause pulmonary infections include *Fusarium* and *Scedosporium* species.

Pneumocystis carinii (jiroveci) *pneumonia (PCP).* The median onset of PCP is 2 months post-transplantation. In the absence of prophylaxis, PCP complicates the course of approximately 16% of allogeneic SCT recipients[159]. Prophylaxis is now recommended from engraftment until 6 months post-transplantation for all allogeneic recipients, but should be extended beyond this point for those receiving ongoing immunosuppressive therapy and those with chronic GVHD[160]. Among patients receiving prophylaxis with oral Trimethoprim–Sulfamethoxasole, the risk of infection has been reduced to a negligible level[161]. Dapsone and inhaled pentamidine are commonly employed alternatives to Trimethoprim–Sulfamethoxasole (poorly tolerated in up to 30%), but are generally less effective in preventing PCP[161-3]. Most disease is therefore seen in this group of patients.

The clinical signs and symptoms are not distinct from other causes of diffuse pneumonia. Compared with patients with the acquired immunodeficiency syndrome (AIDS), SCT recipients generally display a more fulminant onset and course, despite paradoxically appearing to have less pathogens detectable in sputum specimens either obtained non-invasively or by bronchoscopy. This is thought to be due to a greater inflammatory reaction in the non-AIDS population[100].

Chest radiographs usually reveal bilateral interstitial-alveolar infiltrates, although isolated nodules, lobar consolidation, and even normal radiographs have been reported in the literature[164]. The diagnostic yield of bronchoalveolar lavage approaches 80-90% (paradoxically less than in the AIDS population, which is greater than 95%[91]), obviating the need for biopsy in most cases[84, 164].

High-dose Trimethoprim–Sulfamethoxasole for at least 14–21 days is the treatment of choice. Allergic or intolerant patients can be treated with intravenous pentamidine. Clindamycin and Atovaquone have also been used successfully. The use of corticosteroids as an adjunct to antimicrobial therapy in hypoxic patients, of proven efficacy in AIDS-related PCP, is of uncertain benefit in the SCT population.

Despite effective therapy, mortality rates as high as 90% for infections within the initial 6 months and 40% for late-onset infections have been reported[164].

Herpes Viruses. Without routine prophylaxis, the incidence of Cytomegalovirus (CMV) pneumonia is 20-35%[124-6]. The vast majority of episodes of CMV disease result from reactivation of latent virus in seropositive recipients. Seronegative patients who receive stem cells from a seropositive donor have a lower risk of post-transplantation CMV disease than do seropositive recipients, a situation which contrasts markedly with that seen after solid organ transplantation. The high risk is thought due to the delayed reconstitution of cytotoxic T-cell responsiveness. Risk factors for disease development include prolonged and severe neutropenia, T-cell deficiency and the presence of graft versus host disease (GVHD). Therefore, most transplant units routinely use Ganciclovir prophylaxis for at-risk patients, and screen aggressively for asymptomatic disease.

The clinical presentation of CMV pneumonia is not distinctive, but latent pulmonary disease is uncommon. Dry cough, fever, and hypoxemia are typical, and onset of respiratory failure can be rapid. Radiological findings are usually of bilateral interstitial opacities, but a variety of patterns, including focal or diffuse consolidation, ground glass opacities and nodules, can also be seen. Viraemia precedes clinical signs of CMV pneumonia by up to 3 weeks. Use of the diagnostic test for pp65 antigen (Polymerase Chain Reaction/ PCR) is thought to have sensitivity and specificity of greater than 85%, and predeces CXR changes, while also giving quantitative analysis of viral load, but requires sufficient numbers of leukocytes. BAL and transbronchial biopsy have a high yield for CMV, reported as 85-95%[91]. However, diagnosis of actual disease can be difficult. In contrast to invasive pulmonary aspergillosis, the finding of CMV virus in bronchoalveolar lavage fluid does not always suggest invasive disease, and needs to be interpreted with caution in patients without suggestive clinical or radiological features[127-8]. In a study involving 35 immunocompromised patients including SCT recipients, CMV was detected far more often than thought to be causative of pathology based on clinical and radiological findings[10]. However, a study reported in the New England Journal of Medicine in 1991 found that administering Ganciclovir to asymptomatic patients with positive CMV cultures in BAL fluid resulted in significantly less progression to CMV pneumonitis, compared with observation alone[210]. The demonstration of viral inclusion bodies histologically usually secures the diagnosis of CMV disease, but samples are often insufficient to enable this. This also raises important issues regarding screening.

Historically, prior to the commencement of pre-emptive treatment and dual therapy with Ganciclovir and CMV immunoglobulin or Foscarnet together, CMV pneumonia in this patient group led to death in 85% [129-131]. Mortality rates have reduced by over 50% (Agusti et al) with combined treatment, but remain high in patients with CMV pneumonitis requiring mechanical ventilation.

Herpes simplex virus pneumonia typically arises from aspiration or contiguous spread from an oropharyngeal site of infection, and appears rare without concomitant positive virology from the oropharynx. With the use of acyclovir, progression to pneumonia is rare nowadays.

Human herpes virus 6 (HHV6) has been detected in the lungs of some patients with idiopathic pneumonia[132]. However, its role as a pathogen is unclear, as virtually all adults are seropositive for the virus.

Respiratory viruses. Respiratory Syncytial Virus (RSV), influenza A and B, and parainfluenza account for the majority of non-CMV viral respiratory infections and are recovered from almost one-third of SCT recipients hospitalized with acute respiratory illnesses. Outbreaks mirror disease patterns within the general community, and RSV is the most commonly isolated virus[133-4]. Most patients present with fever and usual symptoms of upper respiratory tract infections. CT findings are non-specific. One study used serial thin-section CT scans on 26 SCT recipients who had proven respiratory viral pneumonia, and no other organisms[80]. In all cases, thin-section CT scans were obtained before fiberoptic bronchoscopy and BAL, and assessed for a variety of abnormalities. Areas of ground-glass attenuation were identified in 24 (92%) of 26 patients and were the only finding in 8 patients. Multiple nodules, seen in 17 (65%) of 26 patients, were centrilobular or of random distribution. A tree-in-bud appearance was seen in six of the patients with centrilobular nodules. CT revealed thickening of the bronchovascular bundles in 16 (61%) of the patients, bilateral in 14 and unilateral in 2 patients. Air-space

consolidation was present in 9 (35%) of the cases. Less common findings included bilateral pleural effusion and bronchial dilatation. This shows the enormous range of variation in radiological findings.

Progression to severe pneumonia occurs frequently with RSV (up to 80% in those who are less than 1 month post-transplantation, with mortality approaching 80% if untreated[135]) and parainfluenza infection (mortality considerably lower than RSV), in comparison with influenza. However, post-influenza bacterial pneumonias can become life-threatening[133, 136]. Adenovirus infection is an uncommon cause of pneumonia, but mortality can exceed 50%[137]. There is no treatment with confirmed consistent efficacy, but one uncontrolled study[138] showed favorable results with Cidofovir (nucleotide analogue). Ribavirin has been shown to be effective against RSV if used prior to the onset of respiratory failure. Although early administration of the neuraminidase inhibitors Zanamivir and Oseltamivir can shorten the duration and severity of influenza infection in immunocompetent hosts, their efficacy in SCT recipients has not been established[139-141].

Tuberculosis and non-tuberculous mycobacteria. Tuberculosis is infrequent, aside from in endemic areas. Most patients present with obvious pulmonary disease, marked by fever, cough, and radiographic infiltrates. Testing for Acid-Fast Bacilli is routinely included when respiratory specimens are taken, due to the public health ramifications and catastrophic consequences of undiagnosed disease. Infection with non-tuberculous mycobacteria is uncommon, but can be fatal if untreated. Treatment with standard regimes similar to the non-immunocompromised population has been effective.

Non-infectious Pulmonary Complications

Although beyond the scope of this paper, non-infectious pulmonary insults remain an important cause of morbidity and mortality in the severely immunocompromised patient, in particular, post allogeneic stem cell transplant, as techniques for prophylaxis, early diagnosis and treatment of infectious causes improve. The clinical and radiological picture can closely resemble infectious pulmonary complications, but early diagnosis of non-infective conditions is often possible using clinical and non-invasive methods; many of these conditions are either strongly suggested clinically or by time-course, diagnosed easily by bronchoscopy, or are diagnoses of exclusion and have no treatment and require supportive measures, as opposed to infectious causes. In any case, many diagnostic algorithms recommend FB, to exclude infection. Our discussion will be limited to causes of *early* pulmonary disease, which include:

- Diffuse alveolar haemorrhage
- Pulmonary oedema – cardiogenic and non-cardiogenic
- Idiopathic pneumonia syndrome
- Engraftment syndrome
- Bronchiolitis obliterans/ Graft vs host disease
- Post-transplant lymphoproliferative disorder

Of these, pulmonary oedema and diffuse alveolar haemorrhage and pulmonary oedema are the most common non-infectious causes of pulmonary infiltrates.

Diffuse alveolar hemorrhage (DAH): This is the most common non-infectious bronchoscopic finding in immunocompromised patients with pulmonary infiltrates. Incidence is similar with autologous and allogeneic SCT. Many cases occur at engraftment, usually within first month, but it is not uncommon for cases of DAH to occur later. Clinical presentation is similar to infection, and notably, haemoptysis is rare. Risk factors for DAH include: age greater than 40 years; total body irradiation; transplantation for solid tumors; and the presence of high fevers, severe mucositis, and renal impairment. Platelet levels do not influence the development or course of DAH. The hallmark of this syndrome is the finding of progressively bloodier return from BAL, in the absence of isolation of pathogens. The presence of greater than 20% hemosiderin-laden macrophages in BAL is an alternative diagnostic criterion. Treatment is mostly supportive, but high-dose corticosteroids may improve survival. Death is usually a result of superimposed multisystem organ failure or sepsis rather than respiratory failure from refractory hemorrhage.

Pulmonary oedema: Cardiogenic pulmonary oedema is usually due to the combination of iatrogenic administration of intravenous fluid and transient cardiac dysfunction. Echocardiogram may remain normal, but clinical features of fluid overload are often evident. Non-cardiogenic pulmonary edema can be caused by: drug-induced pulmonary toxicity, transfusion of blood products, or acute GVHD. The latter is most difficult to diagnose.

Idiopathic pneumonia syndrome (IPS): The clinical and radiological picture closely mimics pulmonary infection. Diagnostic criteria were formulated by a working party and published in 1993[216], but despite this, IPS remains primarily a diagnosis of exclusion, requiring a BAL which is negative for infective pathogens. Surgical biopsy shows non-specific changes such as diffuse alveolar damage or interstitial pneumonitis. Treatment is supportive; high-dose corticosteroid therapy appears to have no benefit. Etanercept has been trialed anecdotally with promising results, but has not been approved as standard treatment. In practice, in the presence of fever, neutropenia and diffuse pulmonary infiltrates, patients should be treated for pulmonary infection.

Engraftment syndrome: A clinical entity characterized in its full expression by fever, erythrodermatous rash, and non-cardiogenic pulmonary oedema at the time of neutrophil recovery. This occurs more frequently after autologous than allogeneic SCT; when seen following the latter it must be distinguished from acute GVHD. Clinical and radiological improvement has been seen following discontinuation of colony-stimulating factors, and a prompt response to corticosteroid administration has been reported.

Post-transplant lymphoproliferative disorder: In contrast with solid-organ transplant, onset is typically within the first 6 months. Major risk factors include: Epstein-Barr virus, unrelated or HLA-mismatched related donors, T cell-depleted donor stem cells, and anti-thymocyte globulin or monoclonal anti-T cell antibodies for the prevention or treatment of GVHD. Pulmonary involvement occurs in about 20% of cases[212]. Liver, spleen and lymph node involvement usually occurs concurrently. Definitive diagnosis requires biopsy but quantitative EBV DNA monitoring by PCR techniques is emerging as a noninvasive diagnostic technique[213].

Chronic airflow obstruction/bronchiolitis obliterans: Included in this review for completeness, chronic airflow obstruction is the most common late respiratory complication of allogeneic SCT (incidence reported between 6 and 26% in published series), typically occurring beyond

the third month and mostly associated with underlying GVHD[214-5]. Onset is typically insidious rather than abrupt. Presenting symptoms include non-productive cough, dyspnea, and wheezing. Fever is uncommon. The chest radiograph is commonly normal, but high-resolution CT often demonstrates evidence of air trapping, hypoattenuation, and bronchial dilatation. Diagnosis is established by demonstration of persistent airflow obstruction on simple spirometry, and exclusion of other causes for this. Due to the patchy nature of the process, utility of FB and transbronchial biopsy (TBB) is limited. Treatment is based on augmentation of immunosuppression, which in itself is a significant risk factor for pulmonary infection. Lung transplantation has been performed successfully for this condition on carefully selected patients.

In summary, pulmonary complications in this population can be non-specific and subtle in onset, but are often rapidly progressive, and can occur in a significant percentage of patients with no medical history or findings to suggest high risk. Very little reliable consistency can be found in radiological changes; there are characteristic findings, but these are not sensitive or specific enough to truly dictate treatment. Allogeneic SCT recipients who develop pulmonary infections or infiltrates during treatment can have a mortality rate of 55-90%, while early diagnosis and specific treatment is associated with increased survival [2].

2.3 The role of flexible bronchoscopy in diagnosis of pulmonary infections

In general terms, a diagnostic procedure may benefit a patient by
1. Providing a diagnosis
2. Guiding treatment – acute or ongoing
3. Predicting prognosis.

A flexible bronchoscope is a minimally invasive small tube which can advance either through the nose or mouth, into the subsegmental airways. Instruments such as a forceps or brushes can be inserted into the airways via the bronchoscope, to obtain specimens. Saline lavage can also be performed through the bronchoscope. The flexibility enables this scope to advance further into the airways and the procedure can be performed with minimal analgesia and sedation, or even without anaesthesia if required, as opposed to the rigid bronchoscope, which requires a general anaesthetic in most cases. Unless otherwise specified, the term “bronchoscopy” refers to flexible bronchoscopy (FB) within this paper.

2.3a Bronchoscopy in the non- immunocompromised population

Guidelines published by the Thoracic Society of Australia and New Zealand (TSANZ) do not include pulmonary infection in non-immunocompromised patients as an indication for bronchoscopy[165]. Bronchoscopy is not routinely performed in non-immunocompromised patients who develop classic features suggestive of lower respiratory tract infection/ community-acquired pneumonia, unless there is another indication (Eg therapeutic removal of retained secretions in intubated patients, atypical clinical or radiological features, failure of infection to resolve after a prolonged period with standard treatment, unusual organisms

isolated by non-invasive means, or suspicion of an obstructing endobronchial mass or underlying disease leading to infection[166]).

Some patients with severe community-acquired pneumonia require endotracheal intubation and mechanical ventilatory support. Once this occurs, flexible bronchoscopy becomes relatively straightforward and safe. There have been no randomised controlled trials on whether such patients should be routinely bronchoscoped if no microbiological diagnosis has been made previously, but a significant advantage is that other pathology such as endobronchial obstruction may be discovered, a targeted sample of lower respiratory tract secretions may be obtained, and secretions may be aspirated with therapeutic value. The yield in three studies of intubated patients with severe community-acquired pneumonia already receiving antibiotics was 13–48%.[168-70]. In patients with who fail to respond to initial treatment, BAL identifies pathogens in only 12–30%[172-3].

This differs greatly from current general opinion regarding the severely immunocompromised patient. The reasons for this are severalfold: Firstly, in a patient with intact immunity, lower respiratory tract infections are usually caused by a small and predictable range of pathogens, which respond to standard antimicrobial treatment. Therefore, guidelines for empirical treatment of community acquired pneumonia are simpler and the lack of microbiological diagnosis is not as concerning. A prolonged course of disease or treatment requirements is unusual without other complicating factors, and finally, progression to fulminant respiratory failure in an immunocompetent patient is less likely and generally less severe. Studies have shown that bronchoscopic yield for organisms is generally higher in immunocompromised than immunocompetent subjects, possibly due to the higher pathogen load in specimens[90]..

2.3b Bronchoscopy in severely immunocompromised patients

In many studies to date, flexible bronchoscopy (FB) as used in its current form has not been highly effective when performed on severely immunocompromised febrile neutropenic patients. Bronchoscopy generally offers the advantage of both direct vision and selective sampling from the worst affected regions as seen radiographically. However, in patients with infection, radiological features can lag behind respiratory injury, and earlier, more specific diagnosis may be obtainable if bronchoscopy is performed without awaiting such changes.

Most studies on immunocompromised patients have evaluated the usefulness of FB in patients who have already developed pulmonary infiltrates, where it remains the traditional first-line investigation, although it is not always able to be performed, due to procedural risk. The time lag to bronchoscopy being performed has usually been at least 5-7 days.

Infection is the most common cause of pulmonary infiltrates in all immunocompromised populations. A prospective series of 200 non-HIV-infected immunocompromised patients with infiltrates found infectious agents were recovered from more than 75% of subjects[66]. Bronchoscopic techniques have consistently been shown to have a higher yield for infective causes than non-infectious aetiologies. However, most studies have shown that bronchoscopic yield is lower in the haematology population when compared to other subgroups of immunocompromised patients, such as HIV positive or solid organ transplant recipients.

Numbers of severely immunocompromised haematology patients are limited in literature. Data from the wider range of immunocompromised patients may not be reliably extrapolated, as there are unique considerations in severely immunocompromised haematology patients. These include profound and prolonged neutropenia, the increased risk of thrombocytopenia or platelet transfusion, removal of barrier protection such as mucosal and skin surfaces, malnutrition and the potential need for further severely immunocompromising treatment to occur in the long-term.
Much of the detailed literature focusing on haematology patients is not current. This has important limitations, as standard practice regarding prophylaxis, conditioning regimes and type of bone marrow transplantation has changed significantly in recent decades. For example, prior to the onset of routine PCP and CMV prophylaxis, FB yield was higher, as these organisms are more easily detected in BAL fluid, and infections were more common.

Based on most series, the value of routine diagnostic FB in SCT patients with clinical pneumonia has remained uncertain. The diagnostic yield is generally low, results infrequently alter management, and no study to date has shown that any information gained from bronchoscopy has improved survival. This may, however, reflect problematic timing of FB, traditionally performed during a window when procedural risk is starting to increase, and chance of yield or impact on management decrease. Bronchoscopy in a patient with a declining respiratory state can lead to respiratory failure requiring mechanical ventilation, and there is a large body of evidence showing that mortality in patients who require intubation and ventilation is far higher than those who do not[66].

The ongoing unanswered question is therefore whether bronchoscopy and bronchoscopic findings are of clinical utility, when used in the current widely accepted manner, given that patients are routinely commenced on multiple broad-spectrum antibiotics and commonly antifungal treatments before specimens are obtained, or even irrespective of microbiological findings at bronchoscopy. However, obtaining a more specific diagnosis still remains optimal, as systemic administration of bacterial prophylaxis increases the risk of multi-resistant organisms causing pneumonia. Secondly, rare conditions including non-infectious pulmonary complications must still be considered.

As such, the optimal role and timing of bronchoscopy as a diagnostic tool needs further study to ascertain whether it could be more effectively utilized in the severely immunocompromised population. In addition, the role of each diagnostic test performed during FB (Broncho-alveolar lavage, transbronchial biopsy, protected specimen brushings) needs better clarification. One previous study showed that treatment with standard broad spectrum antibiotics and antifungal regimes would have been ineffective in 41% of patients, if pathogens had not been found in BAL[16]. Bronchoscopic yield for results such as fungal growth may be delayed, but has the potential to affect subsequent treatment, enabling appropriate prophylaxis and assessment of risk for further treatment of underlying disease. There may also be a role for determining antimicrobial sensitivity to current treatment. These benefits need to be balanced against the potential complications of this invasive procedure. Bronchoscopy itself costs a small sum of money compared with the costs of prolonged hospital stay, the need for further ventilatory support and use of more antimicrobials.

2.3c Yield of Flexible Bronchoscopy

i. Overview of literature on bronchoscopic yield compared to other techniques

In neutropenic patients with pneumonia, previous studies have clearly shown the low value of sputum examination for a microbiological diagnosis[43, 187], with the exception of the high positive predictive value when *Aspergillus* is found[146].

Bronchoscopy is the most commonly used invasive diagnostic technique to assess immunocompromised patients with respiratory complications[9, 15, 60, 21, 81-3], but assessment of yield ranges from 20% to 84%[9, 11, 15, 21, 25-6, 34, 78, 82-3]. This vast inconsistency is due to a number of important factors, including the use of differing denominators for yield, low numbers of final diagnoses, as often "gold standard" tests (for example, open lung biopsy) are unsafe to perform in immunocompromised patients, and some diagnoses of exclusion being difficult to interpret in a study setting. Even after open lung biopsy or autopsy, at least 20% of pulmonary infiltrates in immunocompromised patients remain undiagnosed. Therefore, in literature, "yield" needs to be interpreted with caution, as a relatively high yield as percentage may actually indicate lack of overall diagnosis, rather than high clinical utility of a procedure. Furthermore, as mentioned previously, studies from earlier eras may report higher yield, particularly if PCP or CMV disease was prevalent prior to the advent of prophylaxis[82], as these organisms are more easily detectable in bronchoscopically-obtained specimens, compared to the more recent trend towards invasive fungal disease. In fact, with regards to PCP and CMV, in view of the high bronchoscopic yield in the presence of actual disease, ongoing prolonged therapy for these conditions based purely on clinical criteria is considered unacceptable, given the toxicities associated with treatment. The use of FB in detecting invasive fungal disease will be discussed in detail in a separate section of this paper.

Reporting on "bronchoscopic yield" needs to also consider various bronchoscopic diagnostic techniques, including BAL, TBB and Protected Specimen Brushes (PSB). Each of these techniques also has a different adverse event profile. It may be more useful to report on diagnosis achieved, rather than overall yield, and to compare yield from bronchoscopy with *less* invasive means such as sputum microscopy and culture. Table 1 outlines a selection of studies with similar populations, aiming to standardize reported yield and complications.

TABLE 1 – see appendix

One of the earliest studies aimed to define the utility of bronchoscopy in evaluation of immunocompromised patients with diffuse pulmonary infiltrates by reviewing 35 patients between 1980 and 1983 with a wide variety of underlying diseases resulting in immunocompromise[85]. This was an elegant study by Glassroth et al, in which all 35 patients underwent BAL, TBB and protected brush specimens, and 8 also underwent open lung biopsy. An overall diagnosis was made in 29 patients. A diagnosis was made bronchoscopically in 19 patients – 18 of these were infection. For all diagnoses, bronchoscopy had a reported sensitivity of 77%. For all pulmonary infections, bronchoscopy had a sensitivity of 90%, which remains one of the highest reported yields to date. Importantly, the negative predictive value of bronchoscopy for infection was 94.4%.

The first retrospective series which evaluated bronchoscopy in patients with fever and neutropenia was published by Cordonnier et al in 1994[24]. The overall yield from BAL was 53%, with a resultant change in management in 46%. Invasive aspergillosis was the most

frequent infectious pathogen. In an Australian study published in 1987[83], fifty immunocompromised patients with pulmonary infiltrates underwent flexible bronchoscopy, including BAL. The population was heterogenous and included patients with lymphoma, leukemia, other malignancies, steroid treatment and renal failure. A positive diagnosis could be made from analysis of the BAL in 59% (33/56) occasions. Open lung biopsy added additional diagnostic information in three of the four cases in which it was performed. The most common final diagnoses were bacterial, viral or Pneumocystis carinii pneumonia and recurrent malignancy. Reflecting the somewhat higher yield in non-haematological patients, in a study by Kolbe et al[84], 18 immunocompromised patients with recent onset pulmonary disease who had FB and BAL were studied. The underlying diseases were HIV, solid organ transplantation and chemotherapy. Four patients were receiving prophylactic therapy and 12 had been started on empirical therapy for infection. Patients proceeded to FB because of atypical disease presentation or failure to respond to empirical therapy. BAL was diagnostic in 72% of patients and provided clinically useful information in 89%. There was one diagnostic failure (6%) - Pneumocystis carinii pneumonia in an HIV positive patient receiving nebulised pentamidine prophylaxis was missed. In one of the largest studies to date, Stoller et al[1] reported an overall bronchoscopic yield of 56% in 104 non-HIV infected immunocompromised patients with pulmonary infiltrates. Yield was derived as diagnoses made by FB, compared with overall final diagnoses established by open (surgical) lung biopsy, all microbiological techniques, serology testing and clinical response to therapy. They concluded that establishing a diagnosis was more likely when the lung infiltrate was due to an infectious agent. This confirmed the data from Engelhard et al[11], who studied 30 non-AIDS immunocompromised patients, reporting an overall yield of 84%, with non-infective causes being the main missed diagnoses.

Huaringa et al[60] studied 89 patients including 44 recipients of allogeneic SCT, and found BAL to be diagnostic for a variety of conditions in 45% of bronchoscopies, when performed to investigate new pulmonary radiographic infiltrates. The most common complication diagnosed was pneumonia, with diffuse alveolar haemorrhage by far the most common non-infectious cause of infiltrates. In the BAL negative groups, the final diagnoses were: bacterial pneumonia, cytomegalovirus, idiopathic pneumonia syndrome and cancer recurrence. In a study published in Chest in 1997, Dunagan et al[78] retrospectively studied the records of all SCT recipients who underwent FB over a period of 4 years. Indications for FB were suspected pneumonia and/or respiratory failure based on both radiographic and clinical history. FB was performed a mean of 138 days after SCT (median, 66 days). Ninety-four percent of subjects had been treated with broad-spectrum antibiotics prior to the procedure. Despite this, pathogens were identified in 31 (46%) patients overall. Bacteria were most often isolated (27%), with staphylococcal and streptococcal species accounting for the majority. Fungi were isolated from four patients. CMV, identified by either culture or histopathologic study, was noted in nine patients. Multiple organisms were isolated in 11 (15%) patients. There was no significant relationship between the type of bacterial or fungal isolate and the radiographic pattern of infiltrates. Peikert et al[174] studied 35 neutropenic patients with a variety of diseases causing immunocompromise. The overall diagnostic yield from bronchoscopy was 49%, and typically for this era, the most frequent diagnosis was fungal infection. Overall, single bronchoscopy was only responsible for 47% of the probable and definite fungal infections identified at hospital discharge – the others (8 patients) were found by other means - sputum analysis, repeat BAL and surgical lung biopsy. Importantly, they found a relatively high yield in non-invasive sputum analysis - 5 sputum samples correlated with the final clinically significant diagnosis of invasive fungal infection, confirmed by BAL. In 2 further cases, sputum was the only test which produced a diagnosis. In 2 cases, the diagnosis suggested by sputum analysis was confirmed by TBB but not BAL.

Glazer[36] examined the results of 79 bronchoscopies performed between 1991 and 1995 in 62 patients for the evaluation of pulmonary complications after SCT. BAL was performed in all cases, TBB in 10% and open lung biopsy in 13%. Positive results were found in 67% of all bronchoscopies, with fungal infection the most common finding (18%).

In a study design similar to ours, Rañó et al[66] prospectively studied 200 immunocompromised patients with pulmonary infiltrates, with 135 proceeding to bronchoscopy and BAL. The population included 53 SCT recipients and 68 patients with haematological malignancies undergoing chemotherapy, but did not differentiate between the groups in results. They compared diagnoses obtained from bronchoscopic techniques, with non-invasive methods, including induced sputum, naso-pharyngeal aspirate (NPA), tracheal washings if intubated, blood cultures, serological and antigen testing. Nine percent of bronchoscopies were performed prior to commencement of antibiotics, and the rest within 96 hours. A specific diagnosis was obtained by some means in 81% of cases, and infectious aetiogy was responsible for 77%. The most common pathogens were bacterial organisms in 24%, followed by fungi (17%) and viruses (10%). There was no significant difference between aetiology of immunosuppression and diagnosis. NPA was positive in 18%, and all of these were diagnosed also in BAL, except one case of RSV. Sputum was positive in 31%, and importantly, was the only technique to give a definite diagnosis in 8%. The overall diagnosis from non-invasive techniques was 40%. BAL was performed in 135 cases, with an overall yield of 51%, increasing to 69% in those with infectious aetiology. In 12/135 cases, the BAL fluid enabled a diagnosis to be made of a non-infectious cause of the pulmonary infiltrates. In 20%, BAL was the only technique which provided a definite diagnosis. At autopsy of 19 patients, 5 cases of Aspergillosis and 1 of CMV were identified for the first time. Overall, the authors found that in 44% it was possible to achieve the diagnosis using only non-invasive tests. The remaining 66% required the use of various bronchoscopic procedures. Once again, there were no clinical nor radiographic characteristics which predicted likelihood of yield from non-invasive or invasive techniques. In other diagnostic specimens obtained, blood cultures were positive in 16%. Empirical treatment was modified in 93 of the 200 patients (only 35 of those with specific diagnosis did not have treatment modified), and this was as a result of non-invasive procedures in 45 cases, and bronchoscopic techniques in 48. The most common reason for changing treatment was the isolation of a microorganism not covered by the empirical treatment, mostly Aspergillus species, viruses or MTB. In 20%, the reason was isolation of a resistant strain. In a further 20%, empirical antibiotic treatment was able to be discontinued after diagnosis of a non-infectious cause of infiltrates was found from bronchoscopic techniques. However, as a group, those with changes in treatment did not have an improvement in mortality compared to those with no treatment change. Although there was no difference in overall mortality between patients in whom a specific diagnosis was established, and those without a diagnosis for pulmonary infiltrates, the most important finding by Rano et al was a significant difference in mortality between patients in whom the diagnosis was established early (within the first 7 days), compared to late. In subjects in whom there was a greater than 5-day delay in identification of the cause of the pulmonary infiltrates, the risk of death increased independently by more than threefold. One might suggest that this reflects the fact that certain disease states would be more difficult to diagnosis or this subgroup of patients were too ill to tolerate fiberoptic bronchoscopy (FOB), but the authors explain that the delay results from neither of these factors and to date, evidence implicating diagnostic delay as a risk factor for mortality remains lacking although it is an important emerging general theme.

This study suggests that the simultaneous use of non-invasive and bronchoscopic diagnostic procedures improves the yield for specific diagnosis of pulmonary infiltrates, and that a large number of early diagnoses can only be made via bronchoscopic techniques. It makes no recommendations regarding the patients to select for bronchoscopy, or the optimal timing.

However, a study in 1997 from John Hopkins Bone Marrow Transplant Unit challenged the use of FB in this subset of patients, showing a diagnostic rate of only 31%, change in management rate of 24%, no mortality benefit and a high bronchoscopy complication rate of 15%, including 1 death from 58 procedures in 52 patients over 2 years. This resulted in further retrospective data collection on 25 bronchoscopies performed for investigation of pulmonary infiltrates over 30 months at the Lymphoma and Bone Marrow Transplant units at the Royal Marsden Hospital. The yield for positive results was 36%, and 28% led to a change in management. Once again, this had no impact on overall survival. This group consisted of patients with a variety of haematological conditions including both allogeneic and autologous stem cell transplant recipients, which were not differentiated in data analysis. It is widely accepted that bronchoscopic yield is lower in the haematological population. Hillerdal et al[5] performed 67 bronchoscopies in 57 patients with a broad range of disorders causing immunocompromise, and symptoms consistent with pulmonary infection. Diagnosis was achieved in 85% of renal transplant patients and only 28% of subjects with haematological malignancies. The most common pathogens found were CMV and PCP – a pattern also different to usually seen in haematological patients. Of febrile neutropenic patients with respiratory failure in the Intensive Care Unit, Gruson et al[180] prospectively evaluated the utility of FB and found the yield to be 49%, with 28% of patients having resultant management changes. There was a false negative rate of 34%, which is similar to previous studies, but most missed diagnoses were DAH rather than infection.

Overall, it appears that FB with BAL is indeed useful in detecting and diagnosing causes for pulmonary infiltrates in the immunocompromised patient, particularly when these are infective. However, although the procedure adds further diagnostic information to currently used non-invasive techniques, it is by no means the gold standard, and needs to be complemented by other means of diagnoses, and potentially followed by open lung biopsy in certain cases. As current practices, microbiological profile and available laboratory tests change with time, the role and optimal timing of FB as a diagnostic tool requires further investigation. A common factor amongst almost all studies involving bronchoscopy in the immunocompromised patient is the noticeable lack of impact on mortality, regardless of whether positive results are obtained, diagnoses are excluded or management is altered. However, there is some evidence that early positive results may influence outcome.

ii Factors aiding yield:

While clinical and radiographic findings have mostly been shown not to predict yield, one study[101] noted that in 50% of 87 consecutive patients with febrile neutropenia, CT scan revealed a pulmonary lesion not seen on plain chest radiograph. In a recent study by Heussel, CT scanning a similar population identified pneumonia in 60% of cases five days before clinical or CXR abnormalities were apparent[190]. In both of these reports, early CT scanning led to alterations in patient management. They also helped to guide more invasive diagnostic procedures.

Ramila et al[182] reported on 22 patients with haematological malignancies and HRCT guiding BAL: the diagnostic yield was approximately 53%, which is at the higher range for this population. However, again this led to only a 27% management change.

iii. Yield of individual techniques: bronchial washings/ bronchoalveolar lavage, protected specimen brush, transbronchial biopsy

Although flexible bronchoscopy is a frequently used tool in the diagnosis of pulmonary complications in SCT patients, it is often not possible nor advisable to obtain a biopsy, due to concomitant thrombocytopenia. Thus, sampling is often limited to BAL/ washings and protected specimen brushings (PSB). Several studies have assessed the yield and complication rate of individual sampling procedures, including washings, bronchoalveolar lavage (BAL), brushings and transbronchial biopsies (TBB).

Almost all studies show that the yield of either BAL or TBB alone was higher than PSB, and any combination was in general slightly more effective, but BAL alone appears to have an almost equivalent diagnostic yield even with the addition of TBB when the underlying diagnosis is infection[8, 14, 83, 85, 174], in particular PCP and Aspergillus. However, for Aspergillus, the overall diagnostic rate remains only 40-50%. This is important in our study population considering the high clinical probability of these infections. Importantly, the main body of evidence suggests that BAL does not miss many diagnoses for which conventional treatment is available. In a large study of SCT patients by White[15], TBB was found to be safe with aggressive platelet support, but provided only 1 additional diagnosis of infective aetiology, and never in itself changed therapy. These results supported the contention that the routine use of TBB in this setting could not be recommended. Most clinicians believe that BAL is at least as effective but less invasive than TBB for diagnosing infection[240].

In contrast, Stoller et al[1] found 21 exclusive diagnoses by TBB in 104 patients, and recommended TBB be performed "whenever possible". However, of the diagnoses, only 2 were infectious. Furthermore, the study involved a broad range of causes of immunocompromise, including all haematological malignancies, chemotherapy in last six months, bone marrow or solid-organ transplant, and use of high-dose or long-term corticosteroids. A further study found that diagnostic yield was similar for BAL and TBB individually, but the additive yield was significantly higher (45 and 49% vs 70% respectively)[4], and in yet another study involving 157 bronchoscopies on 142 immunocompromised patients including 36 with haematological malignancies[6], TBB provided a diagnostic yield significantly higher than BAL in all categories, including infection. However, the haematological malignancy subgroup had the lowest yield, of 55% for TBB and 20% for BAL. Most series which show high yield from TBB consist of populations with larger rates of non-infective causes for pulmonary infiltrates, but in contrast to more recently published literature, an early study in 1977[69] of nineteen immunocompromised patients with pulmonary infiltrates who underwent TBB found that a specific diagnosis was obtained in 21/25 procedures (10/11 focal lesions and 11/14 diffuse legions). The most common diagnosis was infection, and organisms isolated included bacteria, fungi, Pneumocystis carinii, and herpes simplex. A pneumothroax requiring tube drainage occurred in two cases and mild lung parenchymal bleeding was noted in two others.

There has therefore been ongoing debate regarding the risk-benefit ratio of TBB, particularly when infection is the main diagnostic possibility, as BAL alone avoids some biopsy-related side effects, such as pneumothorax and haemorrhage.

The use of BAL has repeatedly been shown to be more effective than the use of PSB in the diagnosis of bacterial pneumonia in immunocompromised patients[37] – in one study the yield was 59% vs 29% for each technique respectively[83]. An early study in 1980 of 58 bronchoscopies in 49 immunocompromised patients with pulmonary infiltrates[86] found that TBB provided a specific diagnosis in 26%, and the addition of PSB barely increased yield.
This finding was confirmed in a more recent study by Rano[66], PSB was obtained from 125/135 bronchoscopic cases, and yield from this was 24%, but in only one case was PSB the only technique to provide a definite diagnosis. Their yield from BAL was 51% overall, increasing to 69% in those with infectious aetiology. In 12/135 cases, the BAL fluid enabled a diagnosis to be made of a non-infectious cause of the pulmonary infiltrates. In 20%, BAL was the only technique which provided a definite diagnosis. Of all the bronchoscopic diagnoses, BAL yielded 94%, including 100% of non-infectious causes – this is in contrast to previous data suggesting that the main utility of TBB was in non-infectious etiologies which were undiagnosed by BAL. However, Dunagan et al[78] reported the overall yields of BAL and PSB in the isolation of a presumed pathogen were 38% and 15%, respectively. In 4 of 31 (13%) patients in whom a pathogen was isolated, PSB was the exclusive method in which the organism was found. The complication rate was significantly higher in those patients undergoing PSB compared to BAL alone.

The evidence suggests that most information gained bronchoscopically is from BAL alone, particularly when pulmonary infection is the major diagnostic possibility. Furthermore, findings from TBB are less likely to be reversible, and appear to be at the cost of more complications. The performance of TBB should therefore be limited to patients with pulmonary infiltrates on radiological imaging, with acceptable platelet function and stable respiratory status, in whom a non-infective diagnosis is being considered. The addition of PSB is unlikely to be of clinical value in this population.

2.3d Safety of procedure: <u>Complications of FB</u>

In non-neutropenic patients, flexible bronchoscopy is considered a safe intervention, with a quoted complication rate of 0.12% and procedure-related mortality of 0.01-0.04%[20, 221]. Bronchoscopy with BAL is considered minimally invasive even in the immunocompromised patient[89], but there is a significantly higher complication rate, mostly related to clinically significant bleeding. The reasons for this are several-fold, but related mostly to profound thrombocytopenia and multi-organ dysfunction, especially renal failure. Thrombocytopenia, defined as platelets less than 100 x 10^9/L, has been associated with an 8.6% bleeding complication rate, compared to 0.3%[26]. Other major complications include pneumothorax and respiratory failure. The overall complication rate of FB in the immunocompromised population varies widely and is reported as ranging from 0 - 27%[221] (however, one series reported rates up to 40%), increasing if TBB is performed[9, 15, 20-1, 25, 34, 78, 82-4, 184-5]. In general, the complication rate even in the immunocompromised patient is considered acceptable, as most data has shown that BAL is consistently safe and well tolerated even in patients whose general condition is poor[82, 146, 188, 192]. However, fatal outcomes have been reported[2, 186].

This needs to be compared with the reported complication rate of 13% after open lung biopsy in patients with haematological malignancies.

In the haematology/ oncology population, complications are mostly due to bleeding, pneumothorax or respiratory failure secondary to sedation for procedure. FB almost universally reduces pO2, but this is usually transient, unless severe respiratory failure is present prior to the procedure[9]. A study of 30 patients with AIDS and drug-induced immunosuppression found that procedure-related hypoxia was worse in patients with severe CXR abnormalities and reduced FEV1 prior to FB. It has been consistently shown that patients with pre-existing respiratory impairment have a higher complication rate[14, 24-26].

Several studies have assessed the yield and complication rate overall and of individual sampling procedures, including BAL, TBB and PSB. Some of these studies have involved small numbers, and percentage rates of complications differ greatly. This may be due to technical competence, expertise, experience and available facilities at different institutions, but likely also reflects the fact that there is no standardized protocol for describing complications, and there is variation in reporting minor vs significant complications. For the purpose of this paper, we considered significant haemorrhage to be bleeding requiring intervention, blood product support, compromising airway or causing haemodynamic compromise. Respiratory failure was considered to be significant or prolonged hypoxia requiring oxygen by mask, non-invasive ventilation or mechanical ventilation as a result of the procedure. Any admission to an intensive care unit following a procedure was considered a significant complication.

The first retrospective evaluation of bronchoscopy in febrile neutropenic patients[24] reported only 2 complications in 113 procedures. Huaringa[60] reported no complications apart from mild transient hypoxemia. The incidence of major bleeding appears less common than initially thought, with one large study showing no major bleeding, despite 77 out of 95 patients being thrombocytopenic[16]. Minor bleeding has been reported as up to 13%[1], and pneumothorax up to 4%[1] . In one study, pneumothorax occurred *without* TBB in 2/3 patients. However, none required chest tube drainage[15]. Of 35 patients in one study, 2 patients required mechanical ventilation, 1 required observation in ICU, 1 patient had self-limited haemorrhage following TBB[174].

Whittle"s study[54] involved 2 early surveillance bronchoscopies: The first prior to conditioning on 33 subjects, and a second FB after neutropenia on 24 subjects with no respiratory compromise and platelet levels of greater than 40 x 10^9/litre. The adverse event rate was reported as 18% and 33% respectively. However, complications were minor and transient, including fever, minor epistaxis, hypoxia (SaO2 89% on air), vomiting and cough. There was no respiratory failure, major haemorrhage or pneumothorax. Moreover, this study found that neutropenic and thrombocytopenic patients show high rates of fever and minor bleeding regardless of whether invasive procedures are performed. Dunagan et al[78] found in performing later bronchoscopies (mean 138 days after SCT), on high acuity SCT patients, the overall complication rate was 27%. Minor complications occurred in 13 (18%) patients, and most often included mild desaturations or "mild" to "moderate" bleeding. Most patients (85%) experiencing a minor complication had undergone both BAL and PSB. Major complications were less common (8%) and included intubation with mechanical ventilation (3 patients), hypotension requiring pressors (1 patient), and significant bleeding leading to death within 24 h in 2 patients who had undergone BAL and PSB, but not TBB. Complications occurred in 36% of patients who underwent PSB as compared with 14% of patients undergoing BAL alone

($p<0.05$). Importantly, patients who experienced any complication (minor or otherwise) had an associated mortality risk that was 1.8 times higher than patients without complications ($p=0.0148$). Interestingly, in this group, no significant relationship was noted between platelet count or APTT in those who experienced major complications, but the mean INR was significantly elevated. This finding probably reflects routine platelet transfusions before and after FB, and avoidance of bronchoscopy patients with intractable thrombocytopenia.

In those patients already in ICU at the time of bronchoscopy, a higher frequency of complications was noted (16.7%), but most of these were minor[180].

Lethal complications of BAL without TBB seem to be extremely rare, although the possibility of reporting bias must be considered. There was one fatality reported in 1993 in a patient who developed sepsis following BAL[77], and a further case in 1997 of a 45-year old male, 3 years after SCT, with rapidly evolving pulmonary infiltrates and hypoxia requiring bronchoscopy[76]. Unusual resistance to fluid instillation was noticed, and yield was relatively low (20% of instilled volume). Within minutes of the procedure, pneumothorax was diagnosed. Chest tube drainage was performed and the patient was put under mechanical ventilation in the ICU, where he eventually died 2 days later. At autopsy a tear was seen on the visceral pleura of the medium lobe.

The variation in complication rate is not explained by a relationship with yield, and it does not therefore appear that more aggressive procedures produce more diagnoses at the cost of greater complications.

In summary, the standard for diagnosing pulmonary infection is considered to be FB with BAL, which is safe, minimally invasive, reproducible and often leads to a rapid diagnosis, but still has a complication profile which requires careful consideration in potentially unstable patients. The yield of PSB and TBB in the same procedure may be additive, but increases the likelihood of complications[91]. Platelet support should be given prior to bronchoscopy, and institutions providing bronchoscopic service for severely immunocompromised patients should have experienced Respiratory Physicians, Thoracic Surgeons and Intensive Care Units to provide support if needed.

2.3e Timing of bronchoscopy

Very few studies have addressed the specific issue of timing of bronchoscopy, although it seems logical that the sooner this is performed, the higher the yield, as patients will have had less doses of antibiotics.

In one of the first studies addressing timing, Vaughan (1991)[50] hypothesized that pathologic processes involving the lungs could be detected prior to the development of respiratory failure. They devised a surveillance program including bronchoscopy with BAL for patients with leukaemia and pre-SCT, prior to the commencement of chemotherapy or conditioning regimes. Fifty-three patients were bronchoscoped twice - initially prior to treatment, and then at onset of granulocytopenia. Vaughan et al found that patients with pathologies discovered prior to cytotoxic therapy were managed more promptly, while pathologies persisting or discovered only at the onset of aplasia were more ominous. Five of 8 isolates at pre-aplasia BAL were implicated in later fatal respiratory complications. In this study, 12 patients received autopsies,

and no pathology was found which added to the antemortem understanding of pulmonary pathophysiology. They recognized that the information gained by surveillance bronchoscopy with BAL and early repeat bronchoscopy when further respiratory complications developed helped preclude the need for TBB or OLB. However, although surveillance bronchoscopy could identify clinically significant abnormalities in the lung prior to these abnormalities becoming clinically manifest, this was not always sufficient to prevent poor outcomes.

Subsequent to Vaughan's study, Whittle et al[54] performed similar surveillance bronchoscopies. Pre-transplant BAL (B1) prior to conditioning was performed on 33 subjects, followed by post-transplant bronchoscopy and BAL (B2) as soon as feasible after absolute neutrophil count (ANC) fell below 0.5 x 10^9/litre. Platelet support was given to maintain a level greater than 40 x 10^9/litre. Importantly, patients with clinical or radiographical pulmonary abnormalities were excluded. A control group of neutropenic post-transplant recipients was compared. The yield from B1 and B2 were 18% and 13% respectively, but in contrast to Vaughan's study, these authors found that none of the isolates in either group proved to be of clinical significance, and were likely to represent either transient colonization of the respiratory tract without established infection, or contamination from the nasopharynx. The important differences may be two-fold: the very early performance of the diagnostic test – ie, prior to the development of any complications, resulting in lower yield, and the exclusion of patients with radiological abnormalities, although data regarding the latter remains conflicted.

In a Singaporean study of 60 consecutive immunocompromised hosts with pulmonary lesions[4] (but not necessarily febrile neutropenia), bronchoscopies were performed either "early" (40 patients, average 1.6 days after pulmonary infiltrates detected) or "late" (20 patients, average 16.7 days after infiltrates detected), and always within 72 hours of commencement of antimicrobial treatment. Patients bronchoscoped earlier received less empiric antimicrobial therapy overall and had significantly shorter hospitalization. Even with delayed bronchoscopy in the late group, bronchoscopic results influenced clinical management in 85% of patients. Infections accounted for approximately two-thirds of the pulmonary lesions.

The above suggests that the "window of opportunity", during which bronchoscopy is most likely to be diagnostic, and results in least complications, is yet to be determined. Parameters for selection of patients who should proceed directly to bronchoscopy need to be defined with further studies.

2.3f Impact of bronchoscopic results on clinical management and outcome measures

Although it is evident that FB and BAL improves yield for microorganisms compared to non-invasive diagnostic tests alone, the clinical significance of potential pathogens identified in BAL fluid without respiratory impairment is unclear[49, 127]. While detection of pathogens is clearly possible, there remains the additional problem of identifying which organisms are responsible for disease.

Some studies have shown that performing FB earlier led to use of less total antibiotics (less different antibiotics and shorter duration) and less days in hospital[4], but almost all have shown no improvement in mortality rates, even if clinical management was altered based on FB results. While much emphasis is understandably placed on yield, it is important to note that

negative results obtained bronchoscopically may also potentially alter management by excluding diagnostic possibilities. However, this is dependent on negative predictive value of this test, including subsequent microbiological analysis.

Campbell and colleagues[79] reported therapeutic modifications on the basis of FB results in 17 of 27 (63%) SCT patients who had undergone the procedure for further evaluation of pulmonary infiltrates. In the large series reported by Dunagan[78], FB resulted in a change in treatment in 41% of patients. Of these, therapy was changed in 65% of patients when an organism was identified and in 22% of patients in whom all results were negative (p=0.0026). Changes in patients with negative FB were mostly the discontinuation of empiric therapy for PCP in five patients. Despite isolation of an organism and therapeutic changes, the survival of patients in whom a pathogen was isolated (48%) did not significantly differ from that of patients in whom no organism was isolated. Similarly, several other studies have shown that mortality in patients with pneumonia not undergoing FB was similar to those who underwent FB, regardless of results. In one study[174], 28 day mortality was 26%, although management was altered in 18 patients (51%). Furthermore, a non-significant trend toward a higher hospital mortality was observed in patients in whom a specific diagnosis was established. This may be due to mores severe disease leading to a higher burden of pathogenic organisms, or a decreased response to antimicrobial therapy in these patients. In Glassroth"s study[85] as discussed in the previous section, negative results were followed by open lung biopsy in a number of patients. Bronchoscopy had a reported sensitivity of 77% for all diagnoses, and a sensitivity of 90% for pulmonary infections when compared with overall infection yield. The negative predictive value of bronchoscopy for infection was 94.4% - in the face of a negative bronchoscopic procedure, there was very low probability that an infectious process would be found on open biopsy. However, despite this, specific diagnosis still did not improve survival. The finding of lack of mortality benefit even with relatively high yield bronchosopy results has been mirrored in many other studies.

However, one study published in 1995[19] reported that management was changed in 65% of immunosuppressed cancer patients on the basis of BAL results, and this had a significant effect on survival. Those in whom BAL confirmed empirical treatment had the highest overall survival, while those with no positive isolate or other cause for infiltrates found had the lowest.

In summary, the majority of evidence shows that despite potentially enabling significant changes in management, results obtained bronchoscopically, or specific diagnoses have yet to impact on mortality in the severely immunocompromised population.

2.3g Open Lung Biopsy

In terms of "denominator" against which to measure overall yield, surgical or "open" lung biopsy (OLB) is historically considered the gold-standard for diagnosis. However, the complication risk in the severely immunocompromised patient population is far higher than the normal surgical population, and in practice, few proceed to surgery. Reported complication rates are approximately 13% in the immunocompromised population. Prior mechanical ventilation, and thrombocytopenia (platelets less than 50 x 10^9/l were associated with further increased procedural risk. Complications were similar with video-assisted thoracoscopy compared with open thoracotomy[104].

Literature detailing the influence of surgical lung biopsy on outcomes has been retrospective and studies have been of small, heterogenous populations. In general, less than half of the patients sent for surgical lung biopsy yielded results which altered management. Yield has been reported as even lower in patients who were neutropenic[104].

Downey et al[104] assessed the yield and impact of open lung biopsies in 63 patients with haematologic malignancies and unexplained pulmonary abnormalities. A specific diagnosis was found in 62%, resulting in changes in therapy in 57% of patients as a result of biopsy findings. In those in which a specific diagnosis was made, 69% underwent change in therapy. Diagnoses included inflammatory diseases in 23% of cases, infections in 21%, and malignancy in 18%. Fungi and bacteria were the most frequent infectious pathogens. The factor most predictive of finding a specific diagnosis was the presence of a focal rather than a diffuse radiographic abnormality (79% versus 36%, $p = 0.003$). Importantly, neutropenic patients or those on mechanical ventilation had a low chance of finding a specific diagnosis. Having received pulmonary toxic chemotherapy in the 6 months before the biopsy was performed was associated with finding a non-specific lung injury. In a unique study in which patients with acute pneumonitis without neutropenia were randomized to either empiric antibiotic treatment or treatment based on results of open lung biopsy, patients with open lung biopsy had a worse outcome, possibly related to morbidity of open lung biopsy[87]. Other studies have shown that OLB in this population yields only non-infective causes[15]. A Taiwanese study analyzed the etiologies and prognostic factors in 68 SCT recipients with diffuse pulmonary infiltrates and assessed the role of open lung biopsy in management[35]. Thirty-five patients underwent open lung biopsy, resulting in therapeutic changes in 22 (63%) and clinical improvement in 16 (46%). The leading diagnoses were idiopathic interstitial pneumonitis (40%) and cytomegalovirus pneumonitis (20%). Three (9%) patients had miliary tuberculosis. Mortality was 50% of patients with infiltrates overall. Respiratory failure ($p < 0.001$) and acute graft-versus-host disease ($p = 0.016$) were poor prognostic factors. This study confirmed that in those patients who are well enough to tolerate the procedure, open lung biopsy can lead to clinically beneficial treatment changes. A study reported in 1995[14] aimed to compare the diagnostic yield from concurrent open lung biopsy (OLB) and bronchoalveolar lavage (BAL) in febrile neutropenic patients with pulmonary infiltrates and assess the impact of results obtained on clinical outcome. 13 immunocompromised patients (mainly with haematological malignancy or bone marrow transplantation recipients) were investigated. At least one diagnostic finding in 12 of 13 patients was provided by OLB compared to 4 of 13 patients by BAL. Both diagnosed infective and non-infective causes, but 5 patients with non-specific interstitial/alveolar inflammation were diagnosed only by OLB. The concordance between diagnoses present in BAL and OLB was zero. There was one complication in each group – wound infection following OLB, and moderate haemorrhage after BAL. No procedure-related mortality was reported.

The role of open lung biopsy remains to be defined, but in practice, the procedure is rarely performed in immunocompromised patients, due to the inherent risks and often non-specific findings. However, data shows that the presence of a specific diagnosis may result in a change in therapy and reduced mortality, and more recent literature suggests that the complication rate may only be slightly higher than for TBB in selected patients, and there remains a role for OLB particularly in patients with focal pulmonary lesions, suspicion of non-infective causes and stable respiratory state.

2.3h The diagnosis of invasive pulmonary aspergillosis (IPA)

i Role of flexible bronchoscopy

Yield in Fungal disease

Invasive Pulmonary Aspergillosis (IPA) is difficult to diagnose in the immunocompromised patient. The definitive diagnosis is said to only be established by biopsy with substantial tissue. However this can not be achieved in most immunocompromised patients because of coexisting thrombocytopenia and the risks of aggressive biopsy in patients with imminent respiratory failure. The reported yield of bronchoscopy overall for aspergillosis in immunocompromised patients is at best 50%[97, 98, 99].

One small study showed that five (83%) of six cases of invasive pulmonary aspergillosis diagnosed at autopsy were negative for fungi antemortem, despite BAL having been performed[74]. In a recent review article[199] it was reported that the diagnostic sensitivity of BAL was 43% in histologically proven IPA. However, the reported diagnostic sensitivity of BAL for IPA appears to vary with the investigator. Saito et al[188] reported that BAL was diagnostic for pulmonary aspergillosis in zero of nine patients with leukaemia. Most specific diagnoses for pulmonary aspergillosis were established solely from autopsy, so BAL was reportedly useless in most cases. These rather dismal findings were slightly improved when Reichenberger[17] studied 23 neutropenic patients who had received high-dose chemotherapy or SCT. Results were still only positive in 30% of patients with biopsy-proven IPA, and were often negative even with histology showing extensive invasion. This may be due to the recovery of fluid from the bronchoalveloar compartment rather than more central airways. However, only one study has tried to compare bronchial washings and BAL to address this issue, and this patient group was too small for definite conclusions[23]. Patients with positive bronchoscopic results had more changes on CT chest, and a non-significantly lower cumulative dose of Amphoterecin B prior to procedure, but the yield of bronchoscopy was not associated with clinical symptoms or duration of neutropenia. Even when there was a positive bronchoscopy result, there was no association with duration of antibiotic-resistant fever, neutropenia or subsequent antifungal therapy. The authors commented that the low yield could be influenced by the intensity of previous anti-fungal treatment, and therefore recommended diagnostic bronchoscopy with BAL prior to anti-fungal therapy, and discussed the role of pulmonary surveillance by BAL during neutropenia. Peikert et al[174] studied 35 neutropenic patients, yielding 17 bronchoscopic diagnoses, of which 7 were fungal infection. However, single bronchoscopy was only responsible for 47% of the probable and definite fungal infections identified at hospital discharge – the others (8 patients) were found by other means - sputum analysis, repeat BAL and surgical lung biopsy. Importantly, they found a relatively high yield in non-invasive sputum analysis - 5 sputum samples correlated with the final clinically significant diagnosis of invasive fungal infection, confirmed by BAL. In 2 further cases, sputum was the only test which produced a diagnosis. However, in an investigation performed by Stover et al[21] and reported in 1984, BAL had a diagnostic yield in 83% of patients with invasive pulmonary aspergillosis confirmed by open lung biopsy, transbronchial biopsies and autopsy.

The diagnostic sensitivity of TBB for IPA has also mostly been reported as low and prone to sampling error[91].

The specificity of BAL for IPA is good, difficulties remain in distinguishing infection from colonization, as the definitive diagnosis of IPA requires histopathologic demonstration of

septate acute branching hyphae with positive culture results for Aspergillus[200]. Horvath and Dummer[148] performed a retrospective analysis reviewing all respiratory secretions analyzed at Vanderbilt University during a 14-year period, and showed an estimated 72% positive predictive value for invasive aspergillosis if fungal organisms consistent with *Aspergillus* were identified by sputum stain or cultures. Sensitivities were similar for invasively and non-invasively collected respiratory tract specimens. These findings confirmed prior data from Yu et al[183] who concluded that isolation of *Aspergillus fumigatus* and *Aspergillus flavus* from respiratory specimens is highly predictive of invasive aspergillosis in patients with leukemia and/or neutropenia. Therefore, positive results in this population should be treated as IPA, but if bronchoscopic results are negative, and clinical or radiological suspicion of fungal disease exists, treatment should still be considered.

ii Role of CT chest and other methods of detecting invasive fungal disease

CT chest remains one of the most sensitive methods for "diagnosing" invasive pulmonary Aspergillosis rapidly. It is thought that HRCT chest can show characteristic findings of angio-invasive aspergillosis within 30 days of SCT[2]. One study [18] showed that of 56 patients with proven fungal pneumonia investigated with both HRCT and BAL, HRCT had a sensitivity of 84%, compared with 48% for BAL, although the specificity of BAL was 100%. Blood cultures were positive in none of aspergillosis cases. Serological testing and surveillance cultures had only limited value for the early diagnosis of pulmonary mycosis. Another group showed there was a higher chance of achieving diagnosis if pulmonary opacities involved a central third of the lung[7].

In view of the reported increased sensitivity of HRCT even in comparison to bronchoscopy for the diagnosis of IPA, several groups suggested that bronchoscopy and HRCT are mutually complementary diagnostic tools with high sensitivity in patients with haematological malignancies and new pulmonary infiltrates, and facilitate the early and reliable recognition of invasive fungal disease. They recommend the early use of HRCT and antifungal treatment, proceeding to BAL when infiltrates were centrally (inner two-thirds) distributed and were atypical or not responding to treatment, to exclude concurrent or unusual infections. Surgical lung biopsy was recommended in those without central lesions. This protocol was based on the notion that HRCT would diagnose a proportion of patients with aspergillosis, preventing the need for any invasive investigations.. To date, there has been no definitive data regarding the outcomes of this method.

In two other studies[146, 191], the CT scan suggested the diagnosis of invasive aspergillosis by the presence of opacities with a "peripheral halo" at an early stage or an "air crescent formation" at a late stage. Moreover, the CT scan determined precisely the location of the lesions and helped to evaluate the risk of haemoptysis[146]. When local lung resection was performed on basis of imaging, IPA was confirmed histologically in 35/39 patients. The presence of a suggestive lesion as interpreted by an experienced radiologist has on some occasions had 90% positive predictive value.

However, other data has suggested that HRCT findings can be non-specific and in this patient population, in view of the obvious implications of persistent fungal infection, microbiological diagnosis is preferable.

Caillot et al[47, 146] studied new strategies for early diagnosis in 36 neutropenic haematology patients with proven or highly probable IPA. When early CT chest was routinely performed in patients with CXR infiltrates, 92% of patients had halo signs detected, compared with 13% prior to this method. Caillot also found the aspergillus antigen test to be positive in 83% of cases when tested on BAL fluid. Twenty-six patients were reported as cured or improved by early antifungal treatment combined in some cases with surgical resection.

(1AE3)-beta-D-glucan (BDG) assayand PCR

Kami et al[38] also attempted to find a more sensitive marker for invasive pulmonary Aspergillosis in 215 patients undergoing cytotoxic therapy, using the latex agglutination test and plasma (1AE3)-beta-D-glucan (BDG) assay. They found sensitivity tended to be lower in patients with IPA localized to the lung than those with disseminated invasive aspergillosis. However, although some had normal chest xrays, all IPA patients showed abnormal signs on chest CT scans, on average preceding a positive latex agglutination test by 7.1 days and a positive BDG assay by 11.5 days. Hence, CT scanning remained the best diagnostic technique. This group then proceeded to develop a new quantitative system for diagnosis of IPA using real-time automated blood polymerase chain reaction (PCR)[39]. Sensitivity of this test was higher than enzyme-linked immunosorbent assay (ELISA) or BDG plasma measurements, and specificity was 92% compared with 97% for ELISA and 84% for BDG. However, positive findings on PCR only preceded CT changes by 0.3 +/- 6.6 days.

Galactomannan

ELISA detection of serum Galactomannan was found to be sensitive and specific, with positive results prior to clinical or radiographic changes in 68% of cases of Invasive Aspergillosis. However, Galactomannan has still been shown to increase in the absence of invasive disease, when ELISA testing was used[56], and further studies are necessary to define the optimum role and true clinical utility of this serologic tool. Galactomannan detection by Latex Agglutination is now commercially available, and has been suggested as a useful non-culture method. This has been validated in serum testing – positive results preceded clinical suggestion of invasive fungal disease by a mean of 5-8 days (Range 1-27 days). The concentration of circulating Galactomannan is also thought to correspond with the fungal burden in tissue. Reported sensitivity and specificity range from 50-92.6% and 94 – 99.6% respectively, in patients with haematological malignancy. The role of Galactomannan detection has been validated in serum only, to date, but its use in other bodily fluids, including bronchial washings and BAL may have potential and remains to be seen. As the lungs are affected in most cases of invasive aspergillosis, serum results may be translatable into this clinical setting. Importantly, as Galactomannan mainly increases during Aspergillus growth, it is thought to better reflect infection than culture or PCRs, which do not differentiate between colonization and disease.

Other bronchoscopic techniques

Using a unique bronchoscopic technique, Oki et al[195] successfully visualized and sampled cavities caused by IPA in 2 patients immunosuppressed with corticosteroids, with ultrathin bronchoscopy. In both patients, CT findings were of a thick-walled cavity, and in one case, standard bronchoscopy was non-diagnostic. Ultrathin bronchoscopes offer a smaller outer diameter and higher image quality: the external diameter is 2.8mm, working channel 1.2 mm, compared to standard bronchoscope, which has diameters of 6.1 mm 2.0 mm respectively (approximate diameters). This permits the observation and manipulation of more peripheral bronchi than was previously possible with a standard bronchoscope, and has been reported to be

a valuable diagnostic tool for other peripheral pulmonary lesions[196-8]. A cavitatory lesion in the peripheral area of the lung appears to be a good indication for ultrathin bronchoscopy, as once the tip of the ultrathin bronchoscope enters the cavity through a small bronchus, the range of vision becomes broad. Biopsy of a lesion with bronchoscopic visualization should result in higher diagnostic yield. Moreover, a biopsy with direct visualization using mini-forceps may decrease the risks of bleeding. The emergence of bronchoscopy with guide-sheath endobronchial ultrasound may also enable more targeted sampling of lesions.

Overall, it appears that bronchoscopy with BAL has a role in the diagnosis of IPA, especially when supplemented with CT chest. The negative predictive value of currently used fungal detection methods is not sufficient to advise against treatment with anti-fungal medications if BAL is negative and high clinical or radiological suspicion remains. As new techniques are studies and added to diagnostic algorithms, it is expected that this situation will improve.

2.4 CHEMOKINES IN PULMONARY DISEASE

An aim of our study was to further evaluate the local response associated with pulmonary infections in severely immunocompromised patients. We hypothesized that knowledge of the chemokine profile in the lungs of patients with pulmonary infection, as seen in BAL fluid, could enable us to suspect or diagnose such infection with more confidence in a clinical setting, and therefore guide treatment further. We hypothesised that the local chemokine profile could identify those patients who had pulmonary infection but insufficient pathogens present to detect during early bronchoscopy. In addition, the evaluation of the inflammatory response could be useful for deciding the appropriate management of pulmonary complications in immunocompromised patients.

Six closely related subfamilies of chemotactic cytokines, referred to as chemokines, have now been characterized. Of these, members of at least two subfamilies contribute to pulmonary antimicrobial host defense. Members of the C-X-C chemokine family, which include IL-8, have predominant neutrophil stimulatory and chemotactic activities, whereas the C-C family, which includes MCP-1 and RANTES, exerts predominant chemotactic and/or activating effects on macrophages, lymphocytes, and eosinophils. Several lines of evidence, including well-characterized *in vitro* leukocyte-activating and chemotactic activities suggest that C-X-C and C-C chemokines represent integral components of antimicrobial host defence. In addition, a number of infectious agents, or their cellular components, have been shown to induce production of both families of chemokines by macrophages and stromal cells. Lastly, chemokines, in particular IL-8, have been detected in increased amounts within the lungs and blood of patients with a variety of pulmonary infections. The role of chemokines in host defence against invasive pulmonary aspergillosis has not yet been clearly defined, but it is known that animals lacking the chemokine receptor CCR-1 are particularly susceptible to systemic *Aspergillus fumigatus* infection.

Most previous studies of adult populations evaluating the role of the local and systemic inflammatory response in pulmonary infections have been performed in immunocompetent patients. In fact, the first investigations in immunocompromised adults were published by Agusti et al in 2004[238], and there remain few similar studies to date.

Neonatal sepsis is known to be associated with an increase in plasma-derived cytokine levels. Previous studies in the paediatric oncology population have investigated several inflammatory plasma cytokines in an attempt to define suitable markers for bacteraemia in "high-risk" patients. However, the predictive value of individual cytokines has generally been too low to influence management[218]. The time constraints and relatively large volume of specimen required to measure multiple cytokines by conventional enzyme-linked immunosorbent assay (ELISA) techniques is prohibitive. However, the use of Cytometric Bead Array (CBA) kits is a rapid, easy and sensitive method to measure multiple plasma cytokines, and requires comparatively low sample volume. Our laboratory has previously successfully applied cytometric bead arrays to screen multiple plasma cytokines as a novel and effective method of determining bacteraemia in febrile neutropenic paediatric oncology patients (Hodge et al[217, 219]). We have found in the rapid, simultaneous measurement of multiple cytokines that increased interleukin 8 (IL-8) correlates with culture-positive infection[217].

In order to establish direct causal relationships between chemokines and specific biologic events in pneumonia, studies have been performed in murine models. These have found that certain chemokines are frequently increased in BAL when bacterial pneumonia is present, but no corresponding increase is seen in blood. Further studies have confirmed that the cytokine profile is compartmentalized in humans, particularly in the case of pulmonary infection, with poor correlation between BAL and serum levels. Almost all studies have found higher levels in BAL than serum during pulmonary infection. Mathiak et al[230] investigated the levels of several chemokines including MCP-1 in BAL fluid and serum in 20 septic surgical ICU patients, aiming to understand the significance of compartmentalized inflammatory mediator production in an immunologically active organ (lung) in comparison with levels in the systemic circulation. The study group consisted of 20 immunocompetent septic patients and 10 non-septic patients in surgical ICU. At the onset of sepsis, MCP-1 was significantly up-regulated in BAL fluid (as were GRO-alpha, IL-18, and IL-6 levels) compared with non-septic controls. In marked contrast, there was no increase in *serum* levels of these inflammatory mediators determined both at the onset and during the ongoing states of sepsis. These findings further support the notion that mediator measurement in the immunologically active organ might serve as the pivotal indicator of sepsis prior to the actual fulfilment of specific clinical criteria which currently define sepsis.

Lung infections are characteristically associated with a striking inflammatory response aimed at controlling the proliferation of microorganisms and avoiding further derangement of the lung parenchyma. Previous studies have found that the levels of pro-inflammatory cytokines are higher in patients with pulmonary infections, particularly those of bacterial aetiology, than non-infective causes of pulmonary infiltrates. Patients with a more severe pulmonary infection had a more intense local and systemic inflammatory response. Thus, the evaluation of the inflammatory response in patients with pulmonary infections has been relevant for understanding the pathogenesis, as a potential clinical marker of severity, and for designing future immunomodulatory treatments. Both proinflammatory and counter-inflammatory cytokines have been correlated with the severity of the pulmonary infection determined by the APACHE II score and also with prognosis. An elevated BAL fluid IL-6 level of has been shown to be an independent predictor of mortality in immunocompromised patients with pulmonary infiltrates[238]. Conversely, Monton et al[220] assessed the sequential expression of TNF-alpha, IL-1 beta and IL-6 in BAL and blood of 30 immunocompetent patients with severe

pneumonia requiring mechanical ventilation. No significant correlation between BAL fluid cytokine levels and lung bacterial burden was shown in the presence of antibiotic treatment. No clear relationship was found between BAL fluid and serum cytokines and mortality.

The gram-negative bacterium Pseudomonas aeruginosa is an opportunistic human pathogen associated with both an acute lung disease in patients with hospital-acquired pneumonia and a chronic, progressive lung disease in individuals with underlying lung disease. A unique characteristic of this bacterium in its natural environment is the secretion of a wide variety of factors designed for protection against host defences, to ensure its growth and survival. Evidence suggests, however, that when present in the human host, these same factors may contribute to disease. It has been observed that Pseudomonas aeruginosa metalloproteases in bacterial-conditioned medium, as well as purified alkaline protease and elastase, degraded human RANTES, monocyte chemotactic protein-1 (MCP-1), and epithelial neutrophil-activating protein-78 (ENA-78). Under identical conditions, IL-8 was significantly more resistant to proteolysis. Degradation was accompanied by a loss of chemotactic activity. These data suggest that metalloproteases from P. aeruginosa could alter the relative amounts of critical immunomodulatory cytokines in the airway and, thus, could contribute to the pathophysiology observed in P. aeruginosa-associated lung disease.

A role for chemokines in fungal resistance was first suggested by Huffnagle et al. Recent studies of chemokines in cryptococcal infection of human cells have revealed that incubation of primary human endothelial cells with *Crytococcus neoformans* did not induce chemokine synthesis, but resulted in differential inhibition of cytokine-induced IL-8, IP-10, and MCP-1[235].

It has previously been thought that even after treatment with anti-microbials, there may be consistent differences detected in the intensity or pattern of the inflammatory response between bacterial, viral, and fungal pneumonia due to different cell wall components released by killed bacteria such as endotoxin from Gram negative bacilli and lipoteichoic acid and bacterial peptidoglycan from Gram positive bacteria. However, when infectious complications have been divided into bacterial, fungal and viral aetiologies, no inflammatory mediators have been found specific enough to clearly distinguish between these groups – there currently exists no suggestive cytokine profile.

Human interferon-gamma-inducible protein 10 (IP-10) is a member of the chemokine family of cytokines and is induced in a variety of cells in response to interferon gamma and lipopolysaccharide[222]. It differs from most chemokines in its apparent specificity for activated T-lymphocytes. Serum levels of IP-10, IL-2 and IL-6 were found to be significantly elevated during SARS infection, but not other causes of pneumonia[224]. However, in patients with Community acquired pneumonia, but not in those with SARS, the levels of interferon-gamma, IL-10, IL-8 and monokine induced by interferon-gamma (MIG) were significantly elevated compared with the levels in healthy controls[224]. One group recently studied T-cell activity against PCP in mice[225]. Mice who were deficient in CXCR3 but had CD4(+) T-cells intact showed an initial delay in activity, but were eventually able to clear the infectious challenge, indicating that CXCR3 signalling is utilised but not essential for the clearance of PCP. However, CD4-depleted mice had lower levels of MIG, IP-10 and IFN-inducible T cell alpha-chemoattractant at day 7 of infection and were permissive to PCP infection. Over-expression of IP-10 in the lungs by adenoviral gene transfer accelerated PCP clearance by day 28 in mice depleted of CD4(+) T cells, but did not have this effect in control mice. The experiment showed

that IP-10 can direct Tc1 CD8(+) T cell recruitment to the lungs and contribute to the host defence against PCP, even in the absence of CD4(+) T cells.

Intrapulmonary administration of Klebsiella pneumoniae resulted in the local and systemic expression of IP-10, followed sequentially by MIG expression. MIG mRNA expression required the endogenous production of IFN-gamma, whereas IP-10 was expressed in both an IFN-gamma-dependent and independent fashion. Antibody-mediated neutralization of IP-10 resulted in reduced bacterial clearance and decreased survival, whereas bacterial clearance was unaltered in mice treated with anti-MIG antibody[232].

There was no literature showing a relationship between MIG and fungal infection.

Monocyte chemotactic protein-1 (MCP-1), also designated CC chemokine ligand-2 (MCP-1/CCL2), is a pro-inflammatory cytokine responsible for regional monocyte recruitment in acute lung injury. It also promotes collagen synthesis in lungs, and has been implicated in the development of interstitial lung disease[226]. The first demonstration of a role for MCP-1 in clearance of an infection was reported in 1995 by Huffnagle et al[227], who aimed to identify the chemotactic factors which mediate inflammatory cell recruitment into the lungs in the clearance of fungal infection with Cryptococcus neoformans. Huffnagle found that BAL fluid levels of MCP-1 and the recruitment of inflammatory cells both increased following pulmonary infection with *C. neoformans*. The kinetics of MCP-1 production in the lungs correlated most closely with the recruitment of CD4+ T cells and monocytes/macrophages. Administration of neutralizing anti-MCP-1 Antibodies in vivo reduced MCP-1 in BAL, decreased the recruitment of both macrophages and CD4+ T cells and inhibited Cryptococcal clearance. This group also found for the first time that recruitment of neutrophils and B-cells was decreased in anti-MCP-1-treated mice (most likely an indirect effect of reducing the number of CD4+ T cells and macrophages). Neutralization of MCP-1 also resulted in decreased BAL fluid levels of TNF-alpha and IL-6.

The protective mechanisms against invasive aspergillosis in the immunocompromised host remain incompletely understood, but it has been hypothesized that MCP-1 is necessary for effective defence. A rapid and marked induction of MCP-1 has been found in the lungs of neutropenic mice with invasive aspergillosis, while neutralizing MCP-1 resulted in two-fold greater mortality and greater than threefold increase in fungal pathogen burden in the lungs[228, 231]. Neutralization of MCP-1 resulted in reduced recruitment of Natural Killer (NK) cells to the lungs at early time points, but did not affect the number of other leukocyte effector cells in the lungs. These data establish MCP-1-mediated recruitment of NK cells to the lungs as a critical early host defense mechanism in invasive aspergillosis. In studies on mice with unilateral IPA, treatment with Amphotericin B results in decreased fungal load and decreased levels of MCP-1 in the infected lung[229]. MCP-1 is also a protective cytokine expressed in murine endotoxemia. It is thought to function by shifting the balance in favor of anti-inflammatory cytokine expression in endotoxin-challenged animals[232].

Regulated on Activation Normal T-Cell Expressed and Secreted (RANTES) was found by Standiford[237] to be expressed in vivo in response to anaerobic gram-negative endotoxemia. Intrapulmonary levels increased in a time-dependent manner, but blood levels did not.

IL-8 is thought to act predominantly on neutrophils. Agusti[238] found significantly higher levels of IL-8 in immunocompromised patients with proven pulmonary infection, compared to those

with pulmonary infiltrates without pathogens isolated. In other studies, a significant increase in serum IL-8 has been found in blood-culture positive patients, compared with a culture negative group.

In summary, the chemokine profile of severely immunocompromised patients, compared with immunocompetent patients may be different. If within this, sub-profiles of those patients with a variety of pulmonary infections could be defined, this may enable a more rapid and sensitive diagnosis and complement existing diagnostic techniques with negligible added risk or morbidity.

3. AIM

Our aim was to assess difference in yield and safety of early FB when compared to conventional management in severely immunocompromised febrile neutropenic haematology patients, and whether this led to improved outcomes. We hypothesised that proceeding to FB as early as possible after developing febrile neutropenia would improve treatment outcomes by improving yield and reducing complications. As the only prospective, randomized controlled trial involving flexible bronchoscopy as a diagnostic tool in this population, we aim to further define the role of FB with reference to optimal timing of the procedure and its impact on diagnostic yield, future management and complication rate. We also aim to analyse the impact of proven infection on the cytokine profile of immunocompromised patients.

4 METHODOLOGY

Background and setting

The Royal Adelaide Hospital Haematology Unit is the South Australian State Centre for adult allogeneic and unrelated bone marrow transplantation. The unit sees approximately 50 new referrals with Acute Leukaemia per year, with approximately 50 bone marrow transplants over the same period. All patients with febrile neutropenia are treated as inpatients.

Patients

This study was a prospective, randomized trial approved by the Royal Adelaide Hospital/ Institute of Medical and Veterinary Science Human Ethics Committees

Our study population was limited to 3 subgroups containing patients with more severe and prolonged neutropenia, as these groups have most serious consequences if their source of infection is not found and treated as soon as possible. Patients who fall into this category are: those with acute leukemia (either due to disease progression or treatment with induction or consolidation chemotherapy), those on Fludarabine and Mabthera for treatment of chronic lymphocytic leukaemia, or those patients who have received allogeneic bone marrow transplantation. In many other Haematological conditions, such as myelodysplasia or autologous stem cell transplant, patients become febrile and neutropenic during the course of disease or treatment, but the duration is short, and patients usually recover with broad spectrum antibiotics, even if the source of infection or causative organism is never found. They are often also on less nephro- or hepato-toxic medications than our target population, and do not have as large a risk from unnecessary use of antimicrobial agents.

SELECTION AND EXCLUSION CRITERIA

Inclusion criteria: (all of 1,2,3 and 4 required)

1. **Any one of:**
- Acute leukaemia
- Chronic lymphocytic leukaemia being treated with Fludarabine/ Mabthera
- Allogeneic bone marrow transplantation
2. **Fever (Temperature > 38 degrees)**
3. **Neutropenia (neutrophils < 500 x 10^6/L)**
4. **No exclusion criteria**

Exclusion criteria
- Refusal or inability to provide informed consent to study entry
- Categorical refusal to consent for bronchoscopy under any circumstance
- Obvious non-respiratory cause for fever

Patients were identified from the bone marrow transplant database or the hospital admission database, and recruited by Haematology and Respiratory medical and nursing staff. Those potentially meeting the selection criteria were recruited for the study at the point of diagnosis of disease, or prior to commencing treatment (Eg chemotherapy or bone marrow transplantation). The study was explained in detail to the patient and family in an outpatient or consultative manner, to enable full understanding of the trial. Written information was also provided.

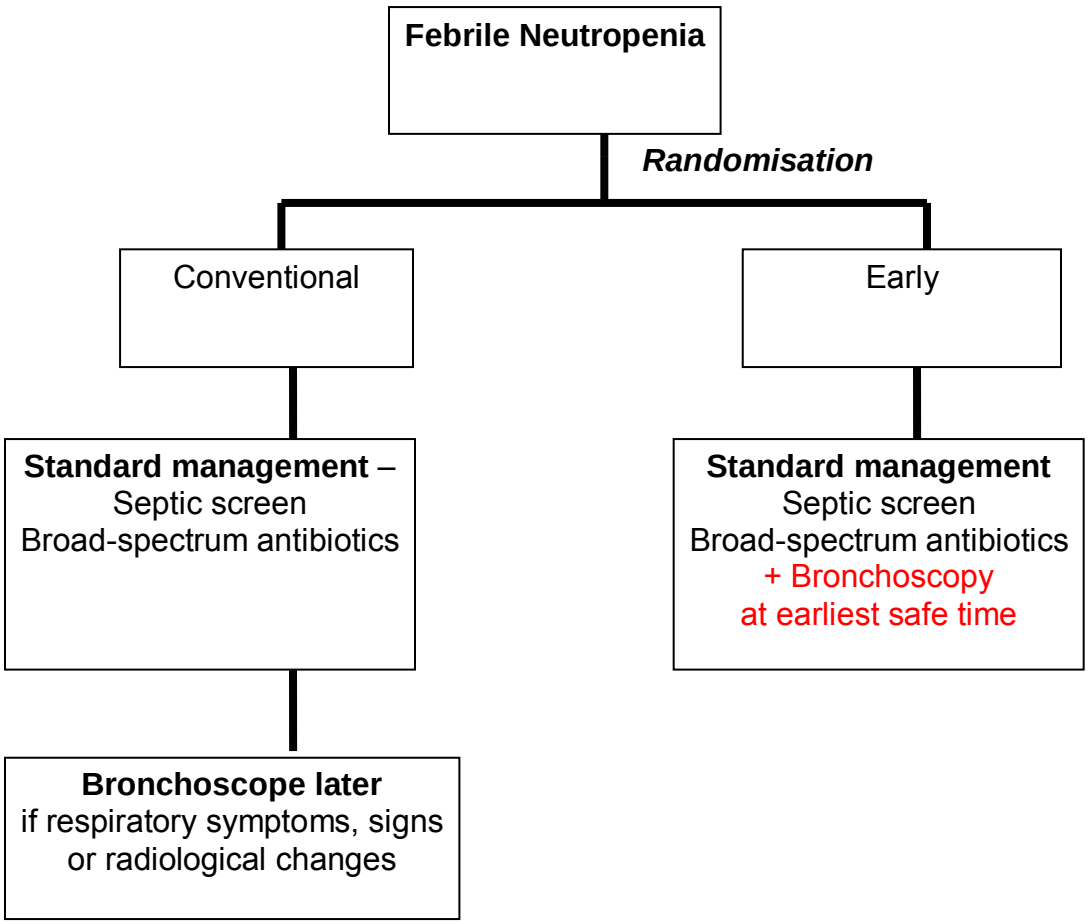
If informed consent was obtained, random allocation software was used to assign patients a group (see below) at the time of development of febrile neutropenia, providing no exclusion criteria had developed.

Conventional Management
The timing of bronchoscopy, if applicable was at the request of the treating Haematologist in consultation with the Respiratory Physician, usually based on respiratory symptoms, signs or radiological changes, or failure to respond to broad-spectrum anti-microbials. The treating Physicians were aware of the study.

Early Bronchoscopy
Early bronchoscopy with Bronchoalveolar lavage (BAL), washings and brushings performed as part of the septic screen, at the earliest safe time, and always within twelve hours of first documented febrile neutropenia. Procedural safety referred to performance of bronchoscopy in the routine procedure suite, or the Intensive Care Unit if the patient was ventilated, with experienced bronchoscopists and support staff.

As part of the protocol, all patients were to have a Chest X Ray, blood, sputum and urine microscopy and cultures, and naso-pharyngeal aspirate, as a standard septic screen. Blood cultures were taken from a peripheral vein and central venous catheter if applicable. Cultures of other body sites were performed as deemed necessary based on clinical findings.

Randomisation:



When febrile neutropenia occurred more than once during the study period, this was included as a separate episode provided the following criteria were met, to ensure resolution of previous febrile neutropenic episode –

- At least two weeks without fever,
- Recovery of neutrophils to at least 2.0 x 10^9 in interim
- Resolution of pulmonary infiltrates and respiratory symptoms/ signs (if previously present),
- No longer on antimicrobial treatment.

These subjects were re-randomised.

Bronchoscopic technique

Bronchoscopic technique was the same for those patients in the early and conventional groups. All bronchoscopies were performed by the primary investigator or a Senior Respiratory Physician with specialist procedural training, using Olympus bronchoscopes. The patient or surrogate signed informed consent prior to the procedure, which was performed in either a dedicated Thoracic Procedure Suite (spontaneously breathing patient) or the Intensive Care Unit (mechanically ventilated patient). Continuous pulse oximetry and heart rate monitor were applied for the duration of the procedure. Blood pressure was measured prior to and immediately post procedure. All bronchoscopies included washings, broncho-alveolar lavage and brushings performed according to a standardized protocol. Oxygen was given via nasal specs or face mask during the procedure to maintain SaO2 above 88%. Unless already intubated at the time of bronchoscopy, all patients were sedated with Fentanyl and Midazolam, titrated according to comfort, level of sedation and oxygen saturation.

The bronchoscope was passed trans-nasally or trans-orally into the trachea after application of local anaestheia. In patients receiving mechanical ventilation, the bronchoscope was passed directly into the trachea through an endotracheal tube by using a connector providing an airtight seal.

After routine inspection of the transbronchial tree to the subsegmental level, general bronchial washings were performed by applying suction via the bronchoscope. Normal saline was instilled in 10ml aliquots until 5ml fluid was recovered. Following this, the bronchoscope was wedged into a subsegmental bronchus either in the left lingular lobe or in the area with most marked radiological abnormality or excess secretions seen under direct vision if applicable. These areas were chosen to maximize yield. BAL was performed according to a standard technique, using 3 x 50ml aliquots of normal saline and low suction after each bolus. The final 10ml recovered was immediately frozen at -70 degrees Celsius for cytokine analysis. Aliquots of lavage fluid were then submitted for microbiology testing. Protected specimen brushing was performed from the right middle lobe. When performed, transbronchial biopsies were guided by using single-plane fluoroscopic control from the area of maximum radiological abnormality.

Although ethics approval was granted for transbronchial biopsies in all study patients, we elected to only perform this in cases with focal radiological abnormality, or suspicion of a non-infectious cause of disease.

Microbiological methods

Specimens were processed urgently by a single microbiological laboratory by senior staff using standardized techniques according to international guidelines, designated as "immunocompromised protocol". Washings and BAL fluid was sent for examination as follows:

Smears and culture prepared for:

- Gram stain and culture for aerobic and anaerobic bacteria
- Zeil-Neilson stain for acid-fast bacilli and culture in MGIT mycobacteriology medium.
- Fungal wet mount and culture as documented below
- Pneumocystis Carinii - Biorad Monofluo™ immunofluorescence assay
- Spare

Bacteriology (sputum panel)
- Chocolate agar
- Blood agar and optochin
- Anaerobic plane (for BAL)
- Glucose cooked-meat (for lung tissue)

Specimens were incubated at 37 degrees in 5% CO2. Anaerobic plates incubated at 37%. All media was reincubated for 7 days, and checked daily for results.

All routine culture material was supplied by OXOID Australia.

Legionella panel:
- Blood agar
- Charcoal yeast extract (CYE) agar
- CYE + Vancomycin, Polymyxin, Pimafucin

Mycology panel
- Blood agar at 35 degrees
- Sabouroud agar
- Sabouroud agar with antibiotics
- Sabouroud broth with antibiotics

Cultures were incubated at 25 degrees in air
KOH Calcofluor stained wet preparation

Virology
- PCR for CMV and other herpes viruses
- Culture for respiratory viruses

Chemokine analysis

Control samples
These were prepared simultaneously with the patient samples and were included with every analysis. Controls included BAL from adults in which the possibility of infection had been excluded. Following bronchoscopy, BAL samples were transported immediately to the laboratory for processing, and stored at -70 degrees celcius until analysis.

Chemokines IP-10, MCP-1, MIG, RANTES and IL-8 were quantified simultaneously using a human Chemokine cytometric bead array (CBA) kit. The CBA kits and CBA software were provided by BD Pharmingen. These assay kits provided a mixture of six microbead populations with distinct fluorescent intensities (FL-3) and were pre-coated with capture antibodies specific for each cytokine. 50μL of BAL fluid or the provided standard cytokines were added to the premixed microbeads in Falcon tubes (BD). After the addition of 50μL of a mixture of conjugated antibodies against the chemokines, the mixture was incubated for 3 hours in the dark at room temperature. This mixture was washed and centrifuged at 500***g*** for 5 minutes, and the pellet re-suspended in 300ml of wash buffer. The FACSCalibur flow cytometer (BD Pharmingen) was calibrated with set-up beads and 3000 events were acquired for each sample.

Individual cytokine concentrations were indicated by their fluorescent intensities (Fl-2) and were computed using the standard reference curve of CELLQUEST and CBA software (BD Pharmingen).

CBA inter- and intra-assay performance
To address intra-assay performance of the CBA chemokine assay, 10 replicate samples of three different levels of the chemokine standards were tested in a single assay. Inter-assay reproducibility was assessed by using two replicate samples of three different levels of the human standards in four separate experiments.

Statistical analysis
Group comparisons were made using the students t-test.

Reference ranges
„Normal" ranges for BAL cytokine levels were established using BAL samples taken from specimens received from known uninfected subjects with no history of haematological disease.

Outcome measures
Major outcome measures were:

- Procedural yield overall (see below)
- Safety of bronchoscopy as measured by complication rate (pneumothorax, minor and major bleeding, respiratory failure or death) and more sensitive parameters including oxygen saturation and requirements pre and post-bronchoscopy, platelet levels pre and post-transfusion
- Impact on clinical management

Yield was established as a positive result with an organism considered responsible for the patient"s clinical presentation. Organisms which were unlikely to cause nosocomial pneumonia (such as coagulase-negative staphylococci) were excluded following the criteria established by the American Thoracic Society[61]. BAL was considered positive for bacterial pneumonia when an organism was isolated by culture, for Aspergillosis when hyphae were identified on smear or cultures were positive, and for virus when rapid antigen was detected or cytopathic effect was observed.

For those with a positive result, we analysed whether this was also detected by less invasive means, and whether this led to a change in management or increased survival.

We also collected data pertaining to alternative sources of yield for respiratory infection, predictors of yield and other sources of infection in febrile neutropenia.

A change in patient management was defined as the withdrawal or addition of a pharmacologic treatment due to either positive or negative microbiological results obtained from bronchoscopy during the relevant episode of febrile neutropenia. The impact of FB on treatment was evaluated from review of all progress notes and physician orders following the procedure, and direct discussion with the treating clinician where clarification was required.

We compared the following: days febrile, total days of antibiotic treatment, days in hospital since onset of febrile neutropenia and survival across the conventional and early groups, as well as survival in those with a diagnosis versus those without.

Statistical Analysis

The Chi-square test was used to analyze rates. Student's t-test was used to compare groups. A log-rank test (Cox-Mantel) was used to analyse survival. A p-value of < 0.05 was considered to indicate statistical significance.

5. RESULTS

- 5.1 Patient characteristics
- 5.2 Bronchoscopic parameters
- 5.3 Safety of bronchoscopy
- 5.4 Yield
- 5.5 Chemokine analysis
- 5.6 Other clinical outcome measures
- 5.7 Survival

5.1 Patient characteristics

Data was collected prospectively between March 2005 and December 2006. There were 33 episodes of febrile neutropenia in 31 severely immunocompromised patients. Two patients were not randomised due to having obvious non-respiratory sources of infection. Therefore, 31 episodes of febrile neutropenia in 29 severely immunocompromised patients were included in the study – 17 were randomised into the conventional group, 14 in the early group (intention to treat). Causes of severe immunosuppression are outlined with baseline patient characteristics in Table 2. The immunosuppressive treatment received was in accordance with the established protocols for chemotherapy or SCT, but may have differed in a particular patient if GvHD was suspected or proven.

Sixty-eight percent of subjects were male, with an overall median age of 52 and 51years in the conventional and early groups respectively. Baseline characteristics were comparable in both groups.

Overall, there were 19 bronchoscopies performed – 12 in the early group and 4 in the conventional group for worsening respiratory clinical status or new changes radiologically. Two patients (1 in each group) were mechanically ventilated at the time of the bronchoscopy. There were 2 procedural refusals in the early group after randomization. Three patients in the early group had repeat bronchoscopies for worsening respiratory clinical status or new changes radiologically. These were analysed with the "conventional" group for yield (see figure), resulting in 7 "conventional bronchoscopies".

All patients received the following routine antibiotic prophylaxis: Nystatin mouthwash, Norfloxacin bowel prophylaxis and a low bacterial diet, Fluconazole, Trimethoprim/ Sulphamethoxazole prophylaxis against PCP, and Famciclovir in patients at risk of Herpes viruses.

After the onset of febrile neutropenia occurred and septic screen was performed, empirical antibiotics were commenced as per protocol: Gentamicin and Timentin were routinely commenced first. If the patient remained febrile and no specific cause was found, Meropenem and then Vancomycin were added.

From 31 episodes of febrile neutropenia, 28 patients had CXR performed as part of septic screen, and 22 had HRCT performed within 2 days prior to or following initial development of fever.

Radiographic reports were available for all patients

	Number of patients	Male: female	Mean age (range)	Underlying disease	Number of episodes
Conventional	17	13:4	52 (29-72)	Acute leukaemia + chemotherapy: 9 Allogeneic PBSCT: 8	20
Early*	14	8:6	51 (29-72)	Acute leukaemia + chemotherapy: 9 Allogeneic PBSCT: 5	12

Table 2: Baseline characteristics of patients
* intention to treat

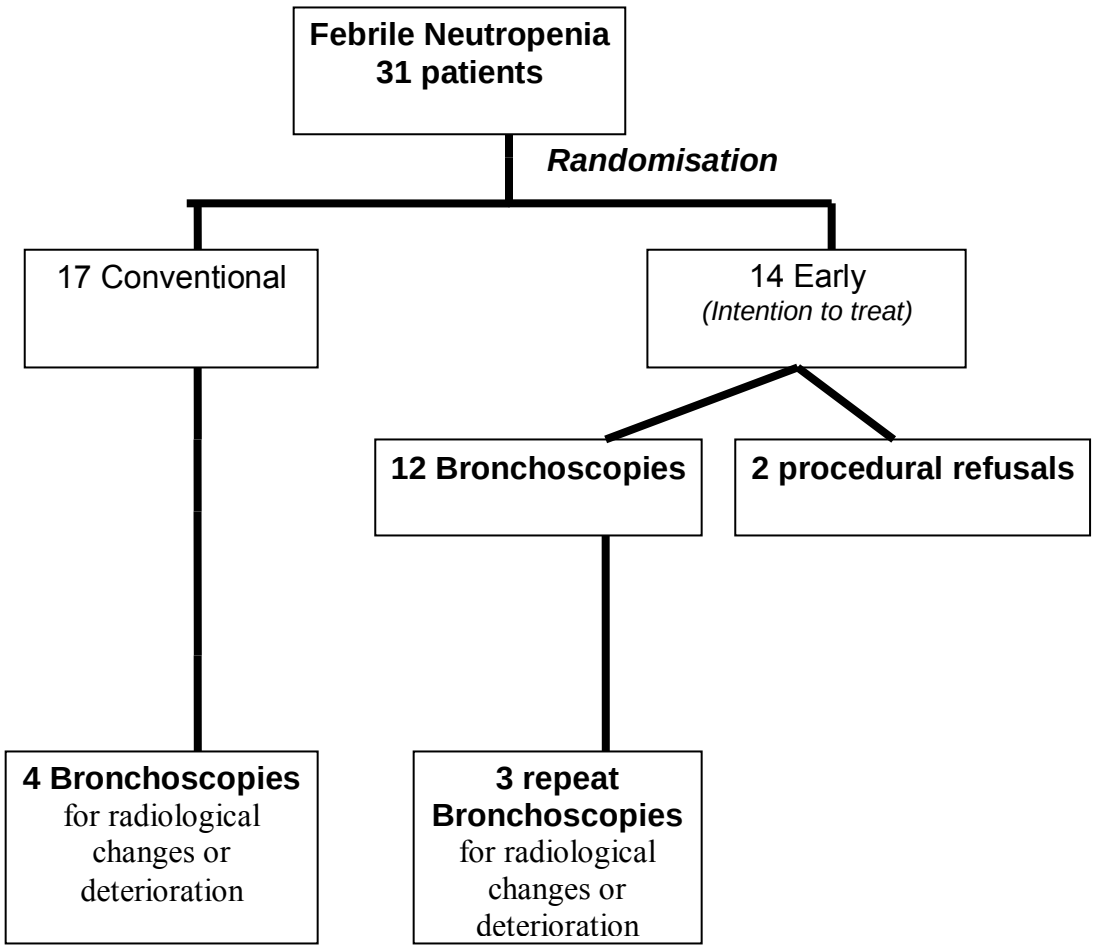


Figure 1: Randomisation and patient numbers

5.2 Bronchoscopic parameters

Median time from development of febrile neutropenia to bronchoscopy was 240 hours in the conventional group, and 10.6 hours in the early group (Figure 2a,b). This equated to significantly higher mean total doses of antibiotics prior to bronchoscopy in the conventional group (Table 5): Gentamicin 10 doses, Timentin 30 doses, Meropenem 6 doses, Vancomycin 6 doses compared with: Gentamicin 1 dose, Timentin 2 doses in the early group. No patients were on anti-fungal agents at the time of bronchoscopy. One patient was being treated with intravenous Aciclovir for oral Herpes lesions. Empirical antibiotics were not delayed while waiting for a safe time to perform early bronchoscopy. Platelet support was given as required to ensure a level of 20 x 10^9 /L prior to bronchoscopy with BAL, or 50 x 10^9 /L if TBB was considered or planned. All patients had bronchial washings, BAL and PSB. Transbronchial biopsies were only performed in 1 patient, for investigation of extensive pulmonary infiltrates on HRCT.

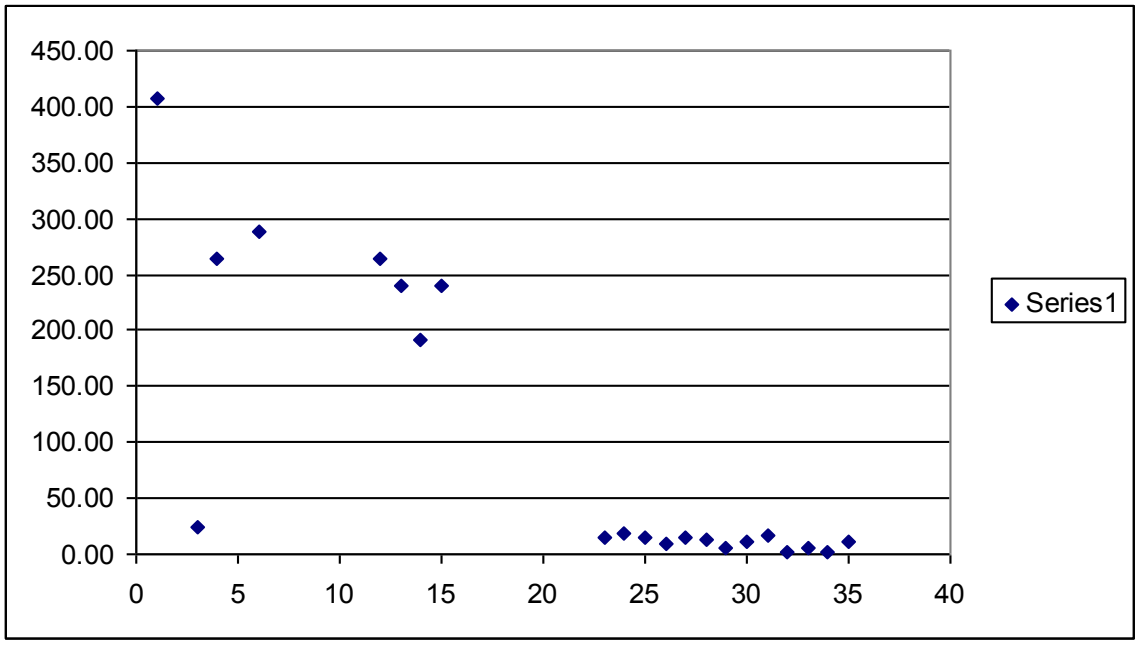


Figure 2a: Dot plot of time to bronchoscopy (hours)

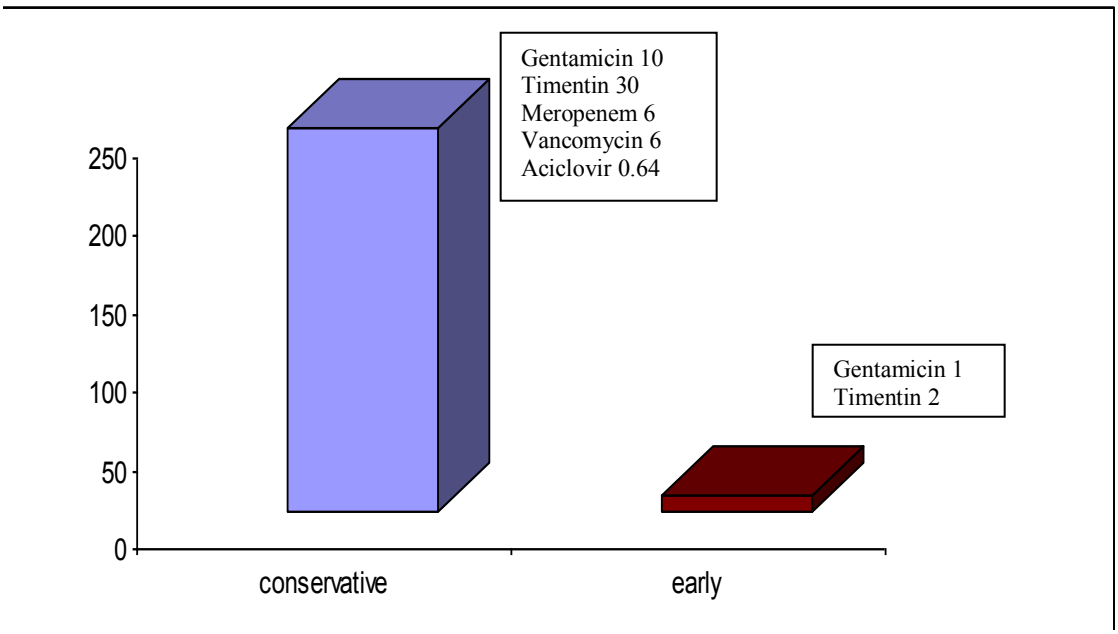


Figure 2b: Mean hours delay until bronchoscopy
The text box shows the mean number of antibiotic doses administered per patient, prior to bronchoscopy

5.3 Safety of bronchoscopy

Comparative data was collected on:

- Oxygen saturation level pre- and post-bronchoscopy,
- Oxygen requirements pre- and post-bronchoscopy
- Platelet levels pre- and post-transfusion

There was a significant difference in oxygen saturation post-bronchoscopy (mean 90.4 vs 96.7, p=0.02) favouring the early bronchoscopy group. However, this was not sustained and did not translate to a difference in need for ventilation or intensive care. There was no significant difference between the groups in any of the other measured parameters.

There was 1 complication in the conventional group: following bronchoscopy at day 7 post-febrile neutropenia, the patient developed severe respiratory failure requiring intubation. No complications related to bronchoscopies in the early bronchoscopy group. There were no procedure-related deaths.

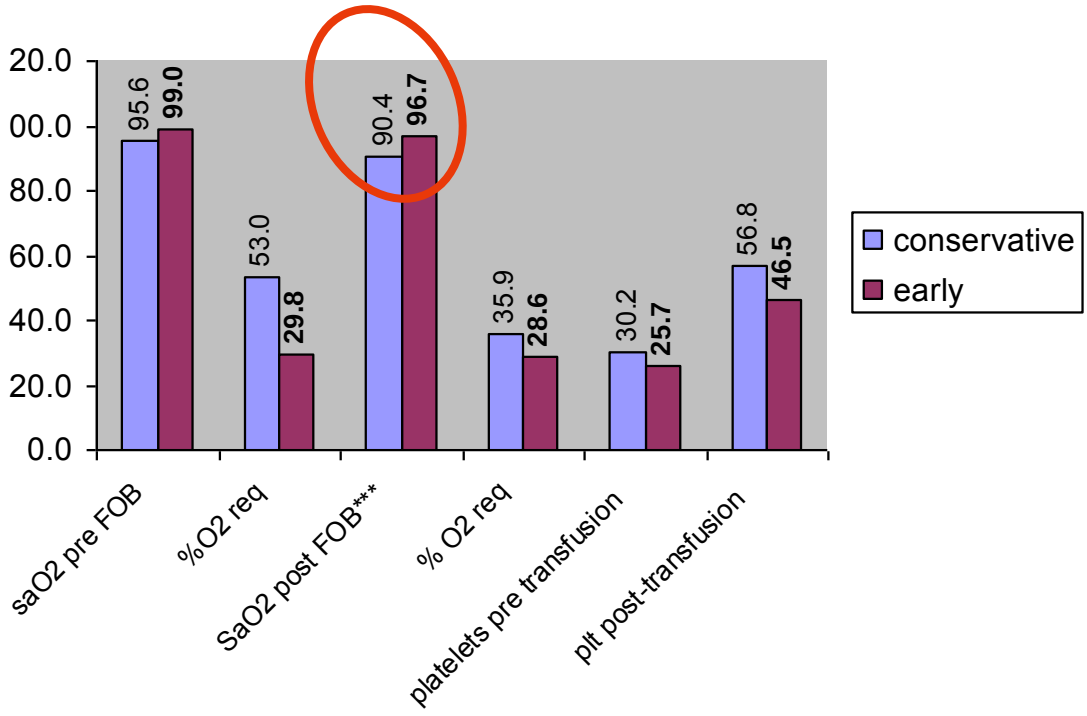


Figure 3: safety of bronchoscopy

5.4 Yield

Seven bronchoscopies out of 19 yielded a positive result (36.8%), with 8 pathogens found. The pathogens isolated were: Herpes Simplex Virus (n=1) and Stenotrophomonas Maltophilia (n=1) in the conventional group, and Aspergillus Spp (n=4), Penicillium (n=1) and Parainfluenza (n=1) in the early bronchoscopy group. There were 2 positive results from the 7 conventional bronchoscopies, and 5 positive results from the 12 early bronchoscopies. These differences were not clinically significant in overall yield. In the conventional group, growth of Stenotrophomonas Maltophilia led to the commencement of additional antibiotics in 1 patient (32%)

There was a significantly higher fungal growth from broncho-alveolar lavage or washings in the early bronchoscopy group (Five fungal results, vs no fungal organisms in conventional bronchoscopy results, $p< 0.05$). However, despite the relatively high fungal yield in the early group, this did not lead to an impact on immediate clinical management during this febrile neutropenic episode, as the mean time delay until fungal growth occurred to a level where standard microbiological techniques detected the results, was 8 days despite being processed urgently, and all patients were already on anti-fungal agents. In the acute febrile neutropenic episode, negative results did not have sufficient predictive value to confidently exclude fungal infection if there was high clinical suspicion.
In the patient in whom HSV was yielded, treatment had already commenced prior to bronchoscopy, for oral HSV lesions. Therefore, this result did not affect subsequent management.

In our patient group, clinical features and chest xray features did not predict the chances of a positive result, and in those with organisms isolated, there was no significant relationship between the type of bacterial or fungal isolate and the radiographic pattern of infiltrates.

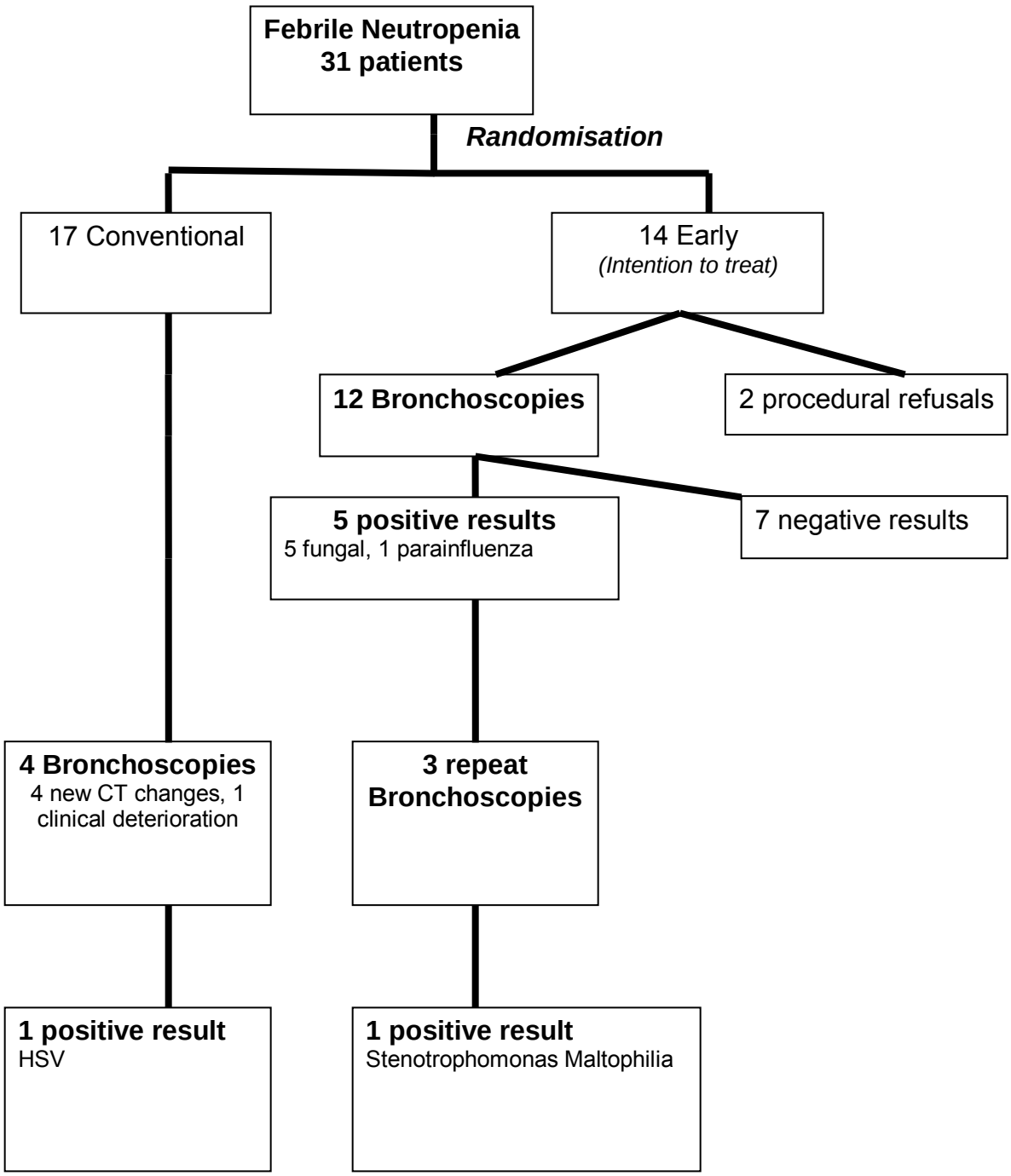


Figure 4: Bronchoscopies and yield

Patient	Randomisation	Time until FB (hours)	Organisms isolated	Specimen type	Yield from other source	Clinical/ Radiological change
Patient 1	Conventional	264	Herpes Simplex Virus	BAL	HSV in mouth	Increasing respiratory distress and hypoxia. Intubated. HRCT: bilateral severe central opacities and R) effusion on CT chest 25th Sept2006.
Patient 2	Conventional	192	*Stenotrophomonas Maltophilia*	BAL	Nil	HRCT: New bilateral infiltrates
Patient 3	Early	15	*Penicillium*	Bronchial Washings	*Strep. Oralis* from blood cultures	HRCT: widespread ground-glass opacity and focal consolidation
Patient 4	Early	18	*Aspergillus Glaucus*	BAL		CXR: normal
Patient 5	Early	15	*Aspergillus non-fumigatus*	BAL		CXR: normal
Patient 6	Early	5	*Aspergillus Fumigatus* *Parainfluenza*	BAL		CXR: normal
Patient 7	Early	2.5	*Aspergillus Fumigatus*	Washings		CXR: minor right basal opacity

Table 3: Positive results obtained by FB

Yield from non-invasive sources:

Nasopharyngeal aspirate was performed in 12 patients in the conventional group and 6 patients in the early group. Positive results were seen as 3 pathogens were isolated in 2 conventional patients: RSV (n=2) and parainfluenza 3 (n=1). None of these were grown during bronchoscopy.

Sputum cultures were positive in 1 patient in the conventional group - Rhinovirus was isolated.

Blood cultures were performed in all patients. Nine organisms were isolated from blood cultures in 8 patients; 6 patients in the conventional group (Streptococcus Mitis, n=2; Enterococcus Faecium, n=2, Streptococcus Salivarius, n=1, Fusobacterium nucleatum, n=1; Vancomycin-resistant Enterococcus, n=1; Staphylococcus Epidermidis, n=1; Corynebac Jeikeium, n=1), and 2 in the early group Streptococcus Oralis, n=2).

There was one positive culture sinus wash-out performed for specific symptoms: Rhizopus oryzae

Overall, 9 subjects (29%) had a non-pulmonary cause of febrile neutropenia. Only 1 patient who had positive bronchoscopy results also had a positive yield from an alternative source: Streptococcus Oralis was isolated from blood cultures.

There was no significant difference between alternative sources of infection in the early vs late groups.

Specimen type	**Number of specimens collected**			**Positive specimens**	**Percentage yield per specimen**
	Conventional	***Early***	***Total***		
Washings	7	12	19	3	15.8
Brushings	7	12	19	1	5.3
BAL	7	12	19	4	21.1
TBBx	1	0	1	0	0
Sputum (non-bronchoscopic)	12	5	17	1	5.9
Naso-pharyngeal aspirate	13	6	19	2	10.5
Blood cultures	19	12	31	8	25.8
Urine	18	11	29	0	0

Table 4: Overall yield from all sources

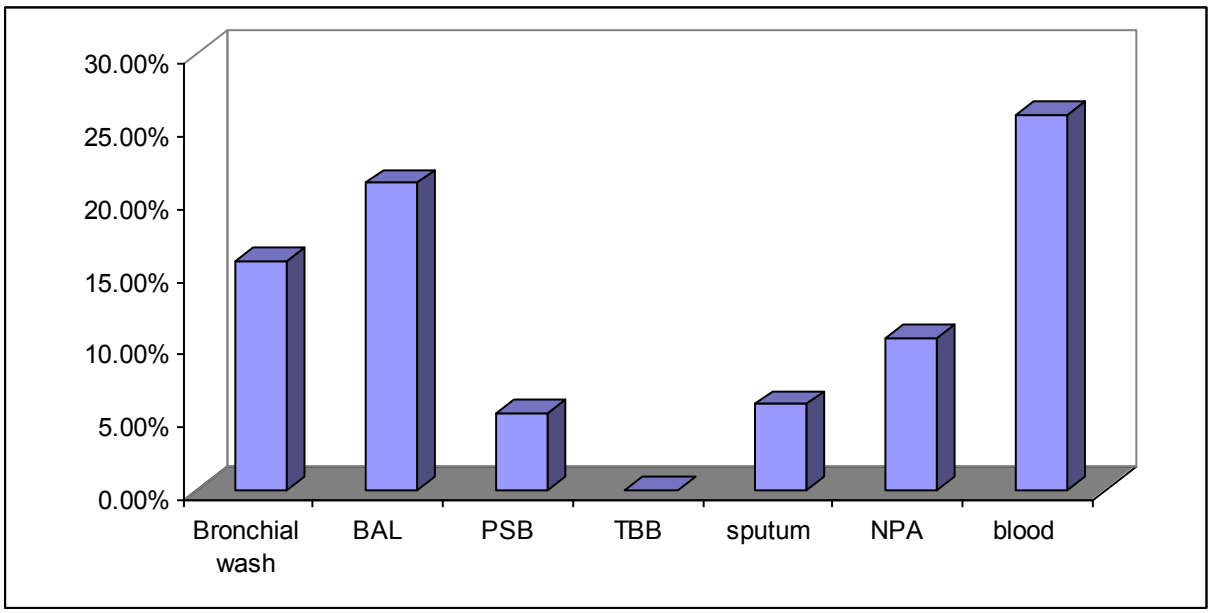


Figure 5: Percentage yield from each diagnostic method

5.5 Antibiotic use

Data was collected on the total days used of each antibiotic in each individual patient.

Antibiotics used included (in order of frequency of use) Ticarcillin and Clavulanate, Gentamicin, Meropenem, Vancomycin, Amphoterecin (or equivalent liposomal formulation), Voriconazole, Piperacillin and Tazobactam, Aciclovir, Pristinamycin, Moxifloxacin, Co-trimoxazole and Amikacin. The mean days of total antibiotic use per patient was not significantly different in each group (15.6 days in the conventional group vs 15.1 days in the early group). There was a trend towards more use of antifungals overall in the early bronchoscopy group, and less use of the broader-spectrum antibiotics.

Antibiotic	Conventional	early
Timentin	3	2.6
Gentamicin	2.6	2.6
Meropenem	3	4.25
Vancomycin	4.2	2.75
Amphoterecin	1.2	2.4
Voriconazole	0.8	1.58
Tazocin	0.29	0
Amikacin	0	0.83
Pristinamycin	0.18	0
Moxifloxacin	0.06	0
Trimethoprim/ Sulfamethoxazole	0	0.083
Aciclovir	0.18	1.25
Total days of antibiotics	15.6	15.1

Table 5: Average days treatment with each antibiotic per patient (total)

5.6 Chemokine analysis

Full results are tabled.
Levels of IP-10, MCP-1, MIG, RANTES and IL-8 were measured in BAL fluid in 4 conventional and 7 early bronchoscopies, and compared with 13 normal controls.

Comparisons were made between conventional and early groups, and between the group of patients with pathogens isolated, versus those without.

A subgroup of patients with fungal pathogens isolated was compared with those without.
Subgroups were compared with normal controls.

Both the IP-10 and MIG were significantly *lower* in those patients who had a fungal pathogen isolated, compared with those study patients who did not (175.17 vs 1157.8, p=0.03, 30.33 vs 247.8, p=0.03 respectively).

IP-10 levels measured in BAL fluid in the conventional group were significantly higher than the early bronchoscopy group (1253.0 vs 261.14, $p = 0.035$). There was also a non-significant trend towards higher RANTES in the conventional group (749.75 vs 28.57 in early group, p=0.06).

The study population had higher MCP-1 (734 vs 2.83, p=0.006) and IL-8 levels (606.9 vs 14.25, p=0.00655) than normal controls

When those cases with fungal infection were compared to normal controls, mean MCP-1, RANTES and IL-8 levels were significantly higher than in normal controls (844.0 vs 2.83, p=0.007; 17.5 vs 2.1, p=0.03; 156.0 vs 14.25, p=0.004).

Patient group	IP-10	MCP-1	MIG	RANTES	IL-8
Conventional (n=4) (mean±SD)	1253± 1029	717± 786	179± 162	750± 941	1014±1 051
Early (n=7) (mean±SD)	261± 334	744± 978	101± 187	29± 37	375± 353
P value: conventional vs early	*0.04*	0.96	0.50	0.06	0.16
Positive bronchoscopy (n=6): (mean±SD)	585± 973	877± 1001	94± 151	175± 380	771± 914
Negative bronchoscopy: (mean±SD)	666± 620	562± 760	172± 208	430± 878	410± 383
P value: positive vs negative bronchoscopy	0.88	0.58	0.49	0.53	0.43
Fungal pathogen isolated (n=6): (mean±SD)	175± 268	845± 1031	30± 22	18± 24	356± 383
No fungal pathogen isolated: (mean±SD)	1158± 908	601± 728	248± 208	619± 866	908± 941
P value: fungal pathogen vs no fungal pathogen	*0.03*	0.67	*0.03*	0.12	0.22
Control group: (n=13) (mean±SD)	764± 754	2.8± 7	265± 331	2.1± 1.5	14± 10
Study population: (mean±SD)	622± 763	734± 844	129± 183	291± 659	607± 738
p-value: early vs control	0.12	0.012	0.26	*0.016*	*0.001*
fungal infection v control	0.09	*0.007*	0.12	*0.03*	*0.004*
all pts v control	0.66	*0.006*	0.25	0.112	*0.007*

Table 6: Chemokine analysis: mean and *p* value.

Positive bronchoscopy: pathogen isolated. Negative bronchoscopy: no pathogen isolated. Fungal infection: fungal pathogen isolated from bronchoscopy. Control population: subjects with no haematological condition, immunocompromise or infection. Study population: all patients in trial, excluding normal controls.

5.7 Other clinical outcome measures

Renal function
The serum creatinine was recorded in all surviving patients at 30 days after the development of febrile neutropenia, aiming to detect any impairment in renal function as a result of cumulative or additive antibiotic doses. The mean creatinine in the conventional group was 114.2µmol/L, compared with 96.3 µmol/L in the early group. This was not significantly different

Total days spent febrile
Patients in the conventional group spent a mean of 6 days febrile (temperature recorded as greater than 38 degrees at any time within the 24 hour period), compared with 8 days in the early group. This was not statistically significant.

Total days spent hospitalized after development of febrile neutropenia
Patients in the conventional group spent a mean of 15.2 days in hospital compared to 24.2 days for the early group, but this did not reach statistical significance (p = 0.1)

Number of bronchoscopies
Three patients who underwent early bronchoscopies still required repeat FB at a later stage after development of worsening pulmonary infiltrates.

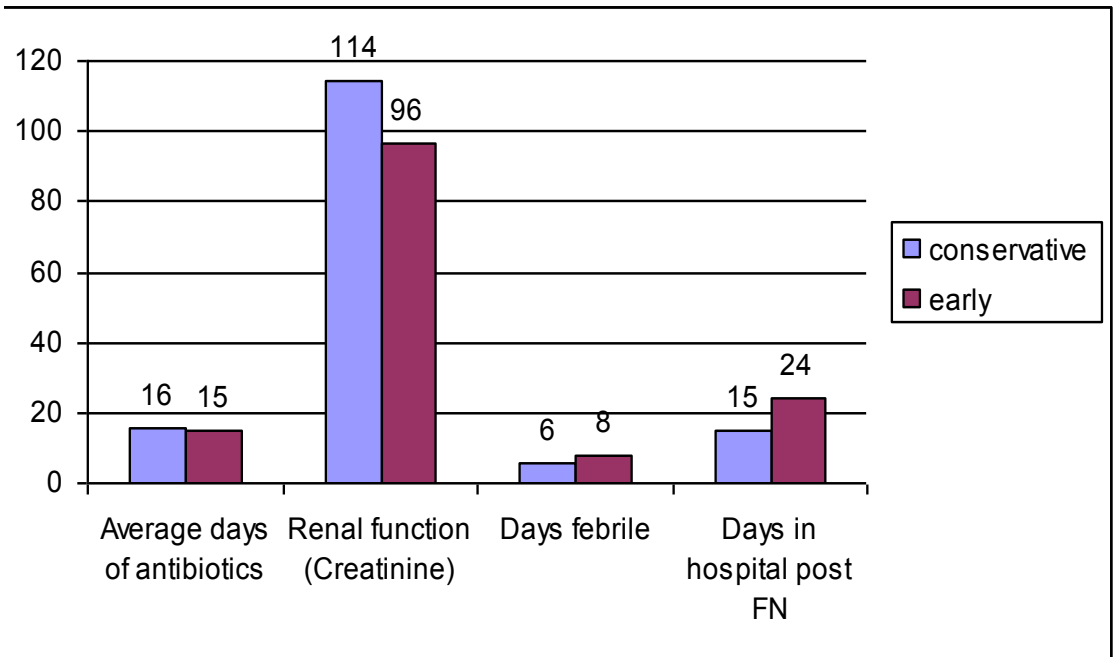


Figure 6: other clinical outcome measures

5.8 Survival

All-cause mortality was similar in both groups, and no deaths were procedure-related. In the conventional group, 1 year survival from time of febrile neutropenia was 8/17 (47%). In the early group, 1 year survival was 8/14 (57%). This was not statistically significant.

The survival at 22 months from commencement of the study was 55% in the conventional group and 32% in the early bronchoscopy group. This was not a statistically significant difference (p = 0.6).

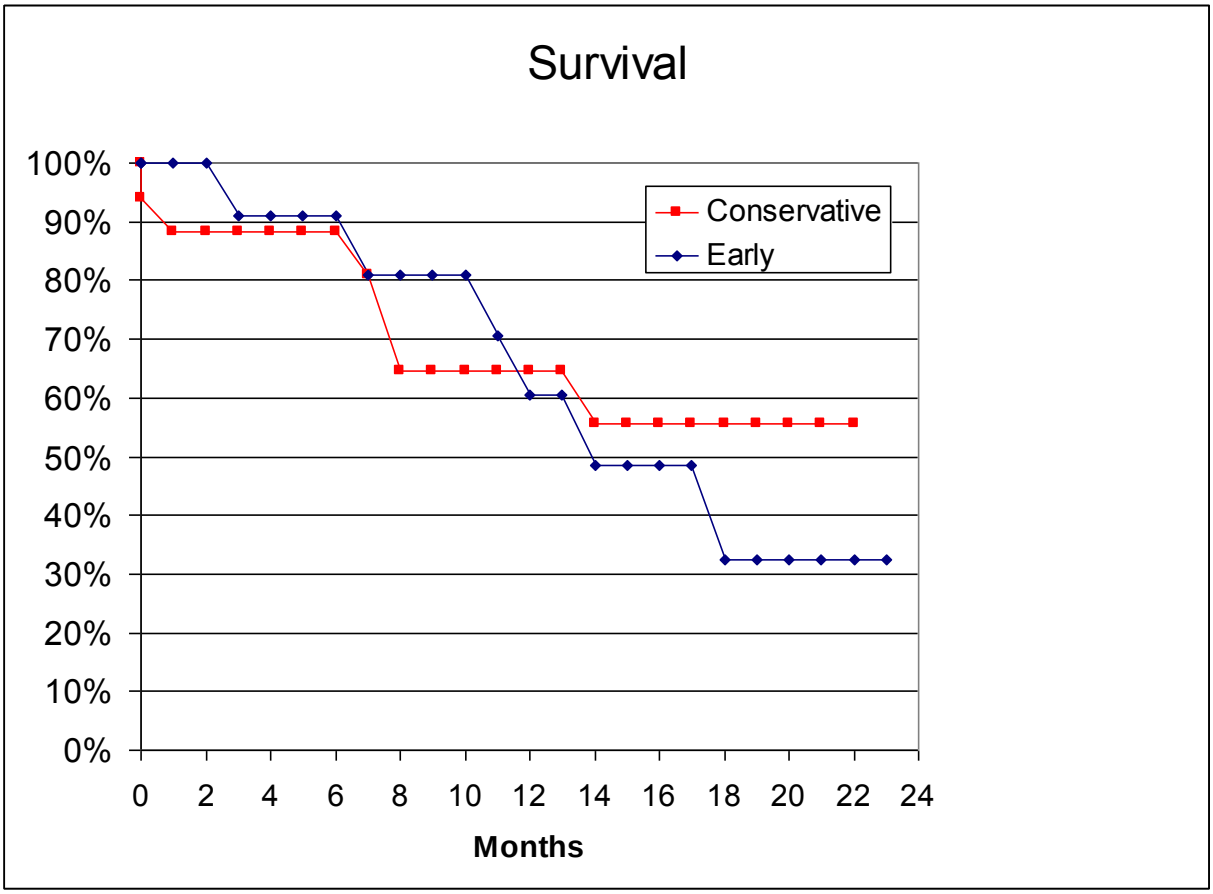


Figure 7: Survival

6. **DISCUSSION**

In this study, we have reappraised prospectively the current diagnostic usefulness and clinical impact of flexible bronchoscopy for the early diagnosis of pulmonary causes of febrile neutropenia in severely immunocompromised patients, as compared to standard non-invasive diagnostic techniques. Our study differs from those in the literature targeting similar populations, as our patients did not require pulmonary infiltrates for inclusion. It was aimed to select more specifically for infective causes of respiratory disease, due to the presence of both fever and neutropenia rather than pulmonary infiltrates in severely immunocompromised patients. Our study, despite its small numbers, is one of the largest prospective, randomized trials investigating the role and optimal timing of flexible bronchoscopy in severely immunocompromised febrile neutropenic patients prior to the development of pulmonary infiltrates, and adds important insights to the field, in the use of this diagnostic tool.

Our bronchoscopic yield was lower than some previous retrospective studies. There are likely to be several reasons for this. Patient population, treatment protocols and patient selection criteria vary greatly between studies and influence the diagnostic yield of FB and patient survival. Most studies selected patients who had distinct abnormalities on CT chest. This could potentially increase yield, although we did not find this in our study as our aim was to bronchoscope prior to CT changes in order to identify patients with pulmonary disease earlier. In studying patients with pulmonary infiltrates, Peikert et al[175] found that blood cultures yielded organisms responsible for febrile neutropenia in only 2 cases (5.7%), while the New Zealand study on febrile neutropenic patients unselected for pulmonary infiltrates reported 27% positive blood cultures, which was similar to our findings of almost one-third of patients having positive blood cultures. We found that there was very little overlap in terms of dual causes of febrile neutropenia including a respiratory source, which highlights the fact that if early bronchoscopy became a routine part of the septic screen, almost one-third of our population would have an unnecessary bronchoscopy, as their actual cause of febrile neutropenia would be bacteraemia rather than pulmonary infection. Furthermore, in those who had early bronchoscopies, 3 patients proceeded to repeat bronchoscopies as pulmonary infiltrates developed and worsened later.

In other trials designed to investigate bronchoscopy prior to pulmonary infiltrates[54], the yield was also lower. This may suggest that our bronchoscopic yield would be higher if only those patients with pulmonary infiltrates were included. In addition, although we tried to bronchoscope patients as early as possible, most patients in the early group had received 1 dose of empirical antibiotics, as it was not ethically possible to delay antibiotic treatment while awaiting bronchoscopy, nor to perform bronchoscopy without appropriate fasting by the patient, or adequate staffing of the procedure suite. The lack of findings of bacterial pathogens may be related to sensitivity of bacteria to current antibiotic regimens, or prevention of bacterial culture in vitro. Although most other studies involved patients who had received antibiotics prior to their procedures, it is difficult to ascertain subtle differences in antibiotic sensitivities across institutions. In Table 6, we aimed to standardize the reported yield as much as possible to avoid the issue of different denominators being used to compare yield – for example, some institutions report bronchoscopic yield as a numerator against total diagnoses made, which overestimates yield, since a large percentage of patients have no diagnosis made by any technique, including post-mortem examination. Another important issue is the low incidence of PCP and CMV disease in our population since the advent of routine prophylaxis. PCP and

CMV are more easily detected bronchoscopically than many other organisms, and earlier studies involving populations with high rates of these infections had higher overall yields. The final reason for our lower yield may be techniques in performing bronchoscopy and analyzing specimens. With more sensitive techniques, our yield may improve further. This highlights the importance of institution-specific protocol development, based on wider evidence. Despite our lower yield, in view of the negative predictive value of bronchoscopy for certain pathogens, it is also important to consider that a non-diagnostic or negative result still has the capacity to alter clinical management.

Unfortunately, although our yield for fungal disease in the early group was far higher than the conventional group, this did not translate to a difference in clinical management, mostly due to the delay in obtaining these results with available assays, despite specimens being processed urgently. The early negative predictive value for detecting fungal disease was inadequate to recommend ceasing anti-fungal therapy, but this highlights the need for better microbiological methods, including non-culture methods, for detection of pathological fungal disease. The use of tests such as Galactomannan detection, as described previously, may produce more rapid results, improve diagnostic yield and enable cessation of anti-fungals earlier if negative microbiological results are obtained. The role of Galactomannan in this population remains to be clearly defined. Furthermore, the results of bronchoscopy had the potential to impact on clinical decision making for subsequent courses of chemotherapy.

In this study, early bronchoscopy had no effect on the total antibiotic use per patient. However, the pattern shown was a trend towards more use of antifungals overall in the early bronchoscopy group, and less use of the broader-spectrum antibiotics. This result may partly be explained by the (delayed) higher yield for fungal organisms resulting in more prolonged treatment.

Part of the dilemma in determining optimal timing for FB has been the understandable aim to avoid unnecessary bronchoscopies in patients who may respond to empirical treatment, and the high risk and potentially low yield in the procedure once it is evident that they do require it, resulting in a fairly narrow diagnostic window. This is particularly so in patients with respiratory failure who are on the borderline of needing intubation and mechanical ventilation; bronchoscopy and the sedation used can cause decompensation in an already fragile patient, and potentially markedly worsen prognosis. A French study[194] reported in 2001 that application of a laryngeal mask airway may be a safe and effective alternative for accomplishing FB with BAL in immunosuppressed patients with suspected pneumonia and severe hypoxemia. This group assessed 46 high-risk hypoxemic immunosuppressed patients. The laryngeal mask airway enabled 100% oxygen to be given during the procedure, without mechanical ventilation. Although transient laryngospasm, hypotension requiring plasma expanders and oxygen desaturation occurred, no patient required intubation, and it was noted that the lowest oxygen saturation recorded during the procedure was still significantly higher than the baseline. In this study, BAL had a diagnostic yield of 65% and treatment was modified in 72% of cases. The use of ultra-thin bronchoscopy as described earlier in this paper may also provide further information for less risk.

In our study, for those patients who had a positive bronchoscopic result, the pathogens were not isolated concurrently from non-invasive methods of obtaining sputum, or nasopharyngeal aspirate. Furthermore, within bronchoscopic techniques, there was no overlap in yield from

bronchial washings, BAL or PSB. Therefore, each separate component of the septic screen can be considered complementary.

The complication rate in this study was very low. This may have been partly due to the small number of total bronchoscopies, but does show that with experienced proceduralists, the complication rate of this procedure was not high, and can be considered as generally well tolerated even in a high risk population. The small numbers of bronchoscopic complications also resulted in the power being inadequate to detect a difference in complication rates between the conventional and early patient groups. It is possible that if transbronchial biopsies had been routinely performed, both the yield and the complication rate may have increased. Ethics approval was given for transbronchial biopsies, but upon further consideration and review of the existing literature, it was decided against performing these as routine investigation. This was due to the significant additional procedural risk, the prediction of lack of radiological changes to guide biopsies, and the evidence showing that additional diagnoses made by TBB were mostly non-infectious. In previous studies, any additional information provided by TBB in the severely immunocompromised haematology population appears to be lower than other groups of immunocompromised patients with pulmonary infiltrates. Importantly, with such a low complication rate and accessibility to specimens, it appears reasonable to perform this procedure to exclude certain forms of disease, especially fungal infection, particularly if more rapidly available and sensitive microbiological testing methods become widely available. It would also be ideal if further information could be gained in patients undergoing bronchoscopy and BAL, by examining their cytokine profile.

In our study, there were significant differences in several chemokine levels between groups of patients. When those with fungal infection were compared with febrile neutropenic patients without fungal infection, IP-10 and MIG were significantly *lower* in the group with fungal infection. IP-10 is specific for activated T-helper-type-1 lymphocytes and has been shown to be induced during SARS and Klebsiella pulmonary infection and to contribute to host defence against both Klebseilla and PCP in a mouse model. Mice with low IP-10 and MIG were permissive to both infections, and conversely, over-expression of IP-10 in the lungs accelerated subsequent PCP clearance. MIG has been elevated in patients with community-acquired pneumonia, but the impact of deficiency of MIG has not been shown as clearly as for IP-10, and there is little previous data on fungal infection. The low levels of both chemokines in those with fungal infection may suggest a subgroup of patients are more prone to such infection, due to lower IP-10 and MIG levels, and low levels may therefore potentially be a marker of increased risk for fungal disease. IP-10 is produced by Th1 T cells in response to IFNγ[239]. Impaired neutrophil activity was observed in susceptible mice with concominant predominant IL-4 production. Measurement of these important regulatory cytokines would have been an important adjunct to this present study in determining the eitiology of reduced IP-10 levels.

When cases with fungal infection were compared with normal controls rather than febrile neutropenic patients, mean MCP-1, RANTES and IL-8 levels were significantly higher in those with fungal infection. It is known that these 3 chemokines are an integral component of defence against bacterial infection, and their role in fungal infection is becoming increasingly understood. Huffnagle reported in 1995 that BAL fluid levels of MCP-1 and the recruitment of inflammatory cells both increased following pulmonary infection with Crytococcus neoformans. Reduced levels of MCP-1 resulted in impaired clearance of the fungus, and in this mouse model, recruitment of neutrophils and B-lymphocytes was also shown to be decreased.

Prior data has established MCP-1-mediated recruitment of NK cells to the lungs as a critical early host defense mechanism in invasive pulmonary aspergillosis. A rapid and marked induction of MCP-1 has previously been found in the lungs of neutropenic mice with invasive aspergillosis, while neutralizing MCP-1 resulted in two-fold greater mortality and greater than threefold increase in fungal pathogen burden in the lungs[228, 231]. Neutralization of MCP-1 resulted in reduced recruitment of NK cells, but not other leukocyte effector cells to the lungs at early time points. In mice with unilateral IPA, treatment with Amphotericin B resulted in decreased fungal load and decreased levels of MCP-1 in the infected lung. There has been no prior firm data regarding RANTES and fungal infection, but IL-8 is thought to act predominantly on neutrophils and has been previously found to increase in immunocompromised patients with pulmonary infection of any type.

MCP-1 and IL-8 were also significantly higher in patients with any pulmonary infection compared with normal controls. However, given the fact that almost all pathogens isolated were fungal, extending these results to other infective causes is not appropriate in this study. Unfortunately, we were not able to examine our data for suggestive chemokine profiles in differentiating between bacterial, viral and fungal causes of infection, for the same reason.

The higher level of IP-10 and trend towards higher RANTES seen in the conventional group compared to the early bronchoscopy group may have reflected an immune response which took some time to develop following the initial insult. For example, since IP 10 is an interferon stimulated gene; it is likely to be higher later in context of a likely infection; this also makes comparisons of levels difficult to interpret.

When all study patients were compared with normal controls, there were significantly higher levels of MCP-1 and IL-8 in the study group. This is likely to be due to the presence of infection and inflammation in the study population, and changes in chemokine profile due to the disrupted immune system. Overall any differences in chemokine levels remain speculative, as our sample size for this part of the study very small, and standard deviations were large. There were a large range of counfounding factors for chemokine changes in this setting, as the immune/ inflammatory responses would be modified by treatment effects and disease itself. In particular, MCP-1, RANTES and IL-8 may reflect any combination of immunosuppression and/or infection, and results must be interpreted with caution.
There was also no control for the degree of neutropenia or exact time post-transplant/ chemotherapy at which FB was performed, aside from early vs conservative, factors which could potentially have a large impact on the chemokine profile. As the yield was mainly fungal pathogens, this may have skewed some of the chemokine profiles. In addition, as samples of BAL fluid samples were taken only once in our study, we cannot be sure whether some patients were able to mount a substantial counter-inflammatory response at an earlier time point (a response that is subsequently downregulated when BAL is performed). However, despite these issues, the chemokine differences in patients with fungal infection are significant, and lends hope to the theory that with a larger sample size for examination, a chemokine profile suggestive of fungal infection could be determined. The analysis of BAL Th1/Th2 cytokines using a Th1/Th2 cytokine CBA may have added further important information regarding alterations in particularly IFNγ and IL-4 levels as possible causes for decreased IP-10 levels noted in patients with fungal infections. These tests would not replace current diagnostic techniques, but would aim to complement and strengthen diagnoses, and further guide treatment with negligible additional risk.

We recognize that a major limitation of this study is the small sample size, and the study was underpowered to detect differences which could lead to early management change. Despite this, we believe that the observations are clinically valid. In addition, this series is one of the larger to study severely immunocompromised patients, and reflects clinical practice and current widely-used diagnostic techniques. Important clinical question regarding the optimal timing of flexible bronchoscopy are also addressed. These results and review of existing literature, together with knowledge of our institution, has enabled the development of a protocol for managing this common medical problem.

Future studies should involve larger patient numbers to increase the power of the study, expansion of our methodology to include a broader range of cytokines and the development of a panel with more diagnostic strength. Serial BAL chemokine measurements would also be useful. The yield and benefit of bronchoscopy may alter in the future, as newer diagnostic techniques for fungal or other infection enable earlier definitive results from specimens obtained, better predictive value, and result in more changes in management. Studies should continue to be performed on this important population to properly incorporate these methods into clinical protocols. Almost every study to date has shown that bronchoscopic findings, regardless of whether these resulted in a change in clinical management, had no impact on overall mortality. However, no study to date has assessed differences in morbidity, aside from procedural complications. This would be an important factor, particularly with the potential side-effects and prolonged hospital stays caused by infection or long-term intravenous antibiotics.

In conclusion, our study found that FB as a diagnostic tool prior to the development of respiratory clinical or radiological indicators did not improve overall microbiological yield. Fungal yield appeared to be significantly better. Results did not impact on immediate clinical management, and early bronchoscopy had minor impact on a broad range of clinical parameters measured. Importantly, the complication rate of bronchoscopy in this population is negligible. In summary, although the procedure is safe, there is little evidence to recommend routine bronchoscopy as a routine part of the septic screen for diagnosis in severely immunocompromised febrile neutropenic patients, but there should be a low threshold for performing bronchoscopy once pulmonary infiltrates develop.

7. PROTOCOL DEVELOPMENT

As seen from the above data and review, management of severely immunocompromised febrile neutropenic patients remains a common and difficult problem, and respiratory infections can lead to significant morbidity if not detected early. With current prophylaxis and empirical treatment regimes, fungal infection is emerging as the main concern.

The practical consequence of the current diversity of new available diagnostic and laboratory procedures is a need for the clinician to return to a strict analysis of the clinical and radiological data in each case. Although some guidelines for management have been developed, it is well recognised that they may be predicated on data from a single institution or depend on diagnostic procedures and laboratory support that are not necessarily available to physicians in all locations[70].

Guidelines or protocols must consider local resources and expertise, as well as previous patterns of infection in the institution. Familiarity with and critical appraisal of the literature on pulmonary disease in immunocompromised patients can contribute to a clear diagnostic and management plan.

The Haematology Unit requires support from a Respirology Unit which has staff with detailed knowledge of diagnostic possibilities, and facilities and expertise for urgent bronchoscopy. There should be recognition that in a subgroup of these patients, there is a "window of opportunity" for bronchoscopy, before which time, yield is low and pulmonary disease may not eventuate or be the source of pathology, and after which time, the patient may be too unwell for bronchoscopy to be performed safely.

From review of the literature as above, and from our institution-specific results, the majority of evidence suggests the following:

1. Early diagnosis of pulmonary complications should be targeted
2. For febrile neutropenic patients, broad-spectrum empirical antibiotics should be commenced without delay
3. The septic screen should consist of specimens sent for culture of: sputum, including induced sputum if possible, nasopharyngeal aspirate, urine and blood, as these are valuable have potentially high yield and are non-invasive
4. There are no specific clinical nor radiographic characteristics which predict likelihood of yield from non-invasive or invasive techniques.
5. High-Resolution CT chest is vastly superior in sensitivity to plain CXR, and should be the pulmonary imaging investigation of choice in all severely immunocompromised febrile neutropenic patients, as part of the initial septic screen, to detect subtle indications of a pulmonary source of infection, provide a baseline and guide bronchoscopic samping if applicable later
6. Clinical and radiological changes provide little reliable information regarding the underlying pathogen causing pulmonary infection, despite reports of some characteristic findings
7. Flexiblebronchoscopy with BAL can be performed safely in the severely immunocompromised patient population, with platelet support, unless respiratory failure

is imminent. Once the patient is intubated, bronchoscopy can be performed with minimal additional risk to the patient

8. TBB can add certain information to BAL at the expense of higher complications, but most commonly yields non-infective diagnoses, which can be often be strongly suspected by clinical and radiological parameters. The most common non-infective cause of pulmonary infiltrates, diffuse alveolar haemorrhage, can usually be diagnosed easily with BAL. Non-infective conditions which can be diagnosed by TBB usually require only supportive management, are suspected on clinical and radiological information, or are not excluded by negative TBB.
9. PSB rarely adds diagnostic information. It may add further time to the procedure, and can potentially increase the complication rate. It should therefore not be performed routinely.
10. Flexiblebronchoscopy with BAL should be a consideration early for any patients with pulmonary infiltrates
11. Bronchoscopically obtained specimens should be sent for:
    a. Urgent gram-stain, microscopy and culture
    b. Fungal microscopy and culture
    c. PCP
    d. CMV
    e. Respiratory viruses
    f. Norcardia

12. Although there appears to be no impact on mortality, regardless of whether positive results are obtained, diagnoses are excluded or management is altered, there is some evidence that early positive results influence outcome.
13. If bronchoscopy findings are negative, open lung biopsy should be considered, especially if ongoing treatment causing further or prolonged immunocompromise is anticipated.

Our guidelines therefore suggest the following course of management for severely immunocompromised neutropenic patients who become febrile:

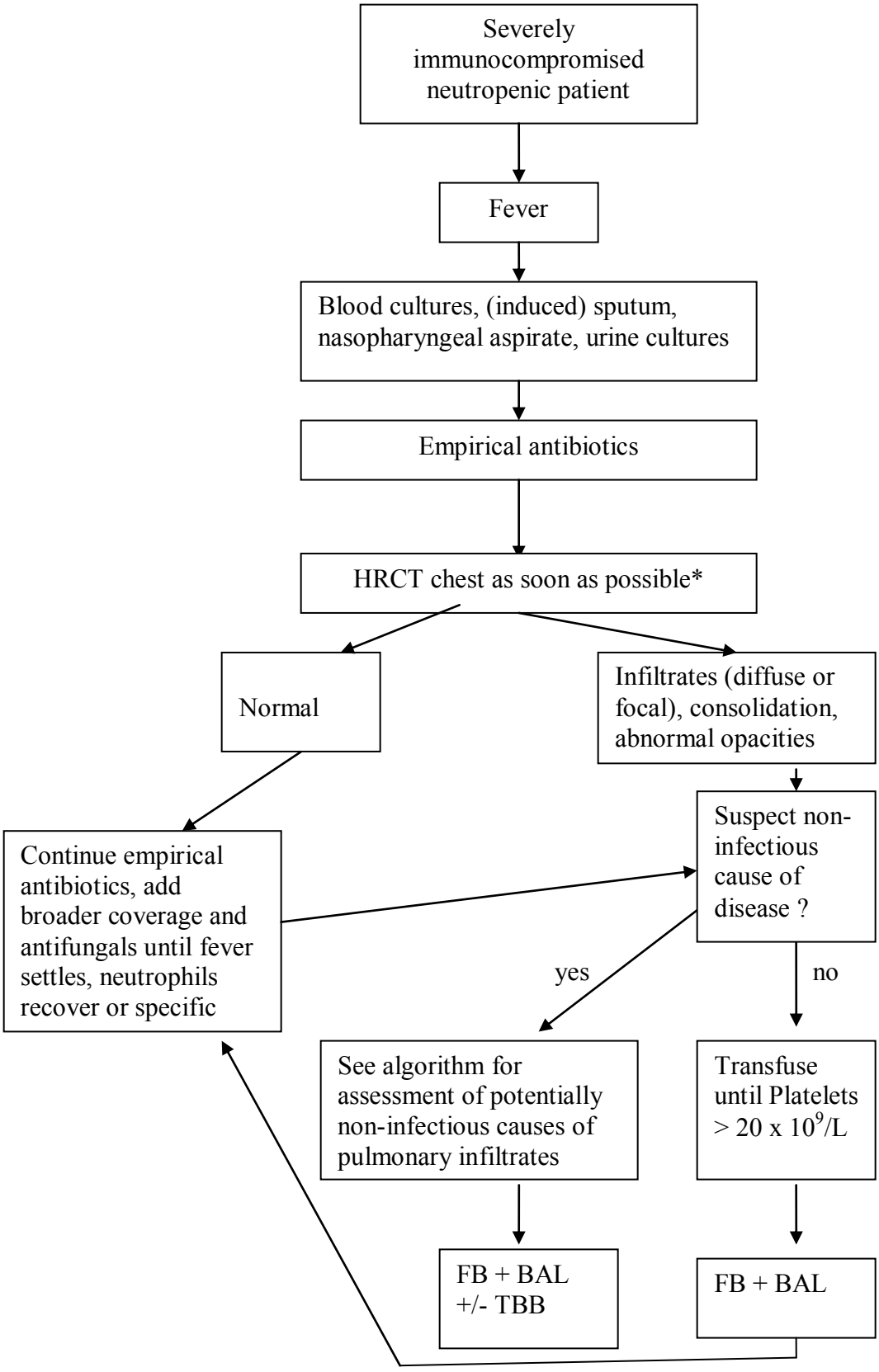
1. Routine septic screen, including blood cultures, sputum cultures (including induced sputum), nasopharyngeal aspirate and urine microscopy and culture (Serology for respiratory viruses is almost always non-diagnostic. Urine cultures are almost always negative in the absence of specific symptoms, but this test is cheap and easily 4performed, and in rare cases of positive results, these impact significantly on clinical management). Specific cultures if symptoms suggest a source of infection (Eg wound swabs, lumbar puncture)
2. Commence broad-spectrum empirical antibiotics with standard regime devised in consultation with local infectious disease unit
3. High-resolution CT chest at earliest possible time (CXR may suffice as initial screen if HRCT will be delayed, but it is far less sensitive):
    a. If pulmonary abnormality detected and cultures negative, consider proceeding to FB with BAL

b. Perform TBB only for specific reasons, eg if suspect non-infectious cause, high yield based on radiological findings, and impact of results on clinical management (Limitation of FB to the performance of BAL without other instrumentation may reduce the likelihood of complications without substantial reduction in yield).
c. Platelet support to keep level > 20 x 10^9/L (> 50 x 10^9/L if TBB being considered)
d. Always send specimens for routine microbiological testing as above, and analyse fo haemosiderin-laden macrophages – diffuse alveolar haemorrhage is the most common non-infective cause of pulmonary infiltrates in this population

4. If a patient is intubated, bronchoscope as soon as possible
5. Continue to add further antimicrobials including anti-fungal antibiotics until patient becomes afebrile or specific organisms isolated

Algorithm for assessment of potentially non-infectious causes of pulmonary infiltrates:

Fig 8: Protocol for bronchoscopy in severely immunocompromised febrile neutropenic patient



* If HRCT unavailable, perform CXR

8. REFERENCES

1. Role of flexible bronchoscopy in immunocompromised patients with lung infiltrates. Jain P, Sandur S, Meli Y, Arroliga A, Stoller J, Mehta A. Chest 2004 Feb; 125 (2) 712-22

2. Use of first line bronchoalveolar lavage in the immunosuppressed oncology patient. Murray PV, O'brien M, Padhani A, Powles R, Cunningham D, Jeans A, Ashley S. Bone Marrow Transplant. 2001 May; 27 (9): 967 – 71

3. Role of flexible bronchoscopy in the management of immunocompromised patients with pulmonary infiltrates. Hussain SF, Malik I, Khan JA. J Pak Med Assoc. 1998 Oct; 48 (10): 311-2.

4. Bronchoscopy in immunocompromised host with pulmonary infiltrates. Hsu A, Allen D, Yeo C, Ang B, Ong Y. Ann Acad Med Singapore. 1996 Nov; 25 (6): 797-803.

5. Diagnosis of pulmonary infections in immunocompromised patients by fiberoptic bronchoscopy with bronchoalveolar lavage and serology. ErikssonB, Dahl H, Wang F, Hillerdal G, et al. Scan J Infect Dis 1996; 28 (5): 479-85

6. Transbronchial biopsy in the diagnosis of pulmonary infiltrates in immunocompromised patients. Cazzadori A, Di Perri G, Todeschini G, Luzzati R, Boschiero L, Perona G, Concia E. Chest 1995 Jan; 107 (1): 101-6.

7. Diagnostic success of bronchoscopic biopsy in immunocompromised patients with acute pulmonary disease: predictive value of disease distribution as shown on CT. Janzen DL, Adler BD, Padley SP, Muller NL. AJR Am J Roentgenol. 1993 Jan;160(1):21-4.

8. Flexible bronchoscopy and bronchoalveolar lavage in the investigation of the immunocompromised lung. Wallace RH. NZ Med J. 1992 Jun 10; 105 (935): 215-217.

9. Bronchoalveoloar lavage in immunocompromised patients. Clinical and functional consequences. Verra F, Hmouda H, Rauss A, Lebargy F, Cordonnier C, Bignon J, Lemaire F, Brochard L. Chest 1992 May; 101 (5): 1215-20.

10. Aetiology of pulmonary diseases in immunocompromised patients. Heurlin N, Brattstrom C, Lonnqvist B, Westman L, Lidman C, Andersson J. Eur Respir J. 1991 Jan; 4 (1): 10-8

11. Utility of bronchoalveolar lavage in the assessment of diffuse pulmonary infiltrates in nonAIDS immunocompromised patients. Breuer R, Lossos I, Lafair J, Engelhard D. Respir Med, 1990 Jul; 84 (4): 313-6.

12. Use of bronchoalveolar lavage to diagnose pulmonary infiltrates in immunocompromised hosts. Saenghirunvattana S, Charoenpan P, Vathesatogkit P et al. J Med Assoc Thai, 1989 Jul; 72 (7): 371-5.

13. Diagnostic value of fiberbronchoscopy in immunocompromised, haematologic patients with pulmonary infiltrates. Langenaeken C, Meirhaeghe J, Tricot G, Boogaerts M et al. Acta Clin Belg. 1987;42(5):323-9.

14. Open lung biopsy provides a higher and more specific diagnostic yield compared to broncho-alveolar lavage in immunocompromised patients. Fungal Study Group. Ellis ME, Spence D, Bouchama A, Antonius J, Bazarbashi M, Khougeer F, De Vol EB. Scand J Infect Dis. 1995;27(2):157-62.

15. Utility of fiberoptic bronchoscopy in bone marrow transplant patients. White P, Bonacum J, Miller C. Bone Marrow Transplantation 1997; 20: 681-687.

16. Role of bronchoalveolar lavage in immunocompromised patients with pneumonia treated with a broad spectrum antibiotic and antifungal regime. Hohenadel I, Kiworr M, Genitasriotis R, Zeidler D et al. Thorax 2001; 56:115-120.

17. Diagnostic yield of bronchoscopy in histologically proven invasive pulmonary aspergillosis. Reichenberger F, Habicht J, Matt P, Frei R, Bolliger CT et al. Bone Marrow Transplantation 1999; 24: 1195-1199.

18. Pulmonary fungal infections in patients with haematological malignancies – diagnostic approches. Von Eiff M, Zuhlsdorf M, Roos N et al. Ann Haematol 1995; 70: 135-141.

19.Pulmonary infiltrates in patients wth haematologic malignancies: clinical usefulness of non-invasive bronchoscopic procedures. Von Eiff M, Zuhlsdorf M, Roos N et al. Eur J Haematol 1995; 54: 157-162.

20. Postal Survey of bronchoscopic practice by Physicians in the United Kingdom. Simpson FG, Arnold AG, Purvis A et al. Thorax 1986; 41: 311-317

21. Bronchoalveloar lavage in diagnosis of diffuse pulmonary infiltrates in the immunosuppressed host. Stover DE, Muhammad BZ, Steven IH et al. Ann Intern Med 1984; 101: 1-7.

22.Epidemiology of Aspergillus infections in a large cohort of patients undergoing bone marrow transplantation. Wald A, Leisenring W, van Burik J, Bowden RA J Infect Dis 1997; 175: 1459-1466.

23. Pulmonary aspergillosis: early diagnosis improves survival. Von Eiff M, Roos N, Schulten R et al. Respiration 1995; 62: 341-347.

24. Bronchoalveolar lavage during neutropenic episodes: diagnostic yield and cellular pattern. Cordonnier C, Escudier E, Verra F et al. Eur Respir J 1994; 7: 114-120

25. Role of bronchoalveolar lavage in the assessment of opportunistic pulmonary infections: Utility and complications. Martin WJ, Smith TF, Sanderson DR et al. Mayo Clin Proc 1987; 62: 549-557

26. Complications of bronchoscopy in thrombocytopenic patients. Weiss SM, Hert RC, Gianola FJ et al. Chest 1993; 104: 1025-1028.

27. Empiric therapy with carbenicillin and gentamicin for febrile patients with cancer and granulocytopenia. Schimpff S, Satterlee W, Young VM, Serpick A. N Engl J Med 1971; 284: 1061–5.

28. Initial therapy with combination of five antibiotics in febrile patients with leukaemia and neutropenia. Tattersall MH, Spiers AS, Darrell JH. Lancet 1972; 1:
162–6.

29. Three antibiotic regimens in the treatment of infection in febrile granulocytopenic patients with cancer. The EORTC International Antimicrobial Therapy Project
Group. J Infect Dis 1978; 137: 14–29.

30. Combination of amikacin and carbenicillin with or without cefazolin as empirical treatment of febrile neutropenic patients. The International Antimicrobial Therapy Project Group of the EORTC. J Clin Oncol 1983; 1: 597–603.

31. EORTC Antimicrobial Therapy project Group. Prospective randomized comparison of three antibiotic regimens for empirical therapy of suspected bacteremic infection in febrile granulocytopenic patients. Klatersky J, Glauser MP, Schimpff SC, Zinner SH, Gaya H. Antimicrob Agents Chemother 1986; 29: 263–70.

32. 2002 guidelines for the use of antimicrobial agents in neutropenic patients with cancer. Hughes WT, Armstrong D, Bodey GP, Bow EJ, Brown AE, Calandra T et al. Clin Infect Dis 2002; 34: 730–51.

33. Evidence-based recommendations for antimicrobial use in febrile neutropenia in Japan: executive summary. Masaoka T. Clin Infect Dis 2004; 39: S49–S52.

34. Fiberoptic bronchoscopy and bronchoalveolar lavage for the evaluation of pulmonary infiltrates after BMT in children. Lanino E, Sacco O, Kotitsa Z et al. Bone Marrow Transplant 1996; 18 (Suppl. 2): 117-120.

35. Diffuse pulmonary infiltrates after bone marrow transplantation: the role of open lung biopsy. Wang JY, Chang YL, Lee LN, Chen JH, Tang JL, Yang PC, Lee YC. Ann Thorac Surg. 2004 Jul; 78(1):267-72

36. Use of fiberoptic bronchoscopy in bone marrow transplant recipients. Glazer M, Breuer R, Or R et al. Acta Haematol. 1998; 99(1):22-6

37. Diagnostic value of protected BAL in diagnosing pulmonary infiltrates in immunocompromised patients. Jolis R, Castella J, Puzo C, et al. Chest 1996; 109: 601-607.

38. Computed tomographic scan of the chest, latex agglutination test and plasma (1AE3)-beta-D-glucan assay in early diagnosis of invasive pulmonary aspergillosis: a prospective study of 215 patients. Kami M, Tanaka Y, Hirai H et al. Haematologica. 2000 Jul;85(7):745-52.

39. Use of real-time PCR on blood samples for diagnosis of invasive aspergillosis. Kami M, Fukui T, Ogawa S, Hirai H et al. Clin Infect Dis. 2001 Nov 1;33(9):1504-12.

40. Invasive pulmonary aspergillosis following bone marrow transplantation: risk factors and diagnostic aspect. Soubani AO, Qureshi MAHaematologia 2002;32(4):427-37.

41. A randomized trial of open lung biopsy versus empiric antimicrobial therapy in cancer patients with diffuse pulmonary infiltrates. Browne M, Potter D, Gress J, *et al*. *J Clin Oncol* 1990; 8: 222-229

42. A double-blind comparison of empirical oral and intravenous antibiotic therapy for low-risk febrile patients with neutropenia during cancer chemotherapy. Freifeld A, Marchigiani D, Walsh T, *et al*. *N Engl J Med* 1999; 341: 305-311

43. Pneumonia in acute leukemia. Sickles E, Young V, Greene W, *et al*. *Ann Intern Med* 1973;79: 528-534.

44. Lung infections in patients on immunosuppressive therapy. Mayaud C, Denis M, Akoun G. In: Akoun G, White J, eds. *Treatment-induced respiratory disorders*. Amsterdam: Elsevier, 1989; 239-257.

45. 1997 guidelines for the use of antimicrobial agents in neutropenic patients with unexplained fever. Hughes W, Armstrong D, Bodey G, *et al*. *Clin Infect Dis* 1997; 25:551-573

46. Oral versus intravenous empirical antimicrobial therapy for fever in patients with granulopenia who are receiving cancer chemotherapy. Kern W, Cometta A, De Bock R, *et al*. *N Engl J Med* 1999; 341:312-318

47. Role of early diagnosis and aggressive surgery in the management of invasive pulmonary aspergillosis in neutropenic patients. Caillot D, Mannone L, Cuisenier B, Couaillier JF. Clin Microbiol Infect. 2001; 7 Suppl 2:54-61.

48. Role of bronchoalveolar lavage in the evaluation of interstitial pneumonitis in recipients of bone marrow transplants Milburn HJ, Prentice HG, Bois, RM. Thorax 1987; 42: 766-72

49. Prediction of cytomegalovirus pneumonia after marrow transplantation from cellular characteristics and cytomegalovirus cultures of bronchoalveolar lavage fluid. Slavin MA, Gooley TA, Bowden RA. Transplantation 1994; 58: 915-919.

50. Pulmonary Surveillance Using Bronchoscopy and Bronchoalveolar Lavage during High-Dose Antineoplastic Therapy. Vaughan WP, Linder J, Robbins R, Arneson M et al. Chest 1991; 99: 105-111.

51. Invasive aspergillosis following hematopoietic cell transplantation: outcomes and prognostic factors associated with mortality. Upton A, Kirby KA, Carpenter P, Boeckh M, Marr KA. Clin Infect Dis. 2007 Feb 15;44(4):531-40.

52. A persistent challenge: the diagnosis of respiratory disease in the non-AIDS immunocompromised host. Mayaud C, Cadranel J. *Thorax* 2000; 55:511-517

53. Management of invasive pulmonary aspergillosis in hematology patients: a review of 87 consecutive cases at a single institution. Yeghen T, Kibbler CC, Prentice HG, Lampe FC, Gillespie S et al. Clin Infect Dis. 2000 Oct;31(4):859-68.

54. The safety and usefulness of routine bronchoscopy before stem cell transplantation and during neutropenia. Whittle AT, Davis M, Johnson PRE, Leonard RCF, Greening A. Bone Marrow Transplantation 1999; 24: 63-67.

55. Epidemiology and Outcome of Mould Infections in Hematopoietic Stem Cell Transplant Recipients. Marr KA, Carter RA, F Crippa F et al. Clinical Infectious Diseases 2002; 34: 909-17

56. Utility of Aspergillus Antigen Detection in Specimens Other than Serum Specimens. Klont RR, Mennink-Kersten MA, Verweij PE. Clinical Infectious Diseases 2004; 39: 1467-74.

57. High-risk febrile neutropenia in Auckland 2003-2004: the influence of the microbiology laboratory on patient treatment and the use of pathogen-specific therapy. Ritchie S, Palmer S, Ellis-Pegler R. Internal Medicine Journal 2007; 37: 26-31.

58. Routine radiography does not have a role in the diagnostic evaluation of ambulatory adult febrile neutropenic cancer patients. Oude Nijhuis CS, Gietema JA, Vellenga E, Daenen SM. Eur J Cancer 2003; 39: 2495-8.

59. Infections in Patients with Febrile Neutropenia: Epidemiology, Microbiology and Risk stratification. Viscoli C, Varnier O, Machetti M. Clin Infect Dis 2005; 40 (Suppl. 4): S240-45).

60. Bronchoalveolar lavage in the diagnosis of pulmonary complications of bone marrow transplant patients. Huaringa AJ, Leyva FJ, Signes-Costa J et al. Bone marrow Transplantation 2000; 25: 975 – 979.

61. Hospital-acquired pneumonia in adults: diagnosis, assessment of severity, initial antimicrobial therapy, and preventive strategies. A consensus statement, American Thoracic Society, November 1005. Am J Respir Crit Care Med 1996; 153: 1711-1725.

62. State of the Art: Pulmonary Complications of Solid Organ and Hematopoietic Stem Cell Transplantation. Kotloff RM, Ahya VN, Crawford SW. Am J Respir Crit Care Med 2004; 140: 22-48.

63. Transplantation of bone marrow as compared with peripheral blood cells from HLA-identical relatives in patients with hematologic cancers. Bensinger WI, Martin PJ, Storer B, et al. N Engl J Med. 2001;344;175–181.

64. Acute myeloid leukemia. Stone RM, O'Donnell MR, Sekeres MA. Hematology Am Soc Hematol Educ Program. 2004; 98-117.

65. Prognostic factors for early severe pulmonary complications after hematopoietic stem cell transplantation. Ho VT, Weller E, Lee SJ, Alyea EP, Antin JH, Soiffer RJ. Biol Blood Marrow Transplant. 2001;7(4):223-9

66. Pulmonary infiltrates in non-HIV immunocompromised patients: a diagnostic approach using non-invasive and bronchoscopic procedures. Rañó A, Agustí C, Jimenez P, Angrill J, Benito N, Danés C, González J, Rovira M, Pumarola T, Moreno A, Torres A. Thorax 2001; 56: 379-387

67. Progress in the diagnosis and management of aspergillosis in bone marrow transplantation: 13 years experience. McWhinney PH, Kibbler CC, Hamon MD et al. Clin Infect Dis 1993; 17: 397 – 404.

68. Therapeutic outcomes in invasive aspergillosis. Denning DW. Clin Infect Dis 1996; 23: 608-615.

69. Diagnostic flexible bronchoscopy in the immunocompromised host with pulmonary infiltrates. Matthay RA, Farmer WC, Odero D. Thorax. 1977 Oct; 32(5):539-45

70. Diffuse pulmonary infiltrates in immunocompromised patients. Reconciling theory and practice. Staszewski H. Postgrad Med. 1993 Jul;94(1):69-72, 75-8

71. Guidelines on the management of acute myeloid leukaemia in adults: British Committee for Standards in Haematology. Milligan DW, Grimwade D, Cullis JO, Bond L, Swirsky D, Craddock C, et al. British Journal of Haematology, 135, 450–474

72. Pulmonary Cytomegalovirus Infection in Immunocompromised Patients. Tamm M, Traenkle P, Grilli B, Cathomas G et al. Chest 2001;119;838-843

73. The Biology and Therapy of Adult Acute Lymphoblastic Leukemia. Faderl S, Jeha S, Kantarjian HM. *Cancer* 2003; 98: 1337–54.

74. Pulmonary complications after bone marrow transplantation: an autopsy study from a large transplantation center. Roychowdhury M, Pambuccian SE, Aslan DL, Jessurun J, Rose AG, Manivel JC, Gulbahce HE. Arch Pathol Lab Med. 2005 Mar; 129 (3):366-71.

75. Pulmonary complications in adult blood and marrow transplant recipients: autopsy findings. Sharma S, Nadrous HF, Peters SG, Tefferi A, Litzow MR, Aubry MC, Afessa B. Chest 2005 Sep; 128(3): 1385-92.

76. Fatal pneumothorax complicating BAL in a bone marrow transplant recipient with bronchiolitis obliterans. Cazzadori A, Di Perri G, Bonora S, Lanzafame M. *Chest;* May 1997; 111, 5; p 1468

77. Sepsis syndrome and death after bronchoalveolar lavage. De Fijter JW, Van der Hoeven JG, et al. Chest 1993; 104:1296-97

78. Bronchoscopic ebvaulation of pulmonary infiltrates following bone marrow transplantation. Dunagan DP, Baker A, Hurd D, Haponik E. Chest 1997; 111: 135-141.

79. Campbell JH, Blessing N, Burnett AK, et al. Investigation and management of pulmonary infiltrates following bone marrow transplantation: an 8-year review. Thorax 1993; 48:1248-51

80. Thin-section CT findings in hematopoietic stem cell transplantation recipients with respiratory virus pneumonia. Franquet T, Rodriguez S, Martino R, Giménez A, Salinas T, Hidalgo A. AJR Am J Roentgenol. 2006 Oct;187(4):1085-90.

81. Bronchoscopic invasive diagnostic techniques in the cancer patient. Ninane V. Curr Opin Oncol. 2001 Jul;13(4):236-41.

82. Fiberoptic bronchoscopy for diagnosis of opportunistic pulmonary infections after bone marrow transplantation. Heurlin N, Lönnqvist B, Tollemar J, Ehrnst A. . Scand J Infect Dis. 1989;21(4):359-66.

83. The role of bronchoalveolar lavage in the diagnosis of suspected opportunistic pneumonia. Abramson MJ, Stone CA, Holmes PW, Tai EH. Aust N Z J Med. 1987 Aug; 17(4):407-12.

84. Flexible bronchoscopy and bronchoalveolar lavage in the investigation of the immunocompromised lung. Wallace RH, Kolbe J. NZ Med J. 1992 Jun

85. The role of fiberoptic bronchoscopy in the evaluation of immunocompromised hosts with diffuse pulmonary infiltrates. Williams D, Yungbluth M, Adams G, Glassroth J. Am Rev Respir Dis. 1985 Jun; 131(6):880-5.

86. Fiberoptic bronchoscopy in the immunocompromised host: the significance of a "nonspecific" transbronchial biopsy. Nishio JN, Lynch JP. Am Rev Respir Dis. 1980 Feb; 121(2):307-12.

87. Diagnosis of pulmonary infections in immunocompromised patients. McCabe RE. Med Clin North Am. 1988 Sep; 72(5):1067-89.

88. The initial pulmonary evaluation of the immunocompromised patient. Walsh FW, Rolfe MW, Rumbak MJ. Chest Surg Clin N Am. 1999 Feb;9(1):19-38.

89. The role of bronchoalveolar lavage in the immunocompromised host. Feller-Kopman D, Ernst A. Semin Respir Infect. 2003 Jun; 18(2):87-94.

90. Bronchoscopic diagnosis of pulmonary infections in a heterogeneous, non-selected group of patients. Ekdahl K, Eriksson L, Rollof J, Miörner H, Griph H, Löfgren B. Chest: 1993 Jun;103(6):1743-8

91. Pulmonary Infiltrates in the Non-HIV-Infected Immunocompromised Patient. Etiologies, Diagnostic Strategies and Outcomes. Shorr AF, Susla GM, O'Grady NP. Chest: 2004 Jan; 125 (1): 260-271.

92. Mechanical ventilation in hematopoietic stem cell transplantation: can we effectively predict outcomes. Shorr AF, Moores LK, Edenfield WJ, et al. Chest 1999: 116: 1012-1018.

93. Stem Cell Transplantation for treatment of severe autoimmune diseases: current status and future perspectives. Jantunen E, Myllykangas-Luosujarvi R. Bone Marrow Transplant 2000; 25: 351-356.

94. Invasive Aspergillosis in transplant recipients. Paterson DL, Singh N. Medicine 1999; 78: 123-138.

95. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. Marr KA, Carter RA, Crippa F, et al. Clin Infect Dis 2002; 34: 909-917.

96. Incidence and risk factors for invasive fungal infections in allogeneic SCT recipients. Jantunen E, Ruutu P, Niskanen L et al. Bone Marrow Transplant 1997; 19: 801-808.

97. Evaluation of pulmonary infiltrates in critically ill patients with cancer and marrow transplant. White P. Crit Care Clin 2001; 17: 647-670.

98. Invasive Aspergillosis: disease spectrum, treatment practices, and oucomes. Patterson TF, Kirkpatrick WR, White M et al. Medicine 2000; 79: 250-260.

99. Opportunistic infections after blood and marrow transplantation. Wingard JR. Transpl infec Dis 1999; 1: 3-20.

100. Pneumocystis pneumonia: clinical presentation and diagnosis in patients with and without acquired immune deficiency syndrome. Thomas CF, Limper AH. Semin Respir Infect 1998; 13: 289-295.

101. Early detection of pneumonia in febrile neutropenic patients: use of thin-section CT. Heussel CP, Kauczor HU, Heussel G, et al. Am J Roentgenol 1997; 169: 1347-1353.

102. Prognostic factors of non-HIV immunocompromised patients with pulmonary infiltrates. Rano A, Agusti C, Benito N, et al. Chest 2002; 122: 253-261.

103. Clinical importance of delays in the initiation of appropriate antibiotic treatment for ventilator-associated pneumonia. Iregui M, Ward S, Sherman G, et al. Chest 2002; 122: 262-268.

104. The utility of open lung biopsy in patients with hematologic malignancies. White DA, Wong PW, Downey R. Am J Respir Crit Care Med 2002; 161: 723-729.

105. Stem cell transplantation for autoimmune diseases. Gratwohl A, Passweg J, Gerber I, Tyndall A. *Best Pract Res Clin Haematol* 2001;14:755–776.

106. Immunologic, clinical, and pathologic aspects of human graft-versus-host disease.Wick MR, Moore SB, Gastineau DA, Hoagland HC. *Mayo Clin Proc 1*983;58:603–612.

107. Idiopathic pneumonia syndrome: changing spectrum of lung injury after marrow transplantation. Kantrow SP, Hackman RC, Boeckh M, Myerson D, Crawford SW. *Transplantation* 1997;63:1079–1086.

108. Pulmonary complications of bone marrow transplantation. Soubani AO, Miller KB, Hassoun PM. *Chest* 1996;109:1066–1077.

109. Outcome of recipients of bone marrow transplants who require intensive-care unit support. Afessa B, Tefferi A, Hoagland HC, Letendre L, Peters SG. *Mayo Clin Proc* 1992; 67:117–122.

110. Long-term survival from respiratory failure after marrow transplantation for malignancy. Crawford SW, Petersen FB. *Am Rev Respir Dis* 1992;145:510–514.

111. Outcome of patients requiring medical ICU admission following bone marrow transplantation. Paz HL, Crilley P, Weinar M, Brodsky I. *Chest* 1993 ;104:527–531.

112. Survival of adult bone marrow transplant patients receiving mechanical ventilation: a case for restricted use. Faber-Langendoen K, Caplan AL, McGlave PB. *Bone Marrow Transplant* 1993; 12:501–507.

113. Outcome of bone marrow transplantation patients requiring mechanical ventilation. Huaringa AJ, Signes-Costa J, Velarde H, Champlin RE et al. *Crit Care Med* 2000; 28:1014–1017.

114. Prognostic indicators for blood and marrow transplant patients admitted to an intensive care unit. Price KJ, Thall PF, Kish SK, Shannon VR, Andersson BS. *Am J Respir Crit Care Med* 1998; 158:876–884.

115. Withdrawing life support from mechanically ventilated recipients of bone marrow transplants: a case for evidence-based guidelines. Rubenfeld GD, Crawford SW. *Ann Intern Med* 1996; 125:625–633.

116. Abnormalities of pulmonary function tests after marrow transplantation predict non-relapse mortality. Crawford SW, Pepe M, Lin D, Benedetti F, Deeg HJ. *Am J Respir Crit Care Med* 1995; 152:690–695.

117. Using outcomes research to improve the management of blood and marrow transplant recipients in the intensive care unit. Crawford SW. *New Horiz* 1998; 6:69–74.

118. Immune reconstitution after hematopoietic stemcell transplantation and its influence on respiratory infections. Matulis M, High KP. *Semin Respir Infect* 2002;17:130–139.

119. Preventing opportunistic infections after hematopoietic stem cell transplantation: the Centers for Disease Control and Prevention, Infectious Diseases Society of America, and American Society for Blood and Marrow Transplantation practice guidelines and beyond. Sullivan KM, Dykewicz CA, Longworth DL, Boeckh M, Baden LR, Rubin RH, Sepkowitz KA. *Hematology (Am Soc Hematol Educ Program)*2001: 392–421.

120. Bacterial pneumonia in recipients of bone marrow transplantation: a five-year prospective study. Lossos IS, Breuer R, Or R, Strauss N, Elishoov H, Naparstek E, Aker M, Nagler A, Moses AE, Shapiro M, *et al. Transplantation* 1995; 60:672–678.

121. Noninvasive ventilation in immunosuppressed patients with pulmonary infiltrates, fever, and acute respiratory failure. Hilbert G, Gruson D, Cardinaud JP et al. *N Engl J Med* 2001; 344:481–487.

122. Nosocomial outbreak of *Legionella pneumophila* serogroup 3 pneumonia in a new bone marrow transplant unit: evaluation, treatment and control. Oren I, Zuckerman T, Avivi I, Finkelstein R, Yigla M, Rowe JM. *Bone Marrow Transplant* 2002; 30:175–179.

123. Legionellosis in a bone marrow transplant center. Harrington RD, Woolfrey AE, Bowden R, McDowell MG, Hackman RC. *Bone Marrow Transplant* 1996; 18:361–368.

124. Current antiviral strategies for controlling cytomegalovirus in hematopoietic stem cell transplant recipients: prevention and therapy. Boeckh M. *Transpl Infect Dis* 1999; 1: 165–178.

125. Cytomegalovirus pneumonia in adult autologous blood and marrow transplant recipients. Konoplev S, Luna M, *et al. Bone Marrow Transplant* 2001; 27: 877–881.

126. CMV pneumonia in allogeneic SCT recipients undergoing early treatment of pre-emptive ganciclovir therapy. Machado CM, Pannuti CS et al. *Bone Marrow Transplant* 2000; 26:413–417.

127. A randomized, controlled trial of prophylactic ganciclovir for cytomegalovirus pulmonary infection in recipients of allogeneic bone marrow transplants.Schmidt GM, Horak DA, Niland JC, Duncan SR, Forman SJ, Zaia JA, City of Hope-Stanford-Syntex CMV Study Group. *N Engl J Med* 1991; 324:1005–1011.

128. Cytomegalovirus is frequently isolated in bronchoalveolar lavage fluid of bone marrow transplant recipients without pneumonia. Ruutu P, Ruutu T, Hovi T et al. *Ann Intern Med* 1990; 112:913–916.

129. Treatment of cytomegalovirus pneumonia with ganciclovir and intravenous cytomegalovirus immunoglobulin in patients with bone marrow transplants. Reed EC, Bowden RA, Dandliker PS, Lilleby KE, Meyers JD. *Ann Intern Med* 1988;109:783–788.

130. Cytomegalovirus pneumonia after bone marrow transplantation successfully treated with the combination of ganciclovir and high-dose intravenous immune globulin. Emanuel D, Cunningham I, Polsky B, *et al. Ann Intern Med* 1988; 109:777–782.

131. Ganciclovir/immunoglobulin combination therapy for the treatment of human cytomegalovirus-associated interstitial pneumonia in bone marrow allograft recipients. Schmidt GM, Kovacs A, Forman SJ et al. *Transplantation* 1988; 46:905–907.

132. Human herpesvirus 6 in lung tissue from patients with pneumonitis after bone marrow transplantation. Cone RW, Hackman RC, Corey L et al. *N Engl J Med* 1993; 329:156–161.

133. Respiratory virus infections after marrow transplant: the Fred Hutchinson Cancer Research Center experience. Bowden RA. *Am J Med* 1997; 102:27–30.

134. Community respiratory virus infections among hospitalized adult bone marrow transplant recipients. Whimbey E, Champlin RE, Yousuf H, *et al. Clin Infect Dis* 1996; 22:778–782.

135. Respiratory syncytial virus upper respiratory tract illnesses in adult blood and marrow transplant recipients: combination therapy with aerosolized ribavirin and intravenous

immunoglobulin. Ghosh S, Champlin RE, Mallik S, *et al. Bone Marrow Transplant* 2000; 25: 751–755.

136. Parainfluenza virus respiratory infection after bone marrow transplantation. Wendt CH, Weisdorf DJ, Jordan MC, Balfour HH Jr, Hertz MI. *N Engl J Med* 1992; 326:921–926.

137. Adenovirus infections in patients undergoing bone-marrow transplantation. Shields AF, Hackman RC, Fife KH, Corey L, Meyers JD. *N Engl J Med* 1985;312:529–533

138. Cidofovir for adenovirus infections after allogeneic hematopoietic stem cell transplantation: a survey by the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation. Ljungman P, Ribaud P, Eyrich M, Gratecos N, *et al. Bone Marrow Transplant* 2003; 31:481–486.

139. US Oral Neuraminidase Study Group. Efficacy and Safety of the oral neuraminidase inhibitor oseltamivir in treating acute influenza: a randomized controlled trial. Treanor JJ, Hayden FG, Mills RG et al. JAMA 2000; 283: 1016-2024.

140. Zanamivir for the treatment of influenza A and B infection in high-risk patients: a pooled analysis of randomized controlled trials.Lalezari J, Campion K, Keene O, Silagy C. *Arch Intern Med* 2001;161:212–217.

141. Efficacy and safety of oseltamivir in treatment of acute influenza: a randomised controlled trial.Nicholson KG, Aoki FY, Ward P et al. Neuraminidase Inhibitor
Flu Treatment Investigator Group. *Lancet* 2000; 355:1845–1850.

142. Late onset of invasive *Aspergillus* infection in bone marrow transplant patients at a university hospital. Grow WB, Moreb JS, Clancy CJ, *et al. Bone Marrow Transplant* 2002; 29:15–19.

143. Diagnostic aspects of invasive Aspergillus infections in allogeneic BMT recipients. Jantunen E, Piilonen A, Volin L, Parkkali T, Koukila-Kahkola P, Ruutu
T, Ruutu P. Bone Marrow Transplant 2000;25:867–871.

144. Pulmonary cavitation and massive hemoptysis in invasive pulmonary aspergillosis: influence of bone marrow recovery in patients with acute leukemia. Albelda SM, Talbot GH, Gerson SL, Miller WT, Cassileth PA. *Am Rev Respir Dis* 1985; 131:115–120.

145. Crescent sign in invasive pulmonary aspergillosis: frequency and related CT and clinical factors. Kim MJ, Lee KS, Kim J, Jung KJ, Lee HG, Kim TS*J Comput Assist Tomogr* 2001; 25:305–310.

146. Improved management of invasive pulmonary aspergillosis in neutropenic patients using early thoracic computed tomographic scan and surgery. Caillot D, Petrella T, Bonnin A, et al. *J Clin Oncol* 1997; 15:139–147.

147. Early diagnosis of invasive aspergillosis. Denning DW. *Lancet* 2000; 355: 423–424.

148. The use of respiratory-tract cultures in the diagnosis of invasive pulmonary aspergillosis. Horvath JA, Dummer S. *Am J Med* 1996;100:171–178.

149. Lung resection for invasive pulmonary aspergillosis in neutropenic patients with hematologic diseases. Reichenberger F, Habicht J, Gratwohl A, *et al. Am J Respir Crit Care Med* 1998; 158:885–890.

150. Biopsy diagnosis and clinical outcome of persistent focal pulmonary lesions after marrow transplantation. Crawford SW, Hackman RC, Clark JG. *Transplantation* 1989; 48:266–271.

151. Radiologically guided fine needle lung biopsies in the evaluation of focal pulmonary lesions in allogeneic stem cell transplant recipients. Jantunen E, Piilonen A, Ruutu T et al. *Bone Marrow Transplant* 2002; 29:353–356.

152. Screening for circulating galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation. Maertens J, Verhaegen J, Lagrou K, Van Eldere J, Boogaerts M. *Blood* 2001; 97:1604–1610.

153. Invasive aspergillosis in allogeneic stem cell transplant recipients: changes in epidemiology and risk factors. Marr KA, Carter RA, Boeckh M, Martin P, Corey L *Blood* 2002; 100:4358–4366.

154. Survival and prognostic factors of invasive aspergillosis after allogeneic bone marrow transplantation. Ribaud P, Chastang C, Derouin F, *et al. Clin Infect Dis* 1999; 28:322–330.

155. Treatment and outcome of invasive *Aspergillus* infections in allogeneic SCT recipients. Jantunen E, Ruutu P, Piilonen A, Volin L, Parkkali T, Ruutu T. *Bone Marrow Transplant* 2000; 26:759–762.

156. Caspofungin acetate for treatment of invasive fungal infections. Pacetti SA, Gelone SP. *Ann Pharmacother* 2003; 37:90–98.

157. Emerging fungal pathogens in patients with hematologic malignancies and marrow/stem-cell transplant recipients. Jahagirdar BN, Morrison VA. *Semin Respir Infect* 2002; 17:113–120.

158. Mucormycosis in allogeneic bone marrow transplant recipients: report of five cases and review of the role of iron overload in the pathogenesis. Maertens J, Demuynck H, Boogaerts MA et al. *Bone Marrow Transplant* 1999; 24:307–312.

159. The value of *Pneumocystis carinii* antibody and antigen detection for diagnosis of *Pneumocystis carinii* pneumonia after marrow transplantation. Meyers JD, Pifer LL, Sale GE, Thomas ED. *Am Rev Respir Dis* 1979;120:1283–1287.

160. Summary of the Guidelines for Preventing Opportunistic Infections among Hematopoietic Stem Cell Transplant Recipients. Dykewicz CA. *Clin Infect Dis* 2001;33:139–144.

161. High rates of *Pneumocystis carinii* pneumonia in allogeneic blood and marrow transplant recipients receiving dapsone prophylaxis. Souza JP, Boeckh M, Gooley TA, Flowers ME, Crawford SW. *Clin Infect Dis* 1999; 29:1467–1471.

162. Aerosolized pentamidine prophylaxis for *Pneumocystis carinii* pneumonia after allogeneic marrow transplantation. Marras TK, Sanders K, Lipton JH, Messner HA, Conly J, Chan CK. *Transpl Infect Dis* 2002; 4:66–74.

163. Aerosolized pentamidine as pneumocystis prophylaxis after bone marrow transplantation is inferior to other regimens and is associated with decreased survival and an increased risk of other infections. Vasconcelles MJ, Bernardo MV, King C, Weller EA, Antin JH. *Biol Blood Marrow Transplant* 2000; 6:35–43.

164. *Pneumocystis carinii* pneumonitis following bone marrow transplantation. Tuan IZ, Dennison D, Weisdorf DJ. *Bone Marrow Transplant* 1992; 10:267–272.

165. Flexiblebronchoscopy in adults: a position paper of The Thoracic Society of Australia and New Zealand. Wood-Baker R, Burdon J, McGregor A, Robinson R, Seal P. *Internal Medicine Journal* 2001; 31: 479–487

166. BTS Guidelines for the Management of Community Acquired Pneumonia in Adults. *Thorax* 2001; 56 (Suppl 4):iv1-iv64 (December)

167. The pulmonary physician in critical care: Critical care management of community acquired pneumonia. Baudouin SV**.** *Thorax* 2002;57:267-271

168. Severe community-acquired pneumonia. Etiology, epidemiology, and prognosis factors. French Study Group for Community-Acquired Pneumonia in the Intensive Care Unit. Moine P, Vercken JB, Chevret S, *et al*. *Chest* 1994;105:1487–95.

169. A new diagnostic approach to the patient with severe pneumonia. Sorensen J, Forsberg P, Hakanson E, *et al*. *Scand J Infect Dis* 1989;21:33–41.

170. Etiology and diagnosis of pneumonia requiring ICU admission. Potgieter PD, Hammond JM. *Chest* 1992;101:199–203.

171. Empiric treatment impairs the diagnostic yield of BAL in HIV positive patients. De Gracia J, Miravitlles M, Mayordomo C et al. Chest 1997: 111: 1180-86

172. Utility of fiberoptic bronchoscopy in non-resolving pneumonia. Feinsilver SH, Fein AM, Niederman MS, *et al*. *Chest* 1990;98:1322–6.

173. Diagnostic fiberoptic bronchoscopy and protected brush culture in patients with community-acquired pneumonia. Ortqvist A, Kalin M, Lejdeborn L, *et al*. *Chest* 1990;97:576–82.

174. Safety, Diagnostic Yield, and Therapeutic Implications of Flexible Bronchoscopy in Patients with Febrile Neutropenia and Pulmonary Infiltrates. Peikert T, Rana S, Edell E. Mayo Clin Proc 2005; 80 (11) 1414-1420.

175. *Pseudomonas* bacteremia: retrospective analysis of 410 episodes. Bodey GP, Jadeja L, Elting L.*Arch Intern Med.* 1985;145:1621-1629.

176 Management of fever in patients with cancer and treatment-induced neutropenia. Pizzo PA. *N Engl J Med.* 1993;328:1323-1332.

177. Initial empirical antibiotic therapy for neutropenic fever: analysis of the causes of death. Rossi C, Klastersky J. *Support Care Cancer.* 1996;4:207- 212.

178. 2000 update of recommendations for the use of hematopoietic colony-stimulating factors: evidence-based, clinical practice guidelines. Ozer H, Armitage JO, Bennett CL, et al, American Society of Clinical Oncology Growth Factors Expert Panel. *J Clin Oncol.* 2000; 18: 3558-3585.

179. Incidence and outcome of pneumonia in patients with acute leukemia receiving first induction therapy with anthracycline-containing regimens. Rossini F, Verga M, Pioltelli P, et al. *Haematologica.* 2000;85:1255-1260.

180. Utility of fiberoptic bronchoscopy in neutropenic patients admitted to the intensive care unit with pulmonary infiltrates. Gruson D, Hilbert G, Valentino R, et al. *Crit Care Med.* 2000; 28: 2224-2230.

181. Diagnostic accuracy of protected specimen brush and bronchoalveolar lavage in nosocomial pneumonia: Impact of previous antimicrobial treatment. Souweine B, Veber B, Bedos JP et al. Critical Care Med 1998; 26: 236-244

182. Bronchoscopy guided by high-resolution computed tomography for the diagnosis of pulmonary infections in patients with hematologic malignancies and normal plain chest X-ray. Ramila E, Sureda A, Martino R, et al. *Haematologica.* 2000; 85: 961-966.

183. Significance of isolation of *Aspergillus* from the respiratory tract in diagnosis of invasive pulmonary aspergillosis: results from a three-year prospective study. Yu VL, Muder RR, Poorsattar A. *Am J Med.* 1986;81:249-254.

184. Safety of the transbronchial biopsy in outpatients. Hernandez Blasco L, Alfaro Abreu J et al. *Chest.* 1991;99:562-565.

185. The safety of outpatient transbronchial biopsy. Ahmad M, Wiedemann HP et al. *Chest.* 1986;90:403-405.

186. Transbronchial biopsy in the thrombocytopenic patient. Papin TA, Lynch JP III, Weg JG. *Chest.* 1985;88:549-552.

187. The role of transtracheal aspiration in the diagnosis of respiratory infection in neutropenic patients with acute leukaemia. Slevin M, Lowes J, Bell R, *et al*. *Leukemia Res* 1981;5:165-168

188. Bronchoalveolar lavage in the diagnosis of pulmonary infiltrates in patients with acute leukemia. Saito H, Anaissie E, Morice R, *et al*. *Chest* 1988; 94:745-749

189. Liposomal amphotericin B for empirical therapy in patients with persistent fever and neutropenia. Walsh T, Finberg R, Arndt C, *et al*. *N Engl J Med* 1999;340:764-771

190. Pneumonia in febrile neutropenic patients, bone marrow and blood stem-cell recipients: use of high-resolution computed tomography. Heussel C, Kauczor H, Heussel G, *et al*. *Am J Respir Crit Care Med* 1999;149(part 2):A561.

191. Invasive pulmonary aspergillosis: MRI, CT and plain radiographic findings and their contribution for early diagnosis. Blum U, Windfuhr M, Buitrago-Tellez C, *et al*. *Chest* 1994;106:1156-1161

192. Inflammatory mediators in BAL fluid as markers of evolving pneumonia in leukocytopenic patients. Kiehl M, Ostermann H, Thomas M, *et al*. *Chest* 1997;112:1214-1220

193. Accuracy of transtracheal aspiration in patients with haematological malignancy and acute pneumonia. Peltier P, Martin M, Barrier J, *et al*. *Med Mal Infect* 1982;3:187-191.

194. Bronchoscopy with bronchoalveolar lavage via the laryngeal mask airway in high-risk hypoxemic immunosuppressed patients. Hilbert G, Gruson D, Cardinaud JP et al. Crit Care Med. 2001 Feb;29(2):249-55.

195. Cavitating Invasive Pulmonary Aspergillosis Visualized and Diagnosed by Ultrathin Bronchoscopy. Oki M, Saka H, Sako C, Minemura N et al. *Chest.* 2006;129:475-479.

196. Diagnosis of pulmonary peripheral lesions using ultrathin bronchoscope. Saka, H, Oki, M, Kumazawa, A, et al. *J Jpn Soc Bronchol* 2000; 22, 617-619

197. CT-guided transbronchial biopsy using an ultrathin bronchoscope with virtual bronchoscopic navigation. Shinagawa, N, Yamazaki, K, Onodera, Y, et al *Chest* 2004; 125, 1138-1143

198. Usefulness of ultrathin bronchoscopy in diagnosis of lung cancer. Yamamoto, S, Ueno, K, Imamura, F, et al *Lung Cancer* 2004;56,43-48

199. Diagnosis and treatment of invasive pulmonary aspergillosis in neutropenic patients. Reichenberger, F, Habicht, JM, Gratwohl, A, et al *Eur Respir J* 2002;19,743-755

200. Guidelines for preventing health-care-associated pneumonia, 2003: recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee. Tablan, OC, Anderson, LJ, Besser, R, et al *MMWR Morb Mortal Wkly Rep Recomm Rep* 2004;53,1-36

201. Survival of acute myelogenous leukemia patients requiring intubation/ventilatory support. Tremblay LN, Hyland RH, Schouten BD, Hanly PJ. *Clin Invest Med*. 1995 Feb;18(1):19-24.

202. Randomisation in Clinical trials. Beller EM, Gebski V, Keech AC. *Med J Aust* 2002; 177:565-567

203. Pulmonary infiltrates in immunocompromised patients: diagnosis by cytological examination of bronchoalveolar lavage fluid. Young JA, Hopkin JM, Cuthbertson WP. *J Clin Pathol* 1984; 37:390–397

204. Diagnostic yield of bronchoalveolar lavage in pneumonitis occurring after allogenic bone marrow transplantation. Cordonnier C, Bernaudin J-F, Fleury J, et al. *Am Rev Respir Dis* 1985; 132:1118–1123

205. Clinical utility of bronchoalveolar lavage in immunocompromised hosts. Pisani RJ, Wright AJ. *Mayo Clin Proc* 1992; 67:221– 227

206. The yield of bronchoalveolar lavage in the etiological diagnosis of pneumonia in leukemia and lymphoma patients. Marra R, Pagano L, Pagliari G, et al. *Eur J Haematol* 1993; 51:256–258

207. Clinical role of bronchoalveolar lavage in adults with pulmonary disease. *American Thoracic Society. Am Rev Respir Dis* 1990; 142: 481-486

208. Pulmonary Complications Occurring After Allogeneic Bone Marrow Transplantation: A Study of 130 Consecutive Transplanted Patients. Cordonnier C, Benaudin J, Bierling P, Vernant J et al. *Cancer* 1986; 58: 1047-1054,

209. NIH Conference. The Laboratory Evaluation of Opportunistic Pulmonary Infections Shelhamer JH, Gill VJ, Quinn TC, Crawford S, Ognibene FP et al. *Annals of Internal Medicine* 1996 ; 124: 585-599

210. A randomized, controlled trial of prophylactic ganciclovir for cytomegalovirus pulmonary infection in recipients of allogeneic bone marrow transplants; The City of Hope-Stanford-Syntex CMV Study Group. Schmidt GM, Horak DA, Zaia JA et al. *New England Journal of Medicine* 1991; 324:1005-1011

211. Value of open-lung biopsy in 87 immunocompromised patients with pulmonary infiltrates. Cheson BD, Samlowski WE, Tang TT, Spruance SL. *Cancer* 1985 Jan 15;55(2):453-9.

212. Anti-B-cell monoclonal antibody treatment of severe post-transplant B-lymphoproliferative disorder: prognostic factors and long-term outcome. Benkerrou M, Jais JP, Leblond V, Le Deist F, Blanche S, et al. *Blood* 1998; 92:3137–3147.

213. Role of Epstein–Barr virus DNA load monitoring in prevention and early detection of post-transplant lymphoproliferative disease. Stevens SJ, Verschuuren EA,Verkuujlen SA,Van Den Brule AJ,Meijer CJ, Middeldorp JM. *Leuk Lymphoma* 2002; 43: 831–840.

214. Airflow obstruction after myeloablative allogeneic hematopoietic stem cell transplantation. Chien JW, Martin PJ, Gooley TA, Flowers ME, Heckbert SR, Nichols WG, Clark JG. *Am J Respir Crit Care Med* 2003; 168: 208–214.

215. Obstructive lung disease after allogeneic marrow transplantation. Clinical presentation and course. Clark JG, Crawford SW, Madtes DK, Sullivan KM. *Ann Intern Med* 1989; 111: 368–376.

216. NHLBI workshop summary. Idiopathic pneumonia syndrome after bone marrow transplantation. Clark JG, Hansen JA, Hertz MI, Parkman R, Jensen L, Peavy HH. *Am Rev Respir Dis* 1993; 147: 1601– 1606.

217. Rapid simultaneous measurement of multiple cytokines in childhood oncology patients with febrile neutropenia: increased interleukin (IL)-8 or IL-5 correlates with culture-positive infection. Hodge G, Osborn M, Hodge S, Nairn J, Tapp H, Kirby M, Sepulveda H, Morgan E, Revesz T, Zola H. *Br J Haematol*. 2006 Jan; 132(2): 247-8.

218. An analysis of interleukin-8, interleukin-6 and C-reactive protein serum concentrations to predict fever, gram-negative bacteremia and complicated infection in neutropenic cancer patients. Engel A, Mack E, Kern P, Ker WV. *Infection* 1998: 126; 213–221.

219. Rapid simultaneous measurement of multiple cytokines using 100 microl sample volumes – association with neonatal sepsis. Hodge G, Hodge S, Haslam R, McPhee A, Sepulveda H, Morgan E, Nicholson I, Zola H. *Clin Exp Immunol*. 2004 Aug; 137(2): 402-7.

220. Cytokine expression in severe pneumonia: a bronchoalveolar lavage study. Montón C, Torres A, El-Ebiary M, Filella X, Xaubet A, de la Bellacasa JP. *Crit Care Med*. 1999 Sep; 27(9): 1745-53.

221. Bronchoscopically induced bleeding: a summary of nine years' Cleveland Clinic experience and review of the literature. Cordasco EM, Mehta AC, Ahmad M. *Chest* 1991; 100: 1141-47

222. The IP-10 chemokine binds to a specific cell surface heparan sulfate site shared with platelet factor 4 and inhibits endothelial cell proliferation. Luster AD, Greenberg SM, Leder P. *J Exp Med.* 1995 Jul 1;182(1):219-31.

223. Human IP-10 selectively promotes dominance of polyclonally activated and environmental antigen-driven IFN-gamma over IL-4 responses. Gangur V, Simons FE, Hayglass KT. *FASEB J*. 1998 Jun; 12(9):705-13

224. Temporal changes in cytokine/chemokine profiles and pulmonary involvement in severe acute respiratory syndrome. Chien JY, Hsueh PR, Cheng WC, Yu CJ, Yang PC. *Respirology*. 2006 Nov; 11(6): 715-22.

225. CXCR3 and IFN protein-10 in Pneumocystis pneumonia. McAllister F, Ruan S, Steele C, Zheng M, McKinley L, Ulrich L, Marrero L, Shellito JE, Kolls JK. *J Immunol.* 2006 Aug 1;177(3):1846-54.

226. A role for MCP-1/CCR2 in interstitial lung disease in children**.** Hartl D, Griese M, Nicolai T, Krauss-Etschmann S et al. *Respiratory Research* 2005, 6**:**93

227. The role of monocyte chemotactic protein-1 (MCP-1) in the recruitment of monocytes and CD4+ T cells during a pulmonary Cryptococcus neoformans infection. Huffnagle GB, Strieter RM, Standiford TJ, McDonald RA, Burdick MD, Kunkel SL, Toews GB. *J Immunol.* 1995 Nov 15;155(10):4790-7.

228. Chemokine-mediated recruitment of NK cells is a critical host defense mechanism in invasive aspergillosis. Morrison BE, Park SJ, Mooney JM, Mehrad B. *J Clin Invest*. 2003 Dec;112(12):1862-70

229. Effect of amphotericin B treatment on kinetics of cytokines and parameters of fungal load in neutropenic rats with invasive pulmonary aspergillosis. Becker MJ, de Marie S, Fens MH, Verbrugh HA, Bakker-Woudenberg IA. *J Antimicrob Chemother.* 2003 Sep; 52(3): 428-34.

230. Chemokines and interleukin-18 are up-regulated in bronchoalveolar lavage fluid but not in serum of septic surgical ICU patients. Mathiak G, Neville LF, Grass G, Bohlen H, Wassermann K, Hoelscher AH et al. *Shock.* 2001 Mar; 15(3): 176-80.

231. Cytokine and chemokine responses following pulmonary challenge with Aspergillus fumigatus: obligatory role of TNF-alpha and GM-CSF in neutrophil recruitment. Schelenz S, Smith DA, Bancroft GJ. *Med Mycol*. 1999 Jun; 37(3): 183-94.

232. MCP-1 protects mice in lethal endotoxemia. Zisman DA, Kunkel SL, Strieter RM, Tsai WC, Bucknell K, Wilkowski J, Standiford TJ. *J Clin Invest*. 1997 Jun 15; 99(12): 2832-6.

233. Interferon-Inducible Protein 10, but Not Monokine Induced by Gamma Interferon, Promotes Protective Type 1 Immunity in Murine Klebsiella pneumoniae Pneumonia. Zeng X, Moore TA, Newstead MW, Standiford TJ et al. *Infect Immun*. 2005 Dec; 73 (12): 8226-36 16299319

234. Molecular Machinations: Chemokine Signals in Host-Pathogen Interactions. Chensue SW. *Clinical Microbiology Reviews*, October 2001; 14 (4): 821-835

235. Inhibition of human endothelial cell chemokine production by the opportunistic fungal pathogen Cryptococcus neoformans. Mozaffarian N, Casadevall A, Berman JW. *J. Immunol*. 2000; 165: 1541-1547

236. Metalloproteases from Pseudomonas aeruginosa degrade human RANTES, MCP-1, and ENA-78. Leidal KG, Munson KL, Johnson MC, Denning GM. *J Interferon Cytokine Res.* 2003 Jun; 23(6): 307-18.

237. Compartmentalized expression of RANTES in a murine model of endotoxemia. VanOtteren GM, Strieter RM, Kunkel SL, Paine R 3rd, Greenberger MJ, Danforth JM, Burdick MD, Standiford TJ. *J Immunol.* 1995 Feb 15; 154(4): 1900-8.

238. Inflammatory response associated with pulmonary complications in non-HIV immunocompromised patients. Agusti C, Rano A, Rovira M, Filella X, Benito N, Moreno A, Torres A *Thorax*. 2004 Dec; 59(12): 1081-8.

239. Th1 and Th2 cytokines in mice with invasive aspergillosis. Cenci E, Perito S, Enssle KH, Mosci P, Latgé JP, Romani L, Bistoni F. *Infect Immun.* 1997 Feb; 65(2):564-70.

240. Pulmonary Complications Following Hematopoietic Stem cell Transplantation: Diagnostic Approaches. Sirithanakul K, Salloum A, soubani A O et al. *Am J Hematol*. 2005. 80: 137-146

TABLE 1: Selected studies of bronchoscopy yield and complications in similar populations

	Patient population	Number of severely immuno-compromised patients (if applicable)	Indications	FOB overall yield and technique	BAL	TBB	Infectious aetiology	Management change/ impact on mortality	PTx	Major Bleeding [t]	Respiratory failure^	Death possibly related to FOB
Peikert[174] 2005	10 BMT, 18 chemotherapy (disease not specified)		Neutropenia, fever and infiltrates	49% BAL/TBB	49%	Addit. diagnoses 3%	76%	51% management change	0	0	5.7	0
Stoller[1] 2004	104 Haematological malignancy, chemotherapy, bone marrow or solid organ transplant, long-term or high-dose corticosteroids	46 haematological malignancy	Infiltrates	51% BAL/ TBB/ PSB	48%	60%	79	NR	0	9.6	2	0
Murray[2] 2001	25 patients with leukaemia or lymphoma, post SCT or high dose chemotherapy	3 allo-SCT, 5 HDC	Pulmonary infiltrates or deteriorating respiratory state	36% BAL	36%	NA	100	Management change 28%, no difference in mortality	0	0	0	0
Hohenadel[16] 2001	95 Neutropenic haematology patients (AML, ALL, CML, NHL, aplastic anaemia and agranulocytosis. 9 BMT)		fever, respiratory symptoms, raised CRP and new radiographic findings of infiltrates	68% -BAL, TBB	65	64% (7/11 patients)	91	Management change 84% but not always due to FOB	0	0	0	0
Rano[66] 2001	200 immunocompromised patients including 53 BMT and 68 haematological malignancy		Pulmonary infiltrates,	59% wash, BAL, TBB, endobronchial Bx.	51%	55% (6/11 cases, 2 infectious)	77%	52% management change, no difference in mortality	0	0.7	0.7	
Huaringa[60] 2000	BMT – allo + auto – 89 patients	44 allo BMT patients[*]	New radiographic infiltrates	45%[*] BAL	45%[*]	NA	66%		0	0	0	0

Gruson[180] 2000	93 patients with neutropenia due to high-dose chemotherapy (for haematological malignancy) or stem cell transplant	71 patients with HDC or allo-BMT	Respiratory failure admitted to ICU (non-intubated) and febrile neutropenia	49%BAL	49%	NA	84% in HDC + allo BMT	28% change in management but no impact on survival	0	3.2	6.5	0
Ramila[182] 2000	Chemotherapy, BMT (auto or allo)	20 (22 FOB + BAL)	fever for 5 days and normal CXR	55% BAL	55%	NA	100%	27%	0	0	0	0
Whittle[54] 1999	33 Auto and allo BMT		Routine prior to conditioning and at neutropenia onset (B1 and B2)	18% B1, 13% B2 (BAL alone)	18% B1, 13% B2	NA			0	0	0	0
Glazer[36] 1998	62 Auto and allo BMT	42 allo BMT	Dyspnoea, hypoxia, new CXR infiltrates and no prior diagnosis	67% BAL	62%	5%	52%	79% management change	0	0	0	0
White[15] 1997	BMT recipients – allo	38 allo BMT	Fever, respiratory symptoms and/ or pulmonary infiltrates	31% BAL/ TBB	20 (14/68)	24% (10/42)	78%	Change in management 24%, no impact on mortality	4%	Epistaxis requiring packing	7%	1.5%, but autopsy indicated sepsis rather than FOB complic-ation.
Dunagan[78] 1997	BMT – auto and allo	81 allo BMT	infiltrates	46% BAL/ PSB TBB (PSB exclusive source of diagnosis in 13%. TBB only in 2 patients)	38%	NR	89%	41% change in management, no difference in mortality		1.4%	4.2%	2.8%
Vaughan[50] 1991	Patients undergoing high dose chemotherapy leukaemia, or BMT.	53	At onset of neutropenia	57% BAL	57%	NA			0	0	0	0

Cordonnier[208] 1987	Allo BMT	58 (66 bronchoscopies)	Chest Xray infiltrates on surveillance	66% BAL	66%	NA	85%	Not reported	NR	NR	NR	NR
Milburn[48] 1987	Allo BMT	30	infiltrates	80 BAL	80%	NA	-	-	0	0	0	0
Cornonnier[204] 1985	Allo BMT	36 patients	Infiltrates	50% BAL	50%	NA	43%	Not reported	0	0	0	NR

Definition of severely immunocompromised is as per our study population: Acute leukaemia, allogeneic bone marrow transplantation, Mabthera + Fludarabine
**results pertain to relevant severely immunocompromised patient population only, not whole study population*
^ Respiratory failure defined as need for intubation or non-invasive ventilation or observation in intensive care unit following procedure, when not previously requiredt
t Major bleeding defined as haemorrhage which was prolonged, difficult to control, causing haemodynamic instability or requiring blood transfusion.
BAL: Bronchoalveolar lavage
TBB: Transbronchial biopsy
PTx: pneumothorax
NR: not reported
NA: not applicable