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Urpo Kinnunen

BLOOD CULTURE FINDINGS DURING NEUTROPENIA IN ADULT PATIENTS WITH ACUTE MYELOID LEUKAEMIA

THE INFLUENCE OF THE PHASE OF THE DISEASE, CHEMOTHERAPY AND THE BLOOD CULTURE SYSTEMS

FACULTY OF MEDICINE,
INSTITUTE OF CLINICAL MEDICINE,
DEPARTMENT OF INTERNAL MEDICINE,
UNIVERSITY OF OULU;
CLINICAL MICROBIOLOGY LABORATORY, OULU UNIVERSITY HOSPITAL,
DEPARTMENT OF INFECTION CONTROL, OULU UNIVERSITY HOSPITAL;
LAPLAND CENTRAL HOSPITAL

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The influence of the phase of the disease, chemotherapy and the blood culture systems

Academic dissertation to be presented with the assent of the Faculty of Medicine of the University of Oulu for public defence in Auditorium 7 of Oulu University Hospital, on 19 November 2010, at 12 noon

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Supervised by
Docent Pirjo Koistinen
Docent Markku Koskela
Docent Hannu Syrjälä

Reviewed by
Docent Olli Meurman
Docent Esa Rintala

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Abstract

In Oulu University Hospital Haematological Ward during the years 1990–1991, a manual blood culture system was able to detect bloodstream infection (BSI) in 23% of febrile episodes of patients with acute myeloid leukaemia (AML), whereas during the years 1992–1993 an automated continuous-monitoring blood culture system (CMBCS) BacT/Alert® detected BSI in 40% of febrile episodes ($p = 0.043$). During the years 1997–2003, regimens containing high-dose cytarabine predisposed patients to laboratory-confirmed BSI (LCBI) with an odds ratio (OR) of 2.3 (with 95% confidence interval (CI) from 1.2 to 4.2). The LCBI risk was lowest after thioguanine-containing regimens (OR 0.26, 95% CI; 0.12–0.58). In the register data (years 1992–2006) from the prospective multi-centre AML -92 trial, when compared to cycle I, the OR for LCBI was significantly higher (from 4.8 to 5.8) in subsequent cycles ($p < 0.001$). In all, 67% of mortality due to BSI occurred in patients with active leukaemia.

An inoculum of microorganisms to produce 10 colony-forming units (cfu)/ml of 10 gram-positive coccal strains, 10 gram-negative bacillar strains and 8 *Candida* yeast strains was cultured in BacT/Alert® blood culture bottles in the presence of several chemotherapeutic drugs. Of the chemotherapeutic drugs tested, the anthracyclines exhibited inhibitory effects on the growth of microorganisms in concentrations corresponding to the therapeutic levels. In the standard bottles, doxorubicin increased the incubation time of gram-positive cocci and idarubicin increased the incubation time of *Candida glabrata*. However, no increase in the incubation time of any microbes was detected in the antimicrobial-neutralizing FAN bottles.

In conclusion, the use of CMBCSs has resulted in an increased LCBI rate in neutropenic AML patients. In general, chemotherapeutic agents have no significant inhibitory effects on the growth of common microbial pathogens in blood culture. The detection of some difficult-to-culture microbial strains – *C. glabrata* for example – in blood cultures may be impaired by the presence of chemotherapeutics in blood. The chemotherapeutics may also affect the LCBI rate in other ways. As a predictor of adverse outcome of infection, the presence of active leukaemia is more important than the type of chemotherapy being administered.

Keywords: acute myeloid leukaemia, barrier function, bloodstream infection, chemotherapy, neutropenia

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Abbreviations

AM	alimentary mucositis
AML	acute myeloid leukaemia
AraC	cytarabine
ATCC	American Type Culture Collection
BHI	brain heart infusion
BSI	bloodstream infection
cfu	colony-forming unit
CD AraC	conventional-dose cytarabine
CDC	Centers for Disease Control and Prevention
CI	confidence interval
C_{max}	maximum plasma concentration
CMBCS	continuous-monitoring blood culture system
CO_2	carbon dioxide
CoNS	coagulase-negative staphylococci
CR	complete remission
CRP	C-reactive protein
DNA	deoxyribonucleic acid
DTIC	dacarbazine
EORTC	European Organisation for Research and Treatment of Cancer
FAB	French-American-British
FLG	Finnish Leukaemia Group
5-FU	5-fluorouracil
FUO	fever of unknown origin
GI	gastrointestinal
h	hour(s)
HD AraC	high-dose cytarabine
HSCT	haematopoietic stem cell transplantation
ICC	intense consolidation chemotherapy
Ida	idarubicin
i.v.	intravenous
LCBI	laboratory-confirmed bloodstream infection
OR	odds ratio
PCR	polymerase-chain reaction
PMN	polymorphonuclear neutrophil

PRRs	pattern recognition receptors
p.o.	*per os*
RNA	ribonucleic acid
ROS	reactive oxidative species
sp.	species
TG	thioguanine
VGS	*viridans*-group streptococci
WHO	World Health Organization

List of original publications

This thesis is based on the following publications, which are referred to in the text and the tables by Roman numerals I to V:

I Kinnunen U, Syrjälä H, Koskela M, Kujala P & Koistinen P (1996) Continuous-monitoring blood culture screening system improves the detection of bacteremia in neutropenic patients. Scand J Infect Dis 28(3): 287–292.

II Kinnunen U, Koistinen P, Ohtonen P, Koskela M & Syrjälä H (2008) Influence of chemotherapy courses on the rate of bloodstream infections during neutropenia in adult acute myeloid leukaemia. Scand J Infect Dis 40(8): 642–647.

III Syrjälä H, Ohtonen P, Kinnunen U, Räty R, Elonen E, Nousiainen T, Jantunen E, Remes K, Itälä-Remes M, Silvennoinen R, Koistinen P & the Finnish Leukaemia Group. Bloodstream infections during chemotherapy-induced neutropenia in adult patients with acute myeloid leukaemia: treatment cycle matters. Eur J Clin Microbiol Infect Dis, DOI 10.1007/s10096-010-0984-1.

IV Kinnunen U, Koistinen P, Syrjälä H & Koskela M (2000) Effect of anti-neoplastic agents on the recovery of bacteria and yeasts in an automated blood culture system. Scand J Infect Dis 32(1): 63–67.

V Kinnunen U, Syrjälä H, Koistinen P & Koskela M (2009) Idarubicin inhibits the growth of bacteria and yeasts in an automated blood culture system. Eur J Clin Microbiol Infect Dis 28(3): 301–303.

Table of contents

Abstract
Acknowledgements **5**
Abbreviations **7**
List of original publications **9**
Table of contents **11**
1. Introduction **15**
2. Review of the literature **17**
2.1 Host defence and neutrophilic granulocytes ... 17
2.1.1 Host defence mechanisms ... 17
2.1.2 Formation of neutrophilic granulocytes ... 17
2.1.3 Role of neutrophilic granulocytes at host defence ... 18
2.2 Acute myeloid leukaemia (AML) ... 19
2.2.1 Etiology and epidemiolgy ... 19
2.2.2 Diagnosis and classification ... 19
2.2.3 Therapy of AML ... 20
2.3 Chemotherapeutic agents used in AML ... 22
2.3.1 Plasma levels ... 22
2.3.2 Effects on microorganisms ... 23
2.4 Factors affecting susceptibility to infections in AML ... 24
2.4.1 Impaired immune defence ... 24
2.4.2 Disruption of barriers ... 25
2.4.3 The phase of the disease ... 27
2.5 Fever during neutropenia in patients with cancer ... 27
2.5.1 Definition ... 27
2.5.2 Clinically documented infectios ... 27
2.5.3 Microbiologically documented infections ... 28
2.5.4 Non-infectious causes of fever ... 31
2.5.5 Fever of unknown origin (FUO) ... 31
2.6 Diagnosis of bloodstream infections (BSI) in neutropenic patients ... 32
2.6.1 Signs and symptoms ... 32
2.6.2 Inflammatory markers ... 32
2.6.3 Blood culture ... 33
2.6.4 Novel techniques to detect BSI ... 39
2.6.5 Observations on blood culture systems ... 39

2.7 Therapy of suspected or detected neutropenic BSI 44
2.7.1 Supportive and preventive measures 44
2.7.2 Initial antimicrobial therapy.. 44
2.8 The outcome of neutropenic BSI... 45
3. Aims of the study 47
4. Materials and methods 49
4.1 Study location 49
4.2 Patients... 49
4.3 Chemotherapy protocols 49
4.3.1 Finnish Leukaemia Group (FLG) AML 86 -protocol 49
4.3.2 FLG AML 92 -protocol.. 50
4.3.3 Other FLG chemotherapy regimens... 51
4.4 Laboratory methods used in clinical studies.. 52
4.4.1 Blood cultures 52
4.4.2 Identification of microorganisms 53
4.4.3 Other laboratory tests 53
4.5 Treatment of infections during neutropenia.. 54
4.6 Collection of the clinical data (I, II and III) 54
4.7 *In vitro* studies using simulated blood cultures (IV and V)................... 55
4.7.1 Microbial strains and chemotherapeutic agents.......................... 55
4.7.2 Preparation of cultures and the study protocol 56
4.8 Statistical analyses 57
5. Results 59
5.1 Study population.. 59
5.2 Neutropenia 60
5.3 The febrile and infection espisodes 61
5.4 Laboratory-confirmed bloodstream infection (LCBI) rate 62
5.4.1 The impact of the blood culture system on LCBI rate................. 62
5.4.2 The associations of the chemotherapy and the phase of the disease with LCBI rate 63
5.5 Blood culture isolates 64
5.6 Inflammatory response and infection data.. 67
5.7 Gastrointestinal (GI) toxicity 68
5.8 Mortality 68
5.9 The effect of chemotherapeutic agents on microbial growth in *in vitro* -studies 69

5.9.1 Microbial growth in the absence of chemotherapeutic agents 69
5.9.2 The effect of the chemotherapeutic agents in the standard bottles.... 69
5.9.3 The effect of the chemotherapeutic agents in the FAN-bottles ... 71
6. Discussion **73**
6.1 The study protocol 73
6.1.1 The clinical studies 73
6.1.2 The *in vitro* -studies 75
6.2 Blood culture results 76
6.2.1 Impact of the blood culture system on recovery of isolates from blood culture........ 76
6.2.2 Impact of the chemotherapy and the phase of the disease on recovery of isolates from blood culture 79
6.2.3 Associations between dose of AraC, GI toxicity and recovery of isolates from blood culture 81
6.3 Mortality due to infection 82
6.4 Fever of unknown origin – what is it? 83
6.5 Future prospects........ 84
7. Conclusions **87**
References
Appendix
Original publications

1. Introduction

Modern therapy of acute myeloid leukaemia (AML) was introduced four decades ago when two new chemotherapeutics cytarabine (AraC) and daunorubicin were introduced into use (Löwenberg *et al*. 1999). A disease that once was incurable, rapidly progressing to death, is nowadays potentially curable. However, a great number of patients still succumb to the illness, either in remission or in refractory or relapsed phases of the disease. Pronounced mortality and morbidity are associated with severe neutropenia caused by the active disease itself or by chemotherapy-induced bone marrow failure. The treatment of infections places a major burden on the health care system. Infections delay scheduled chemotherapy regimens and worsen prognosis.

The most feared infection in neutropenic patients is bloodstream infection (BSI), which significantly increases the mortality rate of these patients (Klastersky *et al*. 2007). Due to impaired host defence mechanisms during the period of neutropenia, it is crucially important to identify the BSI and initiate appropriate antimicrobial treatment. The identification of the BSI by clinical means is complicated due to the fact that in neutropenic patients, the common signs of infection are usually absent and the only clinical sign of BSI is fever (Viscoli *et al*. 1999, Bodey 2009). Accordingly, in the context of neutropenic fever, blood cultures are always obtained and empirical antimicrobial therapy for possible BSI is prescribed. Historically, the rate of laboratory-confirmed BSI (LCBI) – i.e. blood culture positive – has been quite low (Gurwith *et al*.1978, Rintala 1994) but with modern blood culture systems, the rate has increased to a much higher level, from 43 % to 59 % (Harter *et al*. 2006, Hämäläinen *et al*. 2008). However, even in the era of the modern blood culture systems, unexplained fevers in patients with haematological malignancies often respond to antimicrobial therapy (Harter *et al*. 2006), implying that the fever was attributable to a bacterial infection. It seems likely that a considerable share of fever of unknown origin (FUO) in this patient group is caused by infection, compelling clinicians to prescribe and continue broad-spectrum antimicrobial treatment until the fever has resolved or neutropenia has ceased. If the non-infectious causes of fever could be distinguished from infections, especially from BSI, unnecessary antimicrobial treatment could be avoided. Two questions arise: to what extent are the FUOs in patients with chemotherapy-induced neutropenia BSIs and are there some factors that have an adverse effect on results of blood cultures.

Some of chemotherapeutic agents possess antimicrobial properties. This kind of effect has been observed *in vitro* mostly on agar plates (Hamilton-Miller 1984,

Bodet *et al*. 1985) but also in blood cultures (Hopfer *et al*. 1983, Peiris & Oppenheim 1993) although the phenomenon has been considered to have no clinical importance. Nonetheless, some modification of the LCBI rate attributable to chemotherapeutic drugs has been reported. However, instead of direct antimicrobial effects of chemotherapeutic agents, other effects of chemotherapeutics such as mucosal toxicity have been considered to account for these effects on the occurrence of LCBI. (Bochud *et al*. 1994, Cordonnier *et al*. 2003).

Studies on infections of neutropenic patients have been mostly performed on unselected cancer populations, consisting of patients with solid tumours, lymphomas and haematological malignancies. Neutropenic oncohaematologic patients may not be a homogenous group. For example, patients with acute leukaemia exhibit a lower response rate to antibiotic therapy, than patients with lymphomas or solid tumors (Del Favero *et al*. 2001), which could be a reason to examine this special population with more detail. In Finland, adult patients aged 65 years or less with AML have been treated with uniform protocols since 1986 (Elonen *et al*. 1998, Koistinen *et al*. 2007). Thus, this rather homogenous group of patients was used in the present study to investigate the relationship between chemotherapy and LCBI.

2. Review of the literature

2.1 Host defence and neutrophilic granulocytes

2.1.1 Host defence mechanisms

The immune defence mechanisms can be divided to the innate immune response, which is nonanticipatory, non-clonal and less specific and the adaptive immune response, which is induced, highly specific, anticipatory and clonal. The innate immunity attacks infectious deoxyribonucleic acid (DNA)/ribonucleic acid (RNA) carriers from the moment of first contact. Invading pathogens are recognized by diverse germline-encoded pattern recognition receptors (PRRs), which are distributed in three different cellular compartments: extracellular, membrane and cytoplasm. The major membrane-associated PRRs, Toll-like receptors and C-type lectin receptors recognize pathogens and generate inflammation signals to coordinate the innate immune response and they shape adaptive immune response, being assisted in this effect by microvascular endothelial cells (Zänker 2008). In innate immunity, neutrophilic granulocytes are the first and most prominent line of cellular defence (Kobayashi *et al*. 2005).

2.1.2 Formation of neutrophilic granulocytes

All blood cells are derived from haematopoietic stem cells in bone marrow. After stem cell division, daughter cells commit to either the lymphoid or myeloid lineage. Through division, differentiation and maturation, the myeloid progenitor cells give rise to red cells, thrombocytes, monocytes and granulocytes (Yoder 2009). At an early stage in granulopoiesis, the stem cells convert into unipotent myeloblasts, which differentiate and maturate subsequently to promyelocytes, myelocytes and metamyelocytes. The transition to the myelocyte stage is associated with the acquisition of secondary or “specific” granules, which give the characteristic staining that differentiates neutrophils from eosinophils and basophils. Neutrophlic metamyelocytes differentiate into band forms and segmented neutrophils, which constitute more than 50 % of the total granulocyte mass, primarily as a mobilizable pool of cells, in the bone marrow (Khanna-Gupta & Berliner 2009).

In humans, differentiation from myeloblasts to myelocytes normally requires ~ 7.5 days and from myelocytes to mature neutrophils ~ 6.5 days. Since neutrophil turnover is very fast, of the order of 10^{11} cells per day, the major proportion of

haematopoiesis is committed to production of neutrophils. Mature neutrophils are released into the bloodstream where their typical half-life is 6–8 h. Only 5 % of total neutrophils circulate. About 60 % of the neutrophils in the circulation are stored in the spleen and vessel walls (Khanna-Gupta & Berliner 2009).

2.1.3 Role of neutrophilic granulocytes at host defence

Recruitment of neutrophils from the bloodstream into the sites of infection is initiated by chemokines and cytokines for example secreted by infected epithelia. Bacteria also produce molecules that directly recruit neutrophils. Before their transmigration from the bloodstream through the endothelium to tissues, neutrophils begin to roll along vessel walls. This "rolling" is mediated by glycoproteins called selectins. Adhesion to the vessel wall is mediated by leukocyte adhesion molecules such as integrins. Once firmly bound, several neutrophil surface CD-molecules facilitate transmigration into tissues. At the infection site, microorganisms and microbial compounds activate the neutrophils via transmembrane receptors (Kobayashi *et al*. 2005, Ermert *et al*. 2008).

At the site of infection, neutrophils bind and ingest the invading microorganisms by a process known as phagocytosis, forming bacteria-containing phagosomes (Kobayashi *et al*. 2005). The phagocytosis of bacteria triggers the synthesis of neutrophil genes encoding immunomodulatory agents. These cytokines and chemokines recruit more neutrophils, modulate subsequent neutrophil functions and are involved in coordinating early responses of monocytes, macrophages, dendritic cells and lymphocytes in the inflammatory states. The production of these agents by neutrophils serves as a link between the innate and acquired immune responses (Scapini *et al.* 2000).

Phagocytosis of microorganisms triggers also the generation of superoxide radicals and other secondarily derived reactive oxidative species (ROS). Concomitant with the production of ROS, cytoplasmic granules fuse with bacteria containing phagosomes, thereby enriching the vacuole lumen with antimicrobial peptides and proteases (Kobayashi *et al*. 2005) of which the α-defensins are especially important (Rehaume & Hancock 2008).

Activated neutrophils can also eliminate microbes in the extracellular space by releasing chromatin decorated granular antimicrobials (Brinkmann *et al.* 2004), the so-called neutrophil extracellular traps (NETs), which capture and kill microorganisms such as *Shigella flexneri*, *Staphylococcus aureus* and *Candida albicans* (Ermert *et al*. 2008).

However, this attack strategy is not without a disadvantage, the action of degradative enzymes and the production of ROS can also damage host tissues (Kobayashi *et al*. 2005).

2.2 Acute myeloid leukaemia (AML)

AML is a morphologically and genetically heterogeneous clonal disorder of myeloid hematopoietic progenitor cells that lose their normal capacity to differentiate into mature blood cells and that have inappropriate proliferative activity. This leads to the accumulation of immature myeloid blast cells in the bone marrow and impaired normal hematopoiesis. Blast cells are liberated into the circulation and are able to infiltrate extramedullary tissues including liver, spleen, bone, the central nervous system and the lymph nodes (Wernig & Gilliland 2009).

Most patients seek medical attention for symptoms related to bone marrow failure. Anaemia, neutropenia and thrombocytopenia result in fatigue, infection and bleeding. Fever with or without an existing infection can occur. In addition, an elevated white blood cell count can lead to complications, such as leukostasis in the pulmonary and cerebral vasculature (Jabbour *et al*. 2006).

2.2.1 Etiology and epidemiology

The known risk factors for AML account for only a small number of diagnosed cases; most cases of AML develop without any identified leukemogenic exposure (Sandler & Collman 1987)**.** Only three enviromental factors are established causal agents for AML, namely radiation (Kossman & Weiss 2000), chronic benzene exposure (Savitz & Andrews 1997) and some chemotherapeutic agents (Pui *et al.*1991).

AML accounts for 80 percent of the acute leukaemias in adults. In Finland, the incidence of acute leukaemias has varied between 3.1 and 3.8 cases per 100 000 person years in females and between 3.5 and 4.6 cases in males (Finnish Cancer Registry 2009). The incidence of AML in adults in Europe is in the range 5–8 cases per 100 000 (Fey *et al*. 2008), while in the United States it is 2.7 cases per 100 000 (Jabbour *et al*. 2006). The incidence clearly increases with age, the median age at presentation being approximately 65 years (Deschler & Lübbert 2006).

2.2.2 Diagnosis and classification

At the end of 1990s, AML was diagnosed and classified by a combination of morphologic, cytochemical and immunophenotypic features on the basis of criteria devised by the French-American-British (FAB) Co-operative Group (Bennett *et al*. 1976, 1985a, 1985b, 1991). The final FAB classification included altogether eight different subtypes: AML without maturation (M0), AML with minimal maturation

(M1), AML with maturation (M2), acute promyelocytic leukaemia (M3), acute myelomonocytic leukaemia (M4), acute monocytic leukaemia (M5), acute erythroid leukaemia (M6), and acute megakaryoblastic leukaemia (M7).

Currently, the diagnosis of AML is based on morphology, cytochemistry, immunophenotype, cytogenetics and molecular genetics of peripheral blood and bone marrow samples. In adults, acquired clonal chromosomal abnormalities are found in 50 % to 80 % of AML cases (Heim & Mitelman 1992). Furthermore, approximately 30 % of patients with a normal karyotype express clonal genetic mutations (Ravandi *et al*. 2007).

The major limitation of the FAB classification was the absence of cytogenetic and molecular genetic features. These are now taken into account in the classification prepared by the World Health Organization (WHO). In contrast to the FAB classification, in the WHO classification, the blast cell count threshold in the blood or marrow for diagnosis of AML has been reduced from 30 % to 20 % (Vardiman *et al*. 2002). The WHO classification recognizes seven subgroups of AML (Vardiman *et al*. 2009). In the subgroup of AML with recurrent genetic abnormalities**,** patients with some genetic abnormalities should be considered to have AML regardless of the blast perecentage in the peripheral blood or bone marrow.

2.2.3 Therapy of AML

Untreated AML is invariably fatal. The median survival without specific therapy is about 11–20 weeks (Deschler & Lübbert 2006). Chemotherapy for AML is usually administered in two stages, induction therapy followed by post-remission therapy (Jabbour *et al*. 2006).

The purpose of the induction therapy is to achieve complete haematological remission (CR) with no clinical evidence of leukaemia (Jabbour *et al*. 2006). For more than 20 years, the standard induction regimen for adults with AML has been an anthracycline, usually daunorubicin, at a daily dose of 45 to 60 mg per square metre of body-surface area (m^2) for 3 days, combined with a conventional dose (100 to 200 mg /m^2/day) cytarabine (CD-AraC) for 7 days, e.g. 3 + 7 regimen (Vogler *et al.* 1992, Mayer *et al*. 1994). In Finland, thioguanine (TG) is included into the regimen (Elonen *et al*. 1998, Koistinen *et al*. 2007). Another anthracycline, idarubicin (Ida) has been shown to be at least as efficacious as daunorubicin at inducing CR (Wiernik *et al.* 1992) and improving survival (Berman *et al*. 1991, Wiernik *et al*. 1992).

With newly diagnosed AML, the induction therapy results in CR in most adults younger than 60 years (Wiernik *et al*. 1992), but virtually all relapse without

further therapy (Büchner *et al*. 1985). Thus post-remission therapy is essential if one wishes to eliminate residual disease. Cure rates are higher with post-remission dose-intense regimens than with regimens of lesser dose intensities. The options for postremission therapy include allogeneic haematopoietic stem cell transplantation (HSCT) and intense consolidation chemotherapy (ICC). Autologous HSCT has also been used but the results still need to be confirmed (Ravandi *et al*. 2007, Hamadani *et al*. 2008).

ICC is based on high dose (HD) AraC treatment. Originally, AraC was defined to be of HD, when the dose was 3.0 g/m^2 twice daily (Early *et al*.1982), but nowadays HD AraC is generally considered as $\geq$ 1 g/m^2 daily dose (Ravandi *et al*. 2007). Patients who are not candidates for HSCT should receive at least two to four cycles (Döhner *et al*. 2010) and patients eligible for transplantation should receive only one to two cycles of post-remission chemotherapy before transplantation (Morra *et al*. 2009). Increasing the intensity of consolidation chemotherapy is beneficial in younger adults but probably not useful in patients older than 65 years (Cassileth *et al*. 1992, Mayer *et al*. 1994, Bennett *et al*. 1997).

In allogeneic HSCT, very high doses of total body irradiation and chemotherapy (Thomas *et al*. 1977) or chemotherapy alone (Harousseau *et al*. 1997) destroy leukemic cells, normal marrow and the immune system, and an infusion of normal haematopoietic stem cells from an allogeneic histocompatible donor rescues the patient. AML is nowadays the most common indication for allogenous HSCT (Gratwohl *et al*. 2007).

One of the most important prognostic factors is cytogenetics and in this way AML patients can be divided into three prognostic groups: favourable, intermediate and unfavourable (Vardiman *et al*. 2009). Approximately 20 % of patients aged 60 years or younger belong to the favourable prognostic group. Long-term survival without allogeneic HSCT is achievable in the favourable group in 70% of patients, in the intermediate group it is about 40–50% whereas in the adverse group, it is under 10% (Löwenberg 2008).

Age *per se* is a prognostic factor. In all, 60–70% of younger AML patients (< 55 years) eligible for intensive treatment achieve remission (Rowe *et al*. 1994, Bennet *et al*. 1997) and 30–40% are alive at 4–5 years (Bennett *et al*. 1997). Unfortunately among the older patients, long-term survival beyond 5 years is below 20% (Bennett *et al*.1997).

Poor performance status, secondary AML and a previous haematological disorder are all adverse prognostic factors (Löwenberg *et al*. 1999, Estey 2001), as well as a leucocyte count greater than 20 x 10^9/L (Löwenberg *et al*. 1999),

and evidence of multidrug resistance (Leith *et al*. 1999). The haematopoietic cell transplantation comorbidity index is the most influential risk factor for non-relapse mortality and survival in patients with AML in first CR who undergo allogeneic HSCT (Sorror *et al*. 2007).

In the first CR, AML patients with favorable cytogenetics fare better with HD AraC consolidation chemotherapy than with allogenic HSCT (Hamadani *et al*. 2008), whereas patients with unfavourable cytogenetics (Cassileth *et al*. 1998, Yanada *et al*. 2005) and possibly also the patients in the intermediate-risk group (Yanada *et al*. 2005) have a higher 5-year survival with allogeneic HSCT than with ICC.

In Finland, with AML patients aged under 65 years treated with Ida- and Ara C-based intensive chemotherapy, the 5-year survival was 71%, 47%, 37 % and 8 % for the nontransplanted patients with favourable, intermediate/normal, intermediate/abnormal and adverse karyotypes, respectively (Koistinen *et al*. 2007).

2.3 Chemotherapeutic agents used in AML

2.3.1 Plasma levels

Anthracyclines

Ida is characterized by its very rapid metabolic transformation into a 13-alcohol derivative, idarubicinol, which is as active as the parent. Ida has a short terminal half-life of approximately 16 hours (h), whereas idarubicinol has a relatively long half-life of approximately 55 h (Robert 1993). A maximum plasma concentration (C_{max}) of 182 ± 79 nmol/l was observed in AML patients treated at a daily dose of 10 mg/m^2 of Ida (Bauer *et al*. 2005). For doxorubicin, C_{max} may be as high as 200 µmol/l (Greene *et al*. 1983) but a steady-state plasma concentration of only100 nmol/l has been measured in an experimental model (Baurain *et al*.1982).

AraC

The C_{max} of AraC was 74 ± 33 µmol/l during HD AraC treatment with a dose of 3 g/m^2 twice daily (Early *et al*.1982). Mean C_{max} around 60 µmol/l has been observed following administration of HDAraC treatment with a dose of 1g/m^2 twice daily (Sutoh *et al*. 2003). A steady-state plasma concentration of 94 ± 32 µmol/l was reached following a HD AraC treatment consisting of two bolus infusions (3 g/m^2) followed by a continuous infusion (330–3000 mg/m^2/day) in patients with acute or

chronic leukaemia (Liliemark *et al*. 1985). During continuous intravenous infusion of low dose Ara-C (20 mg/m^2/day), the steady state level of plasma AraC was much lower, in the range of 2.5 nmol/l to 122 nmol/l (Kreis *et al.* 1985).

Thioguanine (TG)

In a study with 13 adult AML patients who received 100 mg/m^2 of oral TG every 12 h, the C_{max} reached varied by 30-fold and ranged from 0.03 to 0.94 µmol/l with a mean elimination half-life of 110 min (min–max 45 to 240 min) (Brox *et al*. 1981). In children with acute lymphoblastic leukaemia, mean steady-state plasma TG concentration during continuous intravenous infusion was 2.7 ± 1.4 µmol/l (Lowe *et al*. 2001).

2.3.2 Effects on microorganisms

It has been known for a long time that chemotherapeutic agents have antimicrobial properties. Experimental *in vitro* studies on agar plates by Bodet *et al*. (1985) demonstrated that 5-fluorouracil (5-FU), mitomycin and etoposide at the expected plasma concentrations could inhibit many common aerobic and anaerobic bacteria to grow, whereas doxorubicin inhibited only some gram-positive bacteria like staphylococci, streptococci and *Corynebacterium*-strains but not aerobic gram-negatives like *Escherichia coli* and *Pseudomonas aeruginosa* and anaerobes like *Bacteroides spp*. and *Clostridium spp.*

Hopfer *et al*. (1983) conducted *in vitro* experiments to determine, whether certain commonly used chemotherapeutic drugs delay detection or inhibit microbial growth in Bactec 6B blood culture bottles, and whether resin-containing 16B bottles could alleviate the growth inhibition. The concentrations of chemotherapeutic drugs added to the bottles were calculated to correspond to peak concentrations in serum of patients during therapy. In addition, 10-fold higher and 10-fold lower concentrations of the anti-neoplastic agents were tested. The inocula of microorganisms used were about 1000 colony-forming units (cfu) for bacteria and 100 cfu for *C. albicans*. Methotrexate and acridinyl anisidide did not inhibit microbial growth in 6B bottles at any of the concentrations tested. At the lowest concentration of dacarbazine (DTIC) and 5-FU, all the microorganisms grew but with a delay. At the basic concentrations, DTIC did not inhibit *C. albicans* or *P. aeruginosa*, but it delayed detection of *S. aureus* and completely inhibited the growth of *E. coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*. Using the same drug concentrations, growth of the last four organisms could be detected in the 16B bottles almost as early as

in the 6B control (no-drug) bottles. At the 10-fold higher DTIC concentrations, these four microorganisms failed to grow in either of the test bottles. The results with 5-FU were similar. At the highest 5-FU concentration tested, growth of all microorganisms was inhibited. Only *S. aureus* was able to grow in 16B bottle but the detection was delayed for 72 h. At the basic concentrations of 5-FU, growth of all microorganisms was inhibited. *P.aeruginosa* and *S. aureus* eventually grew with a delay (72 h) regardless of which bottle was used.

Peiris & Oppenheim (1993) studied the effects of cyclophosphamide, doxorubicin and vincristine on the growth of microorganisms *in vitro* in a glass universal container of nutrient broth used as the blood culture bottle. In this experiment, each drug was tested at three concentrations i.e. the expected peak concentrations in adult patients, 10-fold higher and 10-fold lower. At least three consecutive 10-fold dilutions of each microbial isolate were used to inoculate the bottles. Growth of *E. coli* and *P. aeruginosa* was not affected by any of the chemotherapeutic agents. Doxorubicin delayed the growth of *S. aureus*, *Staphylococcus epidermidis*, *Streptococcus sanguis* and *C. albicans*. Considerable delays were mainly shown at high concentrations of drug and using low inocula of organism. The smallest inocula used in this study could be even less than 10 cfu.

Synergism or antagonism with antimicrobial agents has been demonstrated in one-third of combinations with antineoplastic agents against *S. aureus* (Jacobs *et al*. 1979). Nyhlen *et al*. (2002) showed that tobramycin expressed better bactericidal effect against *S. aureus* in the presence of doxorubicin and 5-FU than in the absence of these agents, and that the combination of meropenem and 5-FU had an antagonistic effect against one strain of *S. aureus*.

Kwok *et al*. (2010) showed with a broth microdilution method that aclarubicin was active against *C. albicans* with minimal inhibitory concentration (MIC) values ranging between 0.8 and 7.3 µmol/l. Four other agents, daunorubicin, doxorubicin, Ida and beta-lapachone, affected the morphology of *C. albicans*. The first three agents encouraged the fungus to grow predominantly in its yeast form, whereas beta-lapachone caused hyphal proliferation.

2.4 Factors affecting susceptibility to infections in AML

2.4.1 Impaired immune defence

Several chemotherapeutic agents can impair cell mediated immunity and humoral responses (Young & Weisdorf 2009) and untreated AML *per se* is associated to

impaired mobility and phagocytosis (Hofmann *et al*. 1998) by polymorphonuclear neutrophils (PMNs). However, the profound, even absolute granulocytopenia occurring in patients with AML, either because of the disease itself or else the chemotherapy itself (Young & Weisdorf 2009) is the most important factor that predisposes these patients to infections (Bodey *et al*. 1966).

The inverse relationship between granulocytes and infection was shown in the 1960s in patients with acute leukaemia (Bodey *et al*. 1966). In addition, the duration of granulocytopenia was important. There was a 60 % risk of infection if granulocytopenia persisted for 3 weeks and a 100% certainty of infection, if the granulocyte count was less than 0.1 x 10^9/l. There was a striking decrease in the incidence of infection, when the granulocyte level increased to more than 0.5 x 10^9/l. Lymphocyte levels had also an inverse relationship with the rate of infection, but with a lesser degree than the granulocyte levels. In contrast to the granulocyte levels, the levels of circulating lymphocytes may not accurately reflect the lymphocyte reserve. (Bodey *et al*. 1966). Bacteraemias are also more likely to occur in patients with low PMN counts (EORTC 1983, Velasco *et al*. 2003).

In clinical studies, the usual definition of neutropenia has been a blood PMN count of less than 1.0 x 10^9/l (EORTC 1983, Velasco et al. 2003) or less than 0.5 x 10^9/l (Bow *et al.* 2006) and in profound neutropenia, the PMN count is less than 0.1 x 10^9/l (Young & Weisdorf 2009). An alternative approach has been to use a granulocyte count less than 1.0 x 10^9/l (Klastersky *et al*. 1986, EORTC 1993).

2.4.2 Disruption of barriers

Single cytotoxic agents cause morphological alteration of gastrointestinal (GI) mucosa (Gwavava *et al*. 1981). Increased intestinal permeability is a generally accepted marker of gut disease or damage in patients treated for cancer (Bjarnason *et al.* 1995). Altered intestinal permeability and absorption of sugars have been demonstrated even in patients with AML without treatment (Sundstrom *et al*. 1998, Blijlevens *et al*. 2004). The presence of active leukaemia may be responsible for the increase of intestinal permeability via the production of proinflammatory cytokines (Nitenberg & Raynard 2000). A further increase in intestinal permeability occurs after the start of chemotherapy. The disturbance of permeability and adsorption may last up to 6 weeks from the start of chemotherapy (Blijlevens *et al*. 2004).

In patients with burns, the increased intestinal permeability, which occurs within 30 hours (Deitch 1990), is a significant risk factor for sepsis (Ziegler *et al*. 1988) and the extent correlates with severity of sepsis (LeVoyer *et al*. 1992)

suggesting, that the loss of the intestinal barrier function contributes to infection. On the other hand, intestinal permeability is increased following intravenous injection of endotoxins (O'Dwyer *et al*. 1988) suggesting that infection itself can contribute to impairment of the intestinal barrier function.

AraC infusions have been shown to cause GI necrosis (Slavin *et al*. 1978, Jones & Abramson 1983) and infections (Slavin *et al*. 1978) in patients with acute leukaemia. In an autopsy series, three phases of GI injury during chemotherapy were described (Slavin *et al*. 1978). The initial damage to the GI mucosa was observed during the first three days of AraC infusions. In colonic mucosa, it was characterized by the near total replacement of the normal crypt and surface mucus secreting epithelium by atypical degenerated undifferentiated cells. Progressive injury, characterized by increasing overall cellular necrosis, persisted for as long as one week whereas regeneration started five to nine days after the discontinuation of AraC infusions.

In essence, the alterations observed in the small bowel were similar to those seen in the colonic mucosa. In the gastric mucosa, the gastric glandular compartment was generally spared except in antrum where the alterations involved the pyloric glands. In oesophageal mucosa, the pathological change consisted of atypia and focal epithelial cell necrosis. It was postulated that AraC not only blocked deoxyribonucleic acid (DNA) synthesis but also had a direct effect on gastrointestinal stem cells (Slavin *et al*. 1978).

Mucositis is an important side effect of many anticancer treatments (Rapaport *et al*. 1999). Alimentary mucositis (AM) refers to mucosal lesions in GI tract (Keefe *et al*. 2007). It has been shown that intestinal mucosal surfaces are crucial sites of innate and adaptive immune regulation and immune activation is often related to increased mucosal permeability (Turner 2009). The paracellular pathway in the GI mucosa plays an important role in the regulation of intestinal permeability (Turner 2009).

The increased use of intravascular devices makes patients liable to infections caused by skin contaminants, for example staphylococci (Cordonnier *et al*. 2003). Prolonged use of central venous catheters is almost universal in the treatment of AML, at least in Finland (Koistinen *et al*. 2007). Disruption of barriers can occur also in the genitourinary tract due to urinary catheters (Young & Weisdorf 2009) and in the lung, where HD AraC can cause a toxic effect resembling adult respiratory distress syndrome (ARDS) (Andersson *et al*. 1985). In some studies, the AM and the pulmonary changes caused by HD AraC treatment have been associated with a high rate of *viridans*-group streptococci (VGS) bacteremias (Kern *et al*. 1990, Bochud *et al*. 1994).

GI toxicity of cancer treatment was graded by WHO in 1979. Six parameters – bilirubin, transaminases, alkaline phosphatase, oral findings, nausea/vomiting and diarrhoea – are divided into grades from 0 to 4 (World Health Organization 1979). In grade 0, no evidence of toxicity is found in any of the parameters. Up to grade 2, the laboratory parameters can rise to levels of 5 times higher than the reference values. In grades 3 and 4 toxicity, laboratory parameters rise over 5 times the reference values, oral ulcers are severe enough to allow only liquid diet or alimentation is not possible, vomiting requires therapy or is intractable and diarrhoea is intolerable and requires therapy or is haemorrhagic and results in dehydration.

2.4.3 The phase of the disease

Most of the serious infections of patients with acute leukaemia occur during ICC and relapse (Velasco *et al*. 2003). This may be partly due to changes in PMN function. The mobility of the PMNs improves and their ability to phagocytosis may return to normal, when remission is achieved (Hofmann *et al*. 1998).

2.5 Fever during neutropenia in patients with cancer

Fever is a common finding in patients with haematological malignancies, occurring in more than 80 % of the patients during or after chemotherapy (Klastersky 2004).

2.5.1 Definition

The usual definition of fever in neutropenic patients has been an axial temperature of 38.5°C or more on one occasion or 38°C or more on two occasions within 12 h (Giamarellou *et al*. 2000, Velasco *et al*. 2003).

2.5.2 Clinically documented infectios

The great majority of infections originate from the oral cavity and pharynx, skin and soft tissue, the lung and the intravenous access site (EORTC 1978, EORCT 1983, EORTC 1993 Velasco *et al*. 2003). The number of infections in intravenous access sites has been on the rise (EORTC 1993, Velasco *et al*. 2003, Wisplinghoff *et al*. 2003). AML patients tend to have more skin and soft tissue infections than other cancer patients and they have a disproportionate number of anorectal lesions and infections associated with intravenous catheters and needles (EORTC 1978, Madani 2000).

2.5.3 Microbiologically documented infections

Focal infections

E. coli is usually found in the urinary tract and and anorectal area, *S.aureus* in the skin/soft tissue, *Klebsiella* species *(sp.)* in the urinary tract and oral cavity and *P. aeruginosa* in skin/soft tissues, anorectal area and oral cavity (EORTC 1978). In a recent European study evaluating patients with haematological malignancies (Cattaneo *et al*. 2008), infections with *E. coli* were associated with acute leukaemia and occurred more often in the neutropenic phase, whereas an infection episode caused by *S. aureus* was more frequently observed in patients with an active haematological disease, a neutrophil count > 0.5 x 10^9/l and the absence of antimicrobial prophylaxis. Among non-transplanted patients, those with AML are at highest risk of developing invasive fungal infections. In an Italian study (Pagano *et al*. 2006), about 8 % of AML patients developed mould infections and 4 % had yeast infections; the majority of moulds recovered were *Aspergillus sp.*

Bloodstream infections (BSI)

Bacteraemia and fungaemia refer to the presence of bacteria or fungi in the blood. Today, the concept of bloodstream infection (BSI) is used to refer to an infection with bacteraemia and/or fungaemia. Laboratory-confirmed BSI (LCBI) means that the pathogen in blood has been successfully cultured (Garner *et al* 1996). In neutropenic cancer patients, BSI can originate from all common sites of infection (EORTC 1978). Intravenous site infections and GI infections are the two focal infections that most likely result in bacteraemia (EORTC 1978). The origin of BSI more commonly remains unknown in neutropenic than in non-neutropenic cancer patients (Velasco *et al*. 2006). The so-called primary BSI without any focal source of infection accounts for about one half of all LCBIs during neutropenia (Velasco *et al*. 2003, 2006, Wisplinghoff *et al*. 2003).

The rate of LCBI in febrile episodes in unselected neutropenic cancer populations has generally varied between 20 and 30 % (Table 1). The occurrence of LCBI has been higher in patients with haematological malignancies than in patients with solid cancers (Klastersky *et al*. 2007). In an unselected haematological population, LCBI rates exceeding 40 % has been reported (Table 1). In febrile AML patients with neutropenia, an LCBI rate as high as 59 % has been observed (Hämäläinen *et al*. 2008).

Table 1. The rate of laboratory-confirmed bloodstream infection (LCBI) in febrile neutropenic cancer patients.

Study	Collection of data	Patient No.	Cancer population	LCBI-rate
EORTC 1978	In Europe and USA	625	unselected	22 %
EORTC 1987	In Europe, USA and Middle East	872	unselected	29 %
EORTC 1993	In Europe, USA and Middle East 1988–1990	537	unselected	25 %
Madani 2000[1,2]	In Canada 1992–1994	42	AML	38 %
Cordonnier *et al*. 2003	In France 1995[3]	513	haematological	29 %
Sigurdardottir *et al*. 2005	In Norway 1998–2000	243	haematological	34 %
Harter *et al* 2006[1]	In Germany 2000–2003	161	haematological	43 %
Hämäläinen *et al*. 2008[2]	In Finland 1996–2005	84	AML	59 %

NOTE: AML = acute myeloid leukaemia; EORTC= the European Organization for Research and Treatment of Cancer.

[1]Antibacterial prophylaxis was in use.

[2]Single-centre data.

[3]Personal communication from the author.

While in the 1970s and early 1980s, gram-negative bacteria were most commonly encountered (EORTC 1978, 1987), gram-positive bacteria have become the major cause during the past decade (EORTC 1993, Elting *et al*. 1997, Wisplinghoff *et al*. 2003). In one report, as many as 81 % of BSI was caused by gram-positive organisms (Gonzales-Barca *et al*. 1996). The increase in the incidence of staphylococcal infections has been linked with the use of intravascular devices (Cordonnier *et al*. 2003, Velasco *et al*. 2003) and streptococcal infections with the use of HD AraC therapy (Bochud *et al*. 1994, Cordonnier *et al*. 2003). In addition, the use of trimethoprin/sulfamethoxazole and fluoroquinolones to prevent gram-negative infections has favoured the shift to gram-positive bacteraemias (Engels *et al*. 1998, Klastersky *et al*. 2007). In this decade, a predominance of gram-negative bacteraemia has been observed in some (Velasco *et al*. 2006) but not all studies (Wisplinghoff *et al*. 2003). The use of levofloxacin prophylaxis may have induced increased resistance of *E. coli* to fluoroquinolones and this might be the cause of this recent shift back to gram-negative bacteraemias (Cattaneo *et al*. 2008).

Coagulase-negative staphylococci (CoNS) have been the predominant gram-positive pathogen in neutropenic LCBI (Table 2), representing up to 36 % of isolates in blood cultures. BSI due to VGS seems to be more common in neutropenic patients than in those without neutropenia (Wisplinghoff *et al*. 2004). Nonetheless, the proportion VSG of blood culture isolates has remained small in many cases

Table 2. Species distribution of predominant pathogens in bloodstream infections of patients with malignant diseases in recent studies.

Variable	Hämäläinen *et al*. 2008	Malagola *et al*. 2008	Cherif *et al*. 2003	Hammerstrøm *et al*. 2008	Sigurdardottir *et al*. 2005	Wisplinghoff *et al*. 2003
Country	Finland	Italy	Sweden	Norway	Norway	USA
Study years	1996–2005	1997–2002	1988–2001	1995–2005	1998–2000	1995–2001
Malignancy	AML	AML	haematological	haematological	unselected	unselected
Neutropenia[1]	present	NI	NI	present	present	present
Total no. of isolates	165	77[2]	1402	373	122	798
Gram-positive cocci	57 %	61 %	50 %	48 %	46 %	61 %
CoNS	31 %	36 %	17 %	13 %	12 %	32 %
VGS	5 %[3]	NI	13 %	10 %	16 %	3 %
Staphylococcus aureus	NI	8 %	9 %	9 %	5 %	12 %
Enterococcus sp.	9 %	7 %	7 %	4 %	4 %	6 %
Gram-negative bacilli	42 %	39 %	44 %	48 %	49 %	25 %
Escherichia coli	16 %	NI	16 %	20 %	25 %	7 %
Klebsiella sp.	15 %	NI	10 %	7 %	7 %	5 %
Pseudomonas aeruginosa	3 %	10 %	6 %	8 %	4 %[4]	4 %
Enterobacter sp.	4 %[5]	NI	3 %	6%	5 %	3 %
Anaerobes	NI	NI	4 %	3 %	3 %	5 %
Fungi	0.4 %	NI	0.1 %	1%	2 %	9 %

NOTE: AML = acute myeloid leukaemia; NI = no information; CoNS = coagulase-negative staphylococci; VGS = *viridans*-group streptococci.

[1]Neutropenia = blood neutrophil count < 1.0 x 10^9/l in one of the unselected cancer populations (Wisplinghoff *et al*. 2003), < 0.5 x 10^9/l in the others.

[2]Only bacteraemias reported.

[3]*Streptococcus mitis* reported.

[4]*Pseudomonas sp.* reported.

[5]*Enterobacter cloacae* reported.

(Wisplinghoff *et al*. 2003), though in studies from the Nordic countries its share of the total number of blood culture isolates of patients with haematological malignancies has been as high as 16 % (Table 2). *S. aureus* has been an important pathogen in neutropenic cancer populations (Table 2) but it is recovered more often in the non-neutropenic than neutropenic phase of the cancer (Velasco *et al*. 2006, Cattaneo *et al*. 2008). Enterococci are important pathogens, especially in institutions where there is a predominance of gram-positive organisms (Table 2).

E. coli is the most common gram-negative finding in BSIs of neutropenic cancer patients. Other common gram-negative organisms found in blood cultures are *Klebsiella sp*. and *P. aeruginosa* (Table 2).

In data collected in USA in the late 1990s (Table 2), as many as 9 % of bloodstream isolates of neutropenic cancer patients were *Candida sp*. (Wisplinghoff *et al*. 2003). In the USA, when the *Candida sp*. were examined as a cause of candidemia, *Candida krusei* was most strongly associated with haematological malignancy and neutropenia (Horn *et al*. 2009). In a study from southern Europe, candidemia was observed in 8 % of febrile neutropenic patients with acute leukaemia (Megalakaki *et al*. 2006). Although in some Nordic countries the incidence of *Candida* BSIs has been reported to be increasing in the general patient population (Asmundsdottir *et al* 2002, Poikonen *et al*. 2003), in neutropenic patients, the proportion of fungi as a cause of BSI has remained very small (Table 2).

Polymicrobial BSI is more common in neutropenic than in non-neutropenic cancer patients (29% vs. 17 %, $p < 0.01$; Velasco *et al*. 2006). In a recent Nordic study of patients receiving chemotherapy for haematological malignancies, 12 % of BSIs were polymicrobial (Hammerstrøm *et al*. 2008). In a small study examining AML patients undergoing chemotherapy, 29 % of BSI were polymicrobial (Madani 2000).

2.5.4 Non-infectious causes of fever

In about one-thirds of cases, fever in a neutropenic patient is of a non-infectious origin (EORCT 1978). Fever can be a manifestation of malignancy, transfusion reactions, drug reactions, or endocrine disorders (Young & Weisdorf 2009). For example, AraC is well known for its ability to evoke fever (Slavin *et al*. 1978).

2.5.5 Fever of unknown origin (FUO)

In neutropenic cancer patients, fevers without any obvious infectious source tend to respond to antimicrobial therapy (Harter *et al*. 2006) suggesting that most neutropenic FUOs are infections. Even today, the proportion of FUO of all the febrile episodes of neutropenic patients with acute leukaemia can be quite high (44%; Harter *et al*. 2006). Natural history of neutropenic fever of unknown origin (FUO) tends to be much shorter than for traditional FUOs (Durack & Street 1991). Immediate empiric antimicrobial treatment is usually given.

2.6 Diagnosis of bloodstream infections (BSI) in neutropenic patients

2.6.1 Signs and symptoms

In neutropenic patients with BSI, fever may be the only sign of infection (Viscoli *et al*. 1999, Velasco 2006). For example, the presence of neutropenia hampers the detection of gram-negative bacillary pneumonia in chest X-ray (Valdivieso *et al*. 1977). On the other hand, a neutropenic patient with BSI may develop septic symptoms. Sepsis is a clinical syndrome defined by the presence of both infection and a systemic inflammatory response (Levy *et al*. 2003). This response is manifested by two or more of the following conditions: temperature > 38ºC or < 36ºC, heart rate > 90 beats per minute, respiratory rate > 20 breaths per minute or $PaCO_2$ > 32 mmHg, and white blood cell count > 12.0 x 10^9/l, < 4.0 x 10^9/l, or > 10 % immature forms (Bone *et al*. 1992). Understandably, in neutropenic patients, the white cell count cannot be used in diagnosing sepsis. Severe sepsis is associated with organ dysfunction, hypoperfusion, or hypotension and furthermore lactic acidosis, oliguria and alteration of mental status may develop. Septic shock is characterized by hypotension despite adequate fluid resuscitation along with the presence of perfusion abnormalities (Bone *et al*. 1992).

2.6.2 Inflammatory markers

C-reactive protein (CRP) is an acute phase protein that reflects ongoing inflammation or tissue damage via the increase in its plasma concentration. Due to its nonspecificity, CRP-values cannot be diagnostic on their own, instead they need be interpreted in conjuction with other clinical results. In healthy volunteer blood donors, the median serum concentration of CRP is 0.8 mg/l, the 90th percentile is 3.0 mg/l and 99th percentile is 10 mg/l. Following an acute phase stimulus, values can rise to more than 500 mg/ml (Pepys & Hirschfield 2003).

Febrile neutropenic cancer patients are unlikely to have a serious infection, when their serum CRP-level is below 30 mg/l (Starke *et al*. 1984) to 40 mg/l (Santolaya *et al*.1994, Manian 1995). The underlying malignancy has been shown to cause CRP elevations (Manian 1995) whereas chemotherapy, radiotherapy and transfusion reactions do not cause any elevation of the serum CRP-level over 30 mg/l in patients with acute leukaemia (Starke *et al*. 1984). In febrile patients with haematological malignancies, the median maximum serum level of CRP is higher when the blood culture is positive than when it is negative (Rintala *et al*. 1992), but

positive predictive values of CRP for non-CoNS bacteraemia were only 33–55 % in a recent study (Persson *et al*. 2004). In AML patients, CRP values are not able to predict worsening clinical status, because the rise in CRP tends to coincide with rather than preceding the development of severe sepsis (Hämäläinen *et al*. 2008).

In a German study, procalcitonin and interleukin-6 serum concentrations differed significantly between bacteraemic and non-bacteraemic episodes in neutropenic patients with haematological malignancies (von Lilienfeld-Toal *et al*. 2004). Procalcitonin seem to be able to discriminate fever due to systemic forms of infection from non-infectious causes (Sakr *et al*. 2008) and it may have a high negative predictive value for gram-negative bacteraemia (Prat *et al*. 2008). Compared with CRP, serum vascular endothelial growth factor was a more rapid indicator for sepsis in haematological patients with neutropenic fever (Hämäläinen *et al*. 2009). Nonetheless, these biomarkers have not gained a significant role in clinical practice.

2.6.3 Blood culture

A blood culture is defined as a culture of blood obtained from single venipuncture and inoculated into one or into multiple bottles or tubes (Cockerill *et al*. 2004).

General principles

In the past 20 years, there has been a substantial development in blood culture methods. Automated blood culture methods emerged to complement the manual blood culture methods in the 1980s (Reimer *et al*. 1997) and the automated continuous-monitoring blood culture systems (CMBCSs) were introduced in the 1990s (Thorpe *et al*. 1990, Nolte *et al*. 1993). In the late 1970s and early 1980s, studies were published that defined the optimal volume of blood per blood culture, number of consecutive blood cultures, and incubation time for the detection of bloodstream pathogens (Washington 1975, Ilstrup & Washington 1983). These parameters were established for manual blood culture systems that used basal culture media.

As a culture medium, soybean-casein digest has been adequate for recovering most pathogenic micro-organisms. Brain heart infusion (BHI) is probably as good as or even better than soybean-casein digest for recovering yeasts and some bacteria. Agitating bottles increases microbial recovery from aerobic blood culture bottles (Reimer *et al*. 1997).

The conventional practice has been to obtain blood specimens at or around the time of temperature elevation (Reimer *et al*. 1997) However, some patients with bacteraemia are not febrile (Clemmer *et al*. 1992) and bacteraemia may actually precede temperature elevations by one to two h (Bennett & Beeson 1954). In a recent multi-centre study, the likelihood of confirming BSI was not enhanced by collecting blood specimens at the time that patients experienced temperature spikes (Riedel *et al*. 2008). Nonetheless, since the fever is often the only sign of infection in neutropenic patients (Viscoli *et al*. 1999, Velasco 2006) and the antimicrobial therapy needs to be started as soon as possible (Shimpff *et al*. 1971), the practice of obtaining blood cultures at the first signs of severe infection, i.e. fever, chills or hypotension, is well justified. The current national guidelines recommend serially drawn blood cultures. However, if the clinical picture refers to septic shock, blood culture should be obtained only once and antimicrobial treatment should be started immediately.

The number of microorganisms in blood in adults is miniscule, typically fewer than 10 cfu/ml and often less 1 cfu/ml (Finegold *et al*. 1969, Kellog *et al*. 2000). Thus, the volume of blood cultured is an important variable in optimizing microbial recovery in adult patients. The recommended practice for a single blood culture is to culture ≥ 20 ml blood, divided evenly into an aerobic and an anaerobic blood culture bottle and to obtain two to three blood cultures over a 24 h period (Reimer *et al*. 1997). For adults, increasing the culture volume from 20 to 40 ml increased the yield by 19 % and increasing the volume up to 60 ml increased the yield by an additional 10 % (Li *et al*. 1994). With the modern CMBCSs and media, the increase of yield per ml is smaller and more blood cultures are needed to detect bloodstream pathogens than when manual blood culture method is used (Cockerill *et al*. 2004). With CMBCS, as many as four blood cultures may be needed for a detection rate of 99 % (Lee *et al*. 2007). Especially *P. aeruginosa* and *C. albicans* were often not detected with the initial blood culture.

In the manual blood culture era, the incubation time was at least five days but it could be even longer than two weeks (Washington 1975). The new CMBCS technology permits the use of quite short incubation times (Cockerill *et al*. 2004). In general, automated systems yield almost as many common pathogenic bacteria and yeasts after five days of incubation as after seven days (Wilson *et al*. 1993, Huang *et al*. 1998, Reisner & Woods 1999). Limiting the incubation and test period to five days yields fewer microorganisms ultimately judged as contaminants (Wilson *et al*. 1993).

Concerns about the inability to recover a pathogen due to antimicrobial therapy are well documented in the literature. It has been known that the use of penicillinase

in blood culture (Carleton & Hamburger 1963) can unmask false-negative cultures in patients receiving penicillins. Other means to enhance recovery of microorganisms in the presence of antimicrobials are the dilution of the blood sample in the culture medium, the addition of antimicrobial-inactivating enzymes to the blood culture and a lysis centrifugation method (Krogstad *et al*. 1981). A variety of commercial products have also been developed to increase microbial recovery from the blood of patients receiving systemic antimicrobial therapy (Appelbaum *et al*. 1983, Weinstein *et al*. 1995).

When a blood culture is detected to yield microbial growth, Gram-staining and subculture are performed (Washington 1975). Gram-staining can be very accurate in recognizing and determining some types of microorganisms. For instance, the sensitivity in detecting gram-positive cocci in clusters or chains/diplococci is about 95% (Søgaard *et al*. 2007).

Manual blood culture systems

The simplest blood culture system consists of bottles filled with broth medium and with a partial vacuum in the headspace. To convert these kinds of bottles into aerobic bottles, the oxygen concentration is increased by transiently venting bottles to room air after they are inoculated with blood. Unvented bottles remain relatively anaerobic. After inoculation, the bottles are incubated with or without agitation and inspected periodically for macroscopic evidence of growth, such as haemolysis, turbidity, gas production, darkening of the blood or the presence of visible colonies or a layer of growth on the fluid meniscus. A blind or terminal subculture is required to reliably recover pathogenic microorganisms from this type of bottle (Reimer *et al*. 1997).

Manual blood culture systems also used to be commercially available. Some systems used an agar slant in the bottle as the growth medium, some others utilized a manometric method based on the increasing pressure in the bottle caused by gases liberated by growing micro-organisms and some used a lysis-centrifugation method. Most of these methods were labour-intensive and some of them were not optimal for recovering bacteria (Reimer *et al*. 1997).

Automated detection blood culture systems

Bactec radiometric blood culture system (Bactec 460 model, Becton Dickinson Microbiology Systems) was the first of the automated blood culture systems.

The system detected microbial growth by monitoring the concentration of carbon dioxide (CO_2), more precisely, the increasing amount of ^{14}C in the bottle headspace. In the early 1980s, Becton Dickinson introduced a new generation of automated blood culture systems, the non-radiometric Bactec 660, 730 and 860 systems. Otherwise similar to the radiometric systems, these instruments used infrared spectrophotometry to detect CO_2 in samples of the bottle headspace atmosphere. High volume Bactec PLUS aerobic and anaerobic bottles (launched in 1983) became available, with 25 ml of enriched soybean-casein digest broth. They utilized blood inocula of 10 ml (Reimer *et al*. 1997).

Continuous-monitoring blood culture systems (CMBCS)

The most important technological advance in blood cultures during the past 30 years has been the development of CMBCS. These instruments electronically monitor bottles for evidence of microbial growth on a nearly continuous basis, typically once every 10 min. Data collected from this monitoring system are transmitted to a microcomputer, where they are stored and analyzed. Since many data points are collected per bottle every day, sufficient data are available to permit the use of sophisticated computer algorithms to determine when microbial growth has occurred. The more frequent test cycles and algorithms allow for earlier detection of microbial growth. CMBCSs incorporate the detection system, incubator and agitation mechanism into a single unit. The instruments test each bottle individually, thus obviating the need for manual manipulation of bottles after they have been placed in the instrument and this eliminates the possibility of cross-contamination between bottles. Most bottles of CMBCSs accept blood inocula up to 10 ml. No terminal subcultures are needed with CMBCS (Reimer *et al*. 1997).

BacT/Alert blood culture system. BacT/Alert (developed by Organon Teknika Corp, now manufactured by bioMerioux), introduced in 1990 (Thorpe *et al*. 1990) was the first commercial CMBCS. The base of each BacT/Alert bottle contains a solid sensor separated from the liquid contents of the bottle by a membrane permeable only to CO_2 (Thorpe *et al*. 1990). CO_2 liberated by growing microorganisms causes a colour change in the sensor. A light emitting diode, incorporated in the cell containing the bottle, shines a light into the sensor every 10 minutes and a photodiode generates a voltage signal proportional to the amount of yellow in the sensor and to the amount of CO_2 present in the sensor and blood-broth mixture. The signals are recorded by microcomputer and are analyzed by an algorithm, which recognizes three criteria

as evidence of microbial growth: 1) an initial reading that exceeds a predefined threshold, 2) a sustained linear increase in CO_2 concentration, 3) an increase in the rate of CO_2 production (Wilson *et al*. 1994). To increase microbial recovery from the blood of patients receiving systemic antimicrobial therapy, BacT/Alert FAN bottles contain Ecosorb, a proprietary substance composed, in part, of Fuller's earth and activated charcoal particles (Mirret *et al*. 2001a).

Bactec 9000 Series blood culture systems. Bactec 9240 and 9120 blood culture systems were introduced into clinical practice in 1992. Bactec 9000 uses a fluorescent mechanism to detect the increase in the CO_2 concentration caused by microbial growth. The growth detection algorithm uses 4 criteria to detect microbial growth. Two algorithms, detection of a linear increase in fluorescence and an increased rate of fluorescence are similar to those used in the BacT/Alert system. The other two criteria are not publicly available. In order to increase microbial recovery from the blood of patients receiving systemic antimicrobial therapy, Bactec PLUS and PLUS/F bottles contain resin-like materials (Wilson *et al*.1994).

VersaTREK blood culture system. VersaTREK CMBCS (TREK Diagnostic Systems, Cleveland, OH, US) evolved from and replaced the older Difco ESP CMBCS. As in ESP, also in VersaTREK the detection of positive cultures is based on manometric monitoring of gas consumption and/or production by microorganisms (Wilson *et al*.1994, Mirret *et al*. 2007). VersaTREK CMBCS was commercially introduced in 2003.

Interpretation of blood culture results

A positive blood culture is not a definite sign of LCBI. In unselected patient populations, common blood isolates that always or nearly always (> 90 %) represent true infection include *S. aureus*, *E.coli*, and other members of *Enterobacteriacae*, *P. aeruginosa*, *Streptococcus pneumoniae* and *C. albicans* (Weinstein *et al*. 1997). It is probable that *Strepotococcus pyognenes*, *Streptococcus agalactiae*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Haemophilus influenzae*, members of the *Bacteroides fragilis* group, C*andida sp*. other than *C. albicans* and *Cryptococcus neoformans* always or virtually always represent true infection (Weinstein 2003). Other microorganisms such as *Corynebacterieum* sp., *Bacillus sp*. other than *B. anthracis* and *Probionibacterium acnes* rarely represent true infection in immune-competent patients (Weinstein *et al*. 1997, Weinstein 2003).

More problematic are VGS and enterococci, which represented true bacteraemia in 38 % and 70 % respectively in a study with 843 positive blood cultures (Weinstein *et al*. 1997). Intepretation of a blood culture positive for CoNS is especially difficult and CoNSs are the most frequent blood culture findings. These bacteria are mostly contaminants but they have assumed an increased importance as the etiologic agents of catheter-associated bacteraemia and bacteraemia in patients with vascular and other protheses (Weinstein 2003). CoNSs recovered from blood culture have been estimated to represent true infection in 13 % (Weinstein *et al*. 1997) to 30 % (Finkelstein *et al*. 2002) of incidents in unselected patient populations.

While it seems that the number of positive bottles within a single blood culture set does not predict accurately the clinical significance of isolates (Mirret *et al*. 2001b), the number of blood culture sets that grow microorganisms has proved to be a useful aid for interpreting the clinical significance of positive blood cultures (Weinstein *et al*. 1997). Perhaps the most commonly used definition of LCBI is that devised by the Centers for Disease Control and Prevention (CDC). According to this definition, in adult patients, while a recogonized pathogen cultured from a single blood culture is a definite sign of LCBI, a common skin contaminant should be cultured from more than one blood culture set and clinical signs of infection must be present to warrant a diagnosis of LCBI. A skin contaminant found in one blood culture warrants the diagnosis of LCBI only, if the patient has an intravenous line and the physician institutes appropriate antimicrobial therapy (Centers for Disease Control and Prevention 2002). While most CoNS found in a single blood culture represent contamination, recovery of CoNS from multiple blood cultures is not a confirmation of bacteraemia (Sharma *et al*. 2001). Thus, the intepretation of clinical significance of skin contaminants recovered from blood cultures still remains problematic, and no golden standard exists for differentiating pathogenic CoNSs from contaminants (Weinstein 2003).

In general, patients with neutropenia are more likely ($p < 0.001$) than non-neutropenic patients to have a positive blood culture, which represents a true LCBI (70.2 % versus 51.4 % of positive blood cultures; Weinstein *et al*. 1997). In a study with 31 patients with acute leukaemia who had *S. epidermidis* recovered from their blood (Lyytikäinen *et al*. 1998), the median increase of CRP over 24 h was higher in true bacteraemias than among contaminants (median 35 mg/l vs. 5 mg/l, $p < 0.05$). Presence of a central catheter and clinical signs of catheter infection also favored the existence of true bacteraemia in these patients.

2.6.4 Novel techniques to detect BSI

With CMBCS technology, the time required to detect commonly encountered microorganisms can take as long as 40 h (Mirret *et al*. 2001a, 2007). From the time the blood culture is noted to be positive, identification by Gram-staining and subculture and the susceptility testing can take an additional 24 to 72 hours. Thus, there is a need for more rapid organism identification and susceptibility testing.

To decrease the time to result, molecular methods for rapid identification of microorganisms have been developed, including DNA microarrays (Cleven *et al*. 2006, Tissari *et al*. 2010), RNA-based fluorescence *in situ* hybridization (FISH) (Jansen *et al*. 2000) and polymerase-chain reaction (PCR) -assays (Wellinghausen *et al*. 2004). In addition, serum assays to indicate galactomannan antigenemia in invasive aspergillosis (Herbrecht *et al*. 2002) and mannan antigenemia and antimannan antibodies in invasive candidemia (Prella *et al*. 2005) have been studied. All these tests are aimed at rapid detection of bacteria and yeasts recovered from positive blood culture but they have not become widely used clinical tools.

With respect to the PCR, at first conventional techniques were used to detect bacterial rRNA for the presence of bacterial DNA (Ley *et al*. 1998). Specification was then carried out by sequencing the PCR product. Later a more rapid method, so-called real-time PCR assay, became available. Universal broad-range PCR assays can detect all bacteria. However, the technique is prone to contamination and it detects all bacterial DNA (also degraded bacteria) present in blood (Peters *et al*. 2004) and its performance may be problematic, when there is more than one species of bacteria in the blood.

The so-called multiplex real-time PCR assays have been used to display pathogenic microorganisms even before positive signalling in CMBCS. They have an algorithm involving species and genus specific assays for most the relevant bacterial causes of BSI. The lowest limit of detection has been 10 to 20 cfu per PCR reaction for bacteria (Gebert *et al*. 2008) and 2 cfu/ml for *Candida sp*. (Badiee *et al*. 2009).

2.6.5 Observations on blood culture systems

Comparisons between blood culture systems

Most clinical comparisons of blood culture systems have been performed with unselected patient populations, in which most of the patients are not likely to have been neutropenic.

CMBCSs have mostly been superior to earlier blood culture systems in clinical comparisons. BacT/Alert CMBCS with standard medium recovered more organisms per septic episode (p = 0.003) and more rapidly (p < 0.0001) but also with more false-positive indications and contaminant growth than the Signal (Oxoid Ltd., Hampshire, United Kingdom) manual system (Rohner *et al*. 1995). Bactec CMBCS with PLUS Aerobic/F Resin medium recovered more pathogens overall (p = 0.0006) than the manual Isolator system (Wampole Laboratories, Cranbury, N.J.) in a clinical comparison (Cockerill *et al*. 1997). The Isolator system recovered more contaminant overall (p < 0.0001) and more isolates of *Histoplasma capsulatum* (p = 0.004). BacT/Alert CMBCS with standard medium was equivalent (p > 0.05) to the Bactec 660/730 the non-radiometric blood culture system in overall microbial recovery (Wilson *et al*. 1992). BacT/Alert detected microbial growth earlier (p < 0.001) and with fewer false-positive signals than Bactec. However in a study comparing Bactec 9240 CMBCS with manual Septi-Chek blood culture system (Becton-Dickinson, Sparks, Md.), the manual system detected more pathogens (p = 0.0001) than the CMBCS, although the CMBCS detected the pathogens earlier (p < 0.0001) than the manual system (Rohner *et al*. 1996).

There have been some minor differences in the ability of different CMBCSs to detect microorganisms in direct clinical comparisons. For example, an anaerobic bottle with the ESP CMBCS detected more anaerobic bacteria (p < 0.05) than the BacT/Alert anaerobic bottle and an ESP aerobic bottle detected more *S. aureus* than a BacT/Alert aerobic bottle (p < 0.025; Zwadyk *et al*. 1994). VersaTREK aerobic (REDOX 1) and anaerobic (REDOX 2) media detected more streptococci and enterococci as a group (p < 0.05) and more microorganisms in patients receiving antimicrobial therapy (p < 0.025) than the BacT/Alert 3D system with aerobic (SA) and anaerobic (SN) media (Mirret *et al*. 2007). However, no difference was observed between the two systems in the detection of the 179 unimicrobial bacteremic episodes.

The ability of Bactec 9240 and BacT/Alert3D blood culture systems to detect yeasts was studied in a direct *in vitro* comparison (Horvath *et al*. 2004). In addition to the specialized mycology media Bactec Myco/F Lytic and BacT MB, Bactec PLUS Aerobic/F, Bactec PLUS Anaerobic/F, BacT/Alert FA aerobic and BacT/Alert standard anaerobic media were used. Both mycology media and BacT/Alert FA aerobic medium detected all of the *Candida* pathogens. The five isolates not detected by Bactec aerobic media were all "non-*albicans Candica*" species. *Candida* growth detection was suboptimal in anaerobic media in both blood culture systems. Without use of the mycology media, BacT performed better than Bactec.

The median time to growth detection was overall faster in the BacT (25.62 ± 8.90 h) than in the Bactec (27.30 ± 21.18 h) system ($p < 0.01$), although growth detection with Bactec mycology medium was faster than with the BacT mycology medium. It took longer to detect *Candida glabrata* isolates in aerobic media (Bactec: 106.00 ± 18.99 h, BacT: 51.82 ± 9.93 h) than to detect the other *Candida* spp. ($p < 0.05$).

Effect of resin or charcoal-containing media on the recovery of microorganisms

In most cases, the superiority of resin or charcoal-containing media for recovering clinically significant pathogens from the bloodstream has been established (Weinstein *et al*. 1995, Wilson *et al*. 1995, McDonald *et al*. 1996). Its superiority is considered not simply to be attributable to its ability to bind and inactivate antimicrobial agents, because even in patients not receiving antimicrobial therapy, the charcoal-containing medium has recovered more isolates than the standard medium (Weinstein *et al*. 1995). Nevertheless, especially resins are able to decrease the activity of antimicrobials in blood culture bottles (Spaargaren *et al.* 1998, Flayhart *et al.* 2007).

In 1995, in two companion clinical studies comparing BacT/Alert standard aerobic media to FAN aerobic media (Weinstein *et al.* 1995) and BacT/Alert standard anaerobic media to FAN anaerobic media (Wilson *et al.* 1995) overall recovery of microorganisms was greater in FAN bottles in both studies ($p < 0.001$ and $p < 0.005$, respectively). FAN aerobic bottles grew also significantly more contaminants (especially CoNSs) than standard aerobic bottles. The mean overall detection time of microbial growth was 16.1 h, 16.0 h, 14.2 h and 16.1 h for aerobic standard, aerobic FAN, anaerobic standard and anaerobic FAN bottles, respectively. In a retrospective clinical study (McDonald *et al*. 1996), bacteraemias were detected more often in BacT/Alert FAN bottles than in standard bottles ($p < 0.0001$), and bacteraemias detected only in BacT/Alert FAN bottles more commonly occurred in patients receiving theoretically effective antimicrobial therapy ($p < 0.05$).

Around the turn of the millenium, Organon Teknika introduced a new aerobic nonvented blood culture medium, designated BacT/Alert FA. As the growth medium, FA bottles contain casein-soybean solids instead of BHI. The concentration of activated charcoal was decreased from 8.5 % (w/v) to 6.5 % (w/v). In a controlled *in vitro* comparison, the FA bottle detected more *Burkholderia cepacia* ($p < 0.001$), *C. albicans* ($p < 0.001$) and *C. neoformans* ($p < 0.01$) isolates and all microorganisms combined ($p < 0.05$) than the older vented FAN bottle. There was no difference between the two types of bottles in the numbers of false-positive results (Mirret *et al*. 2001a).

In a controlled clinical laboratory trial, 4402 blood culture sets were taken from immune-competent or immune-compromised patients (Ziegler *et al*. 1998). No differences were found between Bactec PLUS/F resin and BacT/Alert FAN media in microbial recovery when blood cultures (36 % of all the sets) were obtained from patients receiving antimicrobial therapy. More members of the *Enterobacteriacae* group were recovered ($p < 0.01$) from patients without antimicrobial therapy by the BacT/Alert system.

The resin-containing Bactec PLUS bottles were superior to BacT/Alert FA in recovering bacteria in the presence of antibiotics in an *in vitro* study (Flayhart *et al*. 2007). The overall recovery of bacterial isolates from the Bactec PLUS system was 95.1 % (616/648), compared to 43.1 % (279/648) recovered from the BacT/Alert FA system ($p = 0.0001$). Average times to detection for the strains from the growth control bottles were 9.9 h for Bactec PLUS and 15.3 h for BacT/Alert FA. Both systems detected all isolates of *E. coli*, *K. pneumoniae*, and *P. aeruginosa* in the presence of gentamicin. In the presence of ceftriaxone, neither system was able to recover *S. pneumoniae*. Bactec recovered from 89 % to 100 % of the challenge strains in the presence of the other antimicrobials (ampicillin, cefepine, cefoxitin, oxacillin, piperacillin-tazobactam and vancomycin) tested, while the performance of BacT/Alert was considerably poorer and in the presence of ampicillin or oxacillin, the detection rate of the challenge strains was 0 %. Serum levels of vancomycin and cefoxitin measured after 1 h of inoculation tended to be lower in Bactec PLUS than BacT/Alert bottles.

BacT/Alert and BACTEC mostly share the market for CMBCS. Their success is probably based on factors other than their abilities to recover microorganisms. The main advantages of BacT/Alert and Bactec CMBCSs over previous generations of blood culture instruments include full automation, once the bottles are loaded, a shorter time to detection of blood pathogens, considerable labour savings and improved laboratory work flow (Reimer *et al*. 1997).

Deficiencies of CMBCSs

Ideally, blood culture bottles should be entered in a CMBCS as soon as possible. In case of a delay, it has been recommended, that the blood culture bottles should be stored at 36 ºC until arrival in the laboratory. In the late 1990s, it was observed that BacT/Alert aerobic media failed to detect non-fermentative gram-negative bacteria, if they were preincubated at 36°C. The non-fermentative species, when incubated in a clinically relevant colony number, were probably viable but the threshold of

3300 reflection units and the acceleration rate of 32 reflection units/10 min were not reached and no growth signal was generated. The non-fermentative species – especially *P. aeruginosa* and *Acinetobacter baumannii* – when incubated may grow weakly and reach a steady-state concentration where CO_2 acceleration occurs on a relatively low basis, or they may enter a stationary phase after logarithmic expansion (Klaerner *et al*. 2000).

CMBCSs compared to real-time PCR and other novel techniques

The sensitivity of a DNA-based microarray platform (Mobidiag, Helsinki, Finland) to identify bacterial species recovered from blood cultures was 94.7 % (Tissari *et al*. 2010). Its specificity was 98.8 %. Both sensitivity and specificity were 100 % for meticillin-resistant *S. aureus*.

A multiplex real-time PCR assay called MolYsis was able to detect and identify 11 out of 18 microorganisms before positive signalling in CMBCS (Gebert *et al*. 2008). A commercially available multiplex real-time PCR assay SeptiFast (Roche Diagnostics GmbH, Penzberg, Germany) developed to detect 20 different pathogens was evaluated in a study of neutropenic haematological patients (von Lilienfeld-Toal *et al* 2009). In the antimicrobial therapy naive setting, only 11 out of the 34 blood culture-positive episodes yielded a positive result in the PCR. During antimicrobial therapy, positivity in blood culture was detected in only 3 % of cases, but the real-time PCR yielded a positive result in 15 % of cases. It is possible that during antimicrobial therapy, the PCR had detected the remnants of bacteria or non-viable microorganisms. The PCR technique failed to detect *K. pneumoniae*, *C. glabrata* and *Streptococcus sp*. rather often. Thus, blood culture prior to antimicrobial therapy probably cannot be replaced by a molecular method. Nonetheless, PCR can be useful in cases of persistent fever during antibiotic therapy or invasive fungal infection.

In 194 haematological patients (Badiee *et al*. 2009), candidemia was detected by a PCR-ELIZA assay (DIG Detection, Roche, Mannheim, Germany) in 25 patients, but only in one patient by blood culture. *C. albicans* (n = 21) was the most common finding followed by *Candida tropicalis* (n = 3) and *C. krusei* (n = 1). Systemic candidiasis was detected on average 12.6 days earlier with the PCR-Eliza assay of blood than with blood culture.

2.7 Therapy of suspected or detected neutropenic BSI

2.7.1 Supportive and preventive measures

Myeloid growth factors are nowadays used to prevent neutropenic infection (Herbst *et al*. 2009). Neutropenia prophylaxis with granulocyte colony stimulating factors (G-CSF) has been proposed to be prescribed in the first cycle of chemotherapy for cancer patients with more than a 20 % risk of febrile neutropenia, because it improves survival in cancer patients (Dale 2009), but there is no evidence of survival benefit in AML patients. In Finland, the use of myeloid growth factors to shorten chemotherapy-induced neutropenia of AML patients became a common practice during the late 1990s and the early 2000s.

Fluoroquinolone prophylaxis reduces febrile episodes, other infection-related outcomes (Bucaneve *et al*. 2005, Cullen *et al*. 2005) and mortality (Gafter-Gvili *et al.* 2005) in neutropenic cancer patients. The clear benefits of fever prevention have been shown also among patients with acute leukaemia (Bucaneve *et al*. 2005). At present, there is no evidence either for or against the use of antimicrobials compared to myeloid growth factors for preventing of infections in cancer patients (Herbst *et al*. 2009) or in patients with acute leukaemia. In Finland, the use of antibacterial prophylaxis has been avoided due to concerns about the emergence of resistance by microorganisms.

2.7.2 Initial antimicrobial therapy

In the early 1970s, the mortality reducing effect of the empirical treatment of carbenicillin and gentamicin was demonstrated in neutropenic febrile cancer patients (Schimpff *et al*. 1971). Later, third-generation cephalosporins such as ceftazidime of ceftriaxone became the beta-lactams of choice to be used in combinations with the aminoglycosides (EORTC 1993). However, because of the well-known otoxicity and nephrotoxicity of aminoglycosides (EORTC 1993), also other combinations of antimicrobials have been evaluated.

Several studies have been performed with single-agent therapy with the newer extended-spectrum antimicrobials such as third-generation cephalosporins (Pizzo *et al*. 1986, Harter *et al*. 2006, Bow *et al*. 2006), the carbapenems (Cometta *et al*. 1996), piperacillin-tazobactam (Del Favero *et al*. 2001, Harter *et al*. 2006, Bow *et al*. 2006) and even ciprofloxacin (Giamarellou *et al*. 2000). There have been no striking differences between monotherapy and multi-drug combinations in the

empirical treatment of uncomplicated episodes of fever in neutropenic patients (Hughes *et al*. 2002). Monotherapy with a third-generation cephalosporin (or piperacillin-tazobactam) may be successful in institutions where there is a high proportion of gram-positive bacteria as a cause of BSI (Harter *et al*. 2006, Bow *et al*. 2006), especially in the case of "low-risk" neutropenia (Pizzo 2009).

Patients with "low-risk" neutropenia can be characterized by the presence of remission of the underlying cancer, expected duration of neutropenia less than seven days and the absence of prophylactic antibiotics or mucositis or other co-morbidities, such as hypotension or organ failure. These patients can often be treated with third- or fourth-generation cephalosporin monotherapy. For the high-risk patient, the need to add an aminoglycoside to the therapy is more likely. If there are signs of haemodynamic instability, addition of vancomycin is also required. In patients who remain febrile and neutropenic beyond 7 days, the addition of an antifungal agent (amphotericin B, voriconazole, caspofungin) has become the standard practice (Pizzo 2009).

2.8 The outcome of neutropenic BSI

In unselected cancer populations, the reported mortality due to infections is about 5 % (Del Favero *et al*. 2001). Focal infections may increase mortality (Cordonnier *et al*. 2003). Considerably high mortality rates have been reported in patients with pulmonary infiltrates (Velasco *et al*. 2003). The outcome of infections seems to be more severe in leukaemia patients than in other cancer patients (EORTC 1987, Velasco *et al*. 2003) and it is worse during remission induction and especially during relapse (Velasco *et al*. 2003).

A rising granulocyte count during infection is a favourable prognostic sign (EORTC 1978), while the prognosis of a patient with bacteraemia is worse, if neutropenia is persistent (Giamarellou *et al*. 2000). Profound neutropenia (blood PMN count less than 0.1 x 10^9/l) was reported to be an adverse prognostic factor, especially for gram-negative bacteraemia in the past decades (EORTC 1987), but this may no longer be the case (Velasco *et al*. 2003, 2006).

Overall mortality in the presence of candidemia has been high, in the range 24% to 45 % in cancer patient populations (Viscoli *et al*. 1999, Wisplinhoff *et al*. 2003). The prognosis has been worse with *C. glabrata*, *C. tropicalis* and *C. krusei* fungaemias than with other candida fungaemias (Viscoli *et al*. 1999).

3. Aims Of The Study

The specific aims of the present study were:

1. To compare a manual blood culture system to an automated continuous-monitoring blood culture system in diagnosing bloodstream infections (BSI) in neutropenic patients with acute myeloid leukaemia (AML) (I).
2. To analyze the influence of individual chemotherapeutic agents on the BSI rate of neutropenic AML patients (II).
3. To assess the influence of chemotherapeutic regimens and the stage of the disease on the BSI rate of neutropenic AML patients (III).
4. To conduct an *in vitro* study of the possible effect of chemotherapeutic agents on the growth of bacteria and yeasts in blood culture (IV and V).

4. Materials and methods

4.1 Study location

The clinical data of AML patients treated during the years 1990–93 (I) and 1997–2003 (II) in Oulu University Hospital Haematological Departement were retrospectively collected during the years 1993–2003. The register data of the AML -92 trial of the Finnish Leukaemia Group (FLG) from the years 1992–2006 (III) was collected prospectively in the haematological departments of the five Finnish University Hospitals. The *in vitro* -studies were performed during the years 1995–96 (IV) and the year 2004 (V) in Clinical Microbiology Laboratory of Oulu University Hospital.

4.2 Patients

The adult AML patients analysed in the present study were treated according to the two AML protocols planned by the FLG, the AML -86 trial protocol (I) and the AML -92 trial protocol (II and III), and with some other chemotherapy regimens (II) of FLG. The eligibility criteria for FLG chemotherapy protocols were diagnosis of AML, age between 16 and 65 years and absence of severe liver, kidney, heart or other organ failure unrelated to leukaemia *per se*. (Elonen *et al*. 1998, Koistinen *et al*. 2007). All patients had a central venous catheter for prolonged use. A patient was defined as being neutropenic, when blood PMN count was less than 0.5 x 10^9/l. In the absence of a neutrophil count, blood granulocyte count less than 1.0 x 10^9/l was used instead in parts I and II. Blood leukocyte count or PMN count and serum or plasma CRP concentration, axial temperature and blood pressure of the patients were measured daily until recovery from infection and neutropenia.

4.3 Chemotherapy protocols

4.3.1 Finnish Leukaemia Group (FLG) AML 86 -protocol

In the AML -86 trial of the FLG the patients received either four or eight chemotherapy courses. The chemotherapy courses used are presented in Table 3. The 1st course was DAT consisting of daunorubicin, CD AraC and TG. Patients over 60 years of age received solely AraC and TG. In the 2nd course, patients who showed a clear

Table 3. Chemotherapy courses in the AML 86 -protocol (I).

Chemotherapy	Dosage	Administration route	Days of administration
DAT			
daunorubicin	50 mg/m^2	i.v.	1,3,5
AraC	50 mg/m^2	i.v., bolus	1
	100 mg/m^2	i.v., continuously	1–9
TG	75mg/m^2	p.o., twice daily	1–9
Amsa-HDAraC			
M-amsacrine	115 mg/m^2	i.v., 2 h	1–5
AraC[1]	2.0 or 3.0 g/m^2	i.v., 3 h, twice daily	1–2
HDAraC-Daunorubicin			
AraC	2.0 g/m^2	i.v., 3 h, twice daily	1–5
daunorubicin	30mg/m^2	i.v.	6–8
Aclarubicin-Etoposide-Vincristine-Prednisone			
aclarubicin	25 mg/m^2	i.v.	1–7
etoposide	60 mg/m^2	i.v., twice daily	1–5
vincristine	1 mg/m^2	i.v.	1,5
prednisone	60 mg/m^2	p.o.	1–7
Sequential AraC-Daunorubicin			
daunorubicin	30 mg/m^2	i.v.	1–3
AraC	500 mg/m^2	i.v., continuously	1–3,10–12

NOTE: AraC = cytarabine; TG = thioguanine; h = hours; i.v. = intravenously; p.o. = *per os*;
[1] AraC 3.0 g/m^2 in the 2nd and 3rd cycles and 2.0 g/m^2 in the 8th cycle.

decrease in the number of blasts in bone marrow were given DAT (identical to the 1st course) again but for those patients in whom the blasts in bone marrow were not reduced satisfactorily, Amsa-HD AraC (identical to the 3rd course) was prescribed instead of a second DAT. The 4th course was HD AraC-Daunorubicin. The 5th and 6th courses consisted of aclarubicin, etoposide, vincristine and prednisone. The 7th course was Sequential AraC-Daunorubicin. The 8th course was again Amsa-HD AraC. Before starting a new course for a patient in remission, the blood PMN count had to be 1.5 x 10^9/l or more (Elonen *et al*. 1998).

4.3.2 FLG AML 92 -protocol

The AML -92 trial was designed to investigate the long term outcome of Ida and AraC -based intensive chemotherapy in adult AML. The chemotherapy regimens used are presented in Table 4. The 1st course (IAT) was an induction cycle. The 2nd course (HD AraC – Ida) was given irrespective of whether remission was achieved i.e. it was either the second induction or the first post-remission course.

Table 4. Chemotherapy courses in the AML 92 -protocol (II and III).

Chemotherapy	Dosage	Administration route	Days of administration
IAT			
Ida	12 mg/m^2	i.v., 10–15 min	1,3,5
AraC	50 mg/m^2	i.v., bolus	1
	100 mg/m^2	i.v., continuously	1(–7)–9
TG	75 mg/m^2	p.o., twice daily	1(–7)–9
HDAraC-Ida			
AraC	[1]1.5 g (1.0 g)/m^2	i.v., 3 h, twice daily	1–5
Ida	8 mg/m^2	i.v., 30 min	6–8
ME-HDAraC			
etoposide	100 mg/m^2	i.v., 1 h	1–4
AraC	1.0 g (0.5 g)/m^2	i.v., 2 h, twice daily	1–4
mitoxantrone	12 mg (8 mg)/m^2	i.v., 30 min	2–5
Amsa-HDAraC			
M-amsacrine	115 mg/m^2	i.v., 2 h	1–5
AraC	3.0 g/m^2	i.v., 3 h, twice daily	1–2

NOTE: Dosages and days administered in parenthesis indicate patients $\geq$ 56 years old.
AraC = cytarabine ; Ida = idarubicin; TG = thioguanine; i.v. = intravenously; p.o. = *per os*; h = hours; min = minutes.

[1]2.0 g (1.5g)/m^2 for the first 87 patients of the AML 92 trial.

Subsequently, three post-remission courses were given in the following sequence: ME-HD AraC, Amsa – HD AraC and HD AraC – Ida. The 5th course was given only to patients who achieved remission with the 2nd cycle; after CR patients received three postremission cycles. Allogeneic HSCT was offered in the first remission to < 60 years old patients with a histocompatible sibling donor and in the second remission to < 56 years old patients with an unrelated donor (Koistinen *et al*. 2007).

4.3.3 Other FLG chemotherapeutic regimens

In study II AI, ETI, MACE and Mito-HDAraC chemotherapy courses were given to patients aged over 65 years or otherwise ineligible for maximally intensive treatment (Table 5).

Table 5. Chemotherapy courses given to elderly patients (> 65 years) or to patients otherwise ineligible for maximally intensive treatment (II).

Chemotherapy	Dosage	Administration route	Days of administration
AI			
AraC	100mg/m^2	i.v., 15 min, twice daily	1–6(–7)
Ida	12 mg/m^2	i.v., 15 min	4,6 or 5,7
ETI			
etoposide	80mg/m^2	p.o., twice daily	1–5
TG	100mg/m^2	p.o., twice daily	1–5
Ida	15 mg/m^2	p.o.	1–3
MACE			
M-amsacrine	100 mg/m^2	i.v., 2 h	1–4
AraC	100mg/m^2	i.v., continuously	1–5
etoposide	100mg/m^2	i.v., 10 min	1–5
Mito-HDAraC			
mitoxantrone	12 mg/m^2	i.v., 30 min	2–5
AraC	1.5 g/m^2	i.v., 3 h, twice daily	1–4

NOTE: AraC = cytarabine; Ida = idarubicin; TG = thioguanine. i.v. = intravenously; p.o = *per os;* min = minutes; h = hours.

4.4 Laboratory methods used in clinical studies

4.4.1 Blood cultures

Manual blood culture method (I). Manual blood culture was carried out by aseptically drawing venous blood into two Vacutainer agar slant blood culture bottles (10 ml/ bottle; Becton Dickinson Vacutainer Systems, Rutherford, N.J., USA), at the onset of fever and after a 30 minutes interval. One of the bottles was vented with ambient air for aerobic culture and the other remained anaerobic. Both of the bottles were thereafter incubated at + 35 to 36 °C for up to 10 days. The bottles were daily checked visually and shaken manually. The aerobic and anaerobic bottles were monitored by Gram-stain and subcultured on sheep blood agar and chocolate agar plates on day 1 and 2, respectively. If no signs of bacteria or yeasts were seen, incubation of the bottles was continued. The Gram-stain and subcultures were repeated on days 5, 6 or 7, depending on weekend. The subcultures were incubated for up to two days. Thus a final negative blood culture was reported after 7 to 9 days after the blood culture was taken.

CMBCSs (I to III). For a blood culture, venous blood samples (10 ml each sample) were aceptically taken into a standard aerobic and an anaerobic BacT/Alert blood culture bottle (I, II) or into a corresponding aerobic or anaerobic FAN-bottle (II and III), (bioMérieux®, Marcy-l´Etoile, France). The blood cultures were immediately incubated by the automated BacT/Alert® CMBCS (Organon Teknika, Durham, NC, USA; later bioMérieux®, Industry, Hazelwood, MS, USA) up to 7 (I) or to 5 (II and III) days. In addition, clinicians could request that the laboratory staff should continue the incubation (usually the aerobic bottle) for up to 14 days, if no signal was obtained within the standard incubation time. In Part II, the BacT/Alert CMBCS standard blood culture bottles were used if neither antimicrobials nor antineoplastics had been used during the preceding seven days, otherwise the samples were cultured in FAN bottles.

In Part III, of the five participating centres, three hospitals were using Bactec 9240™ CMBCS (BD Diagnostics, Diagnostic Systems, Sparks, MD, USA) with Bactec standard or resin containing bottles.

4.4.2 Identification of microorganisms

Based on the staining results, preliminary strain identification and antimicrobial susceptibility tests were performed directly from the samples taken from the bottles. Isolated bacterial and yeast strains were identified to the species level by routine bacteriological and mycological methods. Their *in vitro* susceptibility to antimicrobials commonly used for treating haematological patients was tested by the disk diffusion method and by E-test (AB Biodisk, Solna, Sweden) and was interpreted using NCCLS (National Committee of Clinical Laboratory Standards, USA, now CLSI i.e. Clinical Laboratory Standard Institute, USA) criteria. The clinician responsible for the antimicrobial treatment of the patient was informed both by the laboratory computer system and by phone when any blood culture of the patient was found to be positive and its Gram-stain, strain identification and antimicrobial susceptibility findings immediately after they were available.

4.4.3 Other laboratory tests

Blood leucocyte count or PMN count and serum or plasma CRP concentration and other common laboratory tests were carried out by the routine methods available in the hospitals and health centres in Finland.

4.5 Treatment of infections during neutropenia

No prophylactic antibacterial therapy for neutropenic patients was used during the study period. Two blood cultures were obtained from the patient after the onset of fever. Empirical antimicrobial treatment followed the generally accepted guidelines (Hughes *et al*. 1990, 1997, 2002). In the haematological ward of Oulu University, empirical antimicrobial therapy in febrile neutropenia was a third-generation cephalosporin combined with an aminoglycoside (I to III). During the AML -86 trial (I), no growth factors were used. Otherwise, the use of growth factors followed the policy of each haematological unit. In Oulu University Hospital, no growth factors were used in the early stages of the AML -92 trial. After 1996, myeloid growth factors were increasingly administered during neutropenias caused by ICC but not during neutropenias attributable to induction treatment.

4.6 Collection of the clinical data (I, II and III)

Data about age at the time of diagnosis, sex, treatment faculty, FAB classification of AML, and whether or not the patient had a positive blood culture were analyzed for every patient. Neutropenia days were measured and peak CRP level (mg/l) was defined for every febrile (I) and infection (II) episode or chemotherapy cycle (III). For the register data of FLG AML -92 trial (III), also the duration of hospital stays and number febrile days (temperature ≥ 38 °C) were measured and grade IV infections (major infection with hypotension) of WHO criteria (World Health Organization 1979), deaths and grade 3 & 4 GI toxicity defined by WHO (World Health Organization 1979) were recorded. In grade 3 & 4 toxicity, laboratory parameters (bilirubin, transaminases, alkaline phosphatase) were elevated over 5 times the reference values, oral ulcers were severe enough to allow only liquid diet or alimentation was not possible, vomiting required therapy or was intractable and diarrhoea was intolerable and required therapy or was haemmorhagic and resulted in dehydration.

In Part I, the clinical data were collected retrospectively into paper blanks to compare the rate of LCBI detected using a manual blood culture method with the rate detected using BacT/Alert CMBCS standard growth medium. All the febrile episodes (see definitions in Appendix) of consecutive patients enrolled in the FLG AML -86 trial were eligible if these episodes occurred during neutropenia and blood culturing had been performed.

In Part II, the clinical data (II) were collected retrospectively into a data file (SPSS Inc, Chicago, IL) to assess the influence of chemotherapeutic agents on the rate of LCBI in the first eligible infection episode (see definitions in Appendix) of each chemotherapy cycle. The Codes of International Classification of Diseases (ICD10) for AML was used to identify the target patients from the hospital's electronic database. An infection episode was excluded from the study if the patient had received bacterial antibiotics within three days before the first blood culture (n = 65), if the first blood sample had been taken before the patient had been given at least one dose of each chemotherapeutic agent belonging to the cycle (n = 24) or if a neutropenic patient had been transferred to another hospital before the resolution of the infection (n = 3). The infection episodes were divided into three groups: LCBI, focal infections with negative blood cultures and FUO (see definitions in Appendix).

The prospectively collected multicentre register data of the FLG AML -92 trial (III) was used to assess the influence of chemotherapeutic regimens on the rate of LBCI during chemotherapy cycles (Appendix). In the FLG AML -92 trial, each part of the study had a structured paper form. These paper forms were filled and stored in each participating centre. Copies of the paper forms were sent to the coordinator of the study who transferred the data into an Excel data file, from which the data were later transferred to a SPSS file for statistical analysis. In the present study, the data of the first four cycles of the AML 92 -protocol were analyzed. A total of 15 cycles had to be excluded because of missing or incomplete infection data.

4.7 *In vitro* studies using simulated blood cultures (IV and V)

4.7.1 Microbial strains and chemotherapeutic agents

The growth inbibitory properties of chemotherapeutic agents were assessed in BacT/Alert standard and FAN bottles. In the first part of the *in vitro* -study (IV) chemotherapeutics commonly used in treatment of haematological malignancies and solid cancers were tested. The chemotherapeutics used were: amsacrine, cisplatin, cyclophosphamide, AraC, doxorubicin, etoposide and vincristine. Cultures were carried out using three American Type Culture Collection (ATCC) control strains and 12 strains originated from blood cultures of patients treated in Oulu University Hospital (Table 6), without any history of chemotherapy. A total of five gram-positive cocci, five gram-negative rods and five yeasts were tested with all the drugs used.

Table 6. Microbial strains predisposed to the effects of chemotherapeutic agents used to treat haematological malignancies and solid cancers (IV).

	Species	No. of strains
Control strains		3
	Enterococcus faecalis ATCC29212	
	Escherichia coli ATCC25222	
	Candida albicans ATCC10231	
Others		12
Aerobic gram-positive cocci		
	Streptococcus mitis	2
	Streptococcus milleri	1
	Streptococcus salivarius	1
Aerobic gram-negative rods		
	Escherichia coli	2
	Enterobacter cloacae	1
	Proteus mirabilis	1
Yeasts		
	Candida albicans	2
	Candida glabrata	2

In the second part of the *in vitro* -study (V), chemotherapeutic agents used in treatment of AML were tested. The chemotherapeutics were Ida, mitoxantrone and TG used as a single agent, and in the following combinations: Ida-AraC and Ida-AraC-etoposide. A total of 13 microbial strains were used (Table 1, V) with ten of the microorganisms being common ATCC control strains. Three of the strains were isolated from blood cultures of patients with haematological malignancies treated in Oulu University Hospital. Altogether five gram-positive cocci, five gram-negative rods and three *Candida sp*. were tested.

4.7.2 Preparation of cultures and the study protocol

The microbial strains were stored at -80ºC until used. They were subcultured on sheep blood agar plates and then inoculated into BHI broth (Difco laboratories, Detroit, Mi, USA) to produce a microbial suspension of 100 cfu/ml in the logarithmic growth phase according to the corresponding standard growth curve prepared for each group of the microbes. Dilutions of all chemotherapeutic agents were prepared aceptically by a laboratory technician (IV) or the hospital pharmacy (V) just before use.

The cultures were carried out aceptically by adding to each bottle 1 ml BHI broth containing 100 cfu of the appropriate bacterium or yeast in the logarithmic growth phase, 1ml of dilution of anti-neoplastic agent and fresh sheep blood to a total volume of 10 ml. In the control cultures, physiological saline was used instead of the anti-neoplastic agent. Immediately after preparation, the bottles were transferred into a BacT/Alert® Blood Culture Cabinet which produced a growth curve reflecting the incubation time. All the cultures were further analyzed as subcultures on sheep blood agar plates. Two laboratory technicians prepared all the bottles which were analyzed in the same BacT/Alert® Cabinet. In each test, the control bottle containing the microbial strain but no anti-neoplastic agent had to result in a positive BacT/Alert signal within an acceptable incubation time and the test strain had to grow in the subculture.

All tests were performed in duplicate and the results were expressed as the mean incubation time of the duplicates. In the case of a negative culture (see definitions in Appendix), the maximal incubation times of 168 h (IV) or 336 h (V) were used as the incubation time.

In Part IV, the median incubation time of each microbial group, i.e. gram-positive cocci, gram-negative rods and yeasts strains, were compared with each other and with the controls. If growth inhibition was detected at the basic concentration of 100 µmol/l of the chemotherapeutic drug, the tests were repeated at lower concentrations.

In Part V, the results were interpreted individually for each microbial strain. In studies at the basic concentration of 100 µmol/l of the chemotherapeutic drug, a lengthening of incubation time of more than 50% compared with control culture was interpreted as a sign of growth inhibition in the test culture and it was followed by studies with lower drug concentrations. On the other hand, if no such lengthening of incubation time was detected, studies with higher drug concentrations were performed and the minimal concentration of the drug required to cause growth inhibition was determined.

4.8 Statistical analyses

Summary statistics for continuous variables are expressed as medians with the 25th and 75th percentiles, median with range or as mean and standard deviation (SD), unless other stated. Comparisons between two groups with continuous variables were utilized with Students's t-test or Mann-Whitney U-test. If the number of groups was > 2 then Analysis of Variance (ANOVA) or Kruskall-Wallis test was

used. If the previous tests for > 2 groups, detected a significant ($p < 0.05$) difference between groups, then pairwise between group comparisons were performed with either t-test or Mann-Whitney U-test. Categorical data was analyzed with Fisher's exact test or Pearson Chi-Square test. The generalized linear mixed model approach was utilized in comparison of repeatedly measured dichotomous (e.g. no/yes) data and the linear mixed model for repeatedly measured continuous data. The Wilcoxon signed rank test was used when comparing two dependent continuous variables. The 95% confidence interval (95% CI) is presented with incidence figures and odds ratios (OR). Two-tailed *p*-values are reported. Analyses are performed using SPSS (SPSS Inc, Chicago, IL) and SAS (SAS Institute Inc, Cary, NC).

5. Results

5.1 Study population

The final study population consisted of 404 adult patients (age from 16 to 85 years at diagnosis) with AML (Table 7). One hundred and twenty six of them were treated in Oulu University Hospital and 278 in other Finnish University Hospitals.

Table 7. Demographic data of the patients and FAB-classification of AML, grouped by the chemotherapy protocols.

Variable	AML -86[1]	Varied	AML -92[1]	AML -92[1]
Period of recruitment	1990–93[2] (I)	1997–2003[2] (II)	1992–2006[3] (III)	1992–2006[3] (III)
Blood culture system	Manual or BacT/ Alert CMBCS	BacT/Alert CMBCS	BacT/Alert CMBCS	Bactec CMBCS
Patient No.	26[4]	76[5]	152	175
Male sex (%)	12 (46)	37 (49)	84 (55)	82 (47)
Age at diagnosis, years	50 (22–65)	59 (16–85)	48(16–66)	48 (17–65)
FAB				
M0	1 (3.8)	5 (6.6)	11 (7.2)	11 (6.3)
M1	3 (12)	17 (22)	29 (19)	49 (28)
M2	6 (23)	30 (39)	52 (34)	53 (30)
M3	1 (3.8)	0	0	0
M4	11 (42)	15 (20)	38 (25)	40 (23)
M5	3 (12)	3 (3.9)	16 (11)	12 (6.9)
M6	1 (3.8)	1 (1.3)	2 (1.3)	6 (3.4)
M7	0	0	2 (1.3)	2 (1.1)
M2/M4	0	1 (1.3)	0	0
Unknown	0	4 (5.3)	2 (1.3)	2 (1.3)

NOTE: Values are presented as median (min–max) or as the number (percentage) of patients. AML = acute myeloid leukaemia; CMBCS = continuous-monitoring blood culture system;.FAB = French-American-British.

[1]AML -86 and AML -92: chemotherapy protocols by the Finnish Leukaemia Group

[2]data collected retrospectively in Oulu University Hospital

[3]The prospectively collected register data of the AML -92 trial by the Finnish Leukaemia Group. BacT/ Alert CMBCS was used in two centres and Bactec 9240 CMBCS in three centres.

[4]4 patients were included both in manual blood culture arm and CMBCS arm of the study and 1 patient was included also into the single centre AML-92 data (II).

[5]25 patients were included both in the retrospectively collected AML -92 data (II) and in the register data of the Finnish Leukaemia Group AML -92 trial (III).

5.2 Neutropenia

The neutropenic episodes of the AML patients in Part I are shown in Table 8. All the chemotherapy cycles were associated with a blood PMN count of less than 0.2 x 10^9/l. Of the 91 neutropenic episodes (Table 8), 26 (28.6 %) episodes were associated with induction treatment. The median duration of neutropenia was 19 days (with the 25th percentiles 13–23) during the manual blood culture period and 20 days (with the 25th percentiles 13–27; $p = 0.569$) during the CMBCS period.

Table 8. Febrile episodes (Feb) of neutropenic patients (Pat) with acute myeloid leukaemia treated with AML -86 chemotherapy protocol[1], according to the blood culture system (I).

Pat	Age[2] (years) /sex	Manual blood culture system In 1990–91			CMBCS In 1992–93		
		Neutropenia		Feb	Neutropenia		Feb
		No.	Duration (*days*)	No.	No.	Duration (*days*)	No.
1	26/F	1	27	1	2	26.5 (25–28)	3
2	60/F	7[3]	18 (9–22)	7			
3	39F	3	29 (17–57)	5	4	16 (10–35)	5
4	51/F	2	24 (20–28)	2			
5	49/F	7	19 (8–23)	9			
6	45/F	3	22 (12–24)	3	2	31 (22–40)	2
7	65/M	1	9	1			
8	25/M	2	9 (4–14)	5			
9	62/F	4	22.5 (16–58)	6			
10	45/M	2	20.5 (13–28)	5	6	21.5 (6–28)	8
11	23/M	1	9	1			
12	47/M	4	18.5 (11–23)	8			
13	57/M				4	18 (11–36)	6
14	41/M				3	20 (8–22)	4
15	65/F				1	12	1
16	56/M				4	24.5 (13–27)	6
17	48/M				3	22 (15–33)	5
18	29/F				4	24.5 (13–36)	4
19	58/M				4[3]	17 (12–42)	7
20	22/M				2	19 (13–25)	2
21	46/M				3	12 (11–31)	7
22	64/F				4	15.5 (13–19)	4
23	63/F				2	27 (22–32)	3
24	52/F				2	23.5 (18–29)	2
25	65/F				2	25 (19–31)	2
26	63/F				2	18 (16–20)	2

NOTE: Values are presented as median (min–max) or as the number of patients or cases. CMBCS = continuous-monitoring blood culture system.
[1]AML 86-protocol= a chemotherapy protocol by the Finnish Leukaemia Group.
[2]Age at diagnosis.
[3]Includes a double induction resulting in a single neutropenia.

Table 9. Duration of neutropenia (blood neutrophil count less than 0.5 x 10^9/l) and peak serum CRP-values in patients with acute myeloid leukaemia treated in Oulu University Hospital during the years 1997–2003 (II).

Chemotherapy regimen	No.	Neutropenia (days) $p = 0.34$[1]	CRP (mg/ml) $p = 0.63$[1]
HD AraC-Ida	37	19 (16–24)	180 (123–222)
Amsa-HD AraC	17	15 (14–21)	121 (102–212)
Mito-HD AraC	5	14 (12–19)	136 (105–240)
ME-HD AraC	25	17 (14–24)	187 (145–238)
MACE	3	19 (16.5–21.5)	240 (199.5–270)
ETI	14[2]	19 (12–22)	178 (90–199)
AI	44	24 (16–28)	166 (105–214.5)
IAT	37	20 (16–26)	159 (85–238)
All	182		

NOTE. Values are presented as median (interquartile range from 25% to 75%).

[1]Determined by Kruskall-Wallis Test.

[2] In one case of ETI, the patient died when still neutropenic. That case of neutropenia was not included in the data.

The neutropenic episodes of the AML patients in Part II are shown in Table 9. All but four of the 182 chemotherapy cycles were associated with a blood PMN count count of less than 0.1 x 10^9/l. A total of 74 neutropenias (40.7 %) occurred in association with induction courses and 108 neutropenias (59.3 %) in association with consolidation courses. In 22 cases (29.7 %), the patient was already neutropenic when the induction treatment began. The median duration of neutropenia associated to HD AraC-containing courses (n = 84) was 18 days (with the 25th percentiles 14–22.5), compared to 21 (14–27; $p = 0.471$) days of the courses not containing HD AraC. The median duration of neutropenia associated with TG-containing courses (n = 51) was 19.5 days (13–25) compared to 19 days (14–26; $p = 0.245$) in courses not containing TG.

The neutropenic episodes of the AML patients in the FLG AML -92 trial of years 1992–2006 are shown in table 1 of Part III. The median duration of neutropenia associated with chemotherapy varied between 22 to 26 days ($p < 0.001$).

5.3 The febrile and infection espisodes

In Part I, a total of 126 febrile episodes (Appendix) occurred in 26 neutropenic patients (Tables 8 and 10). All except three patients had more than one febrile

Table 10. The detection of bloodstream infections by a manual Becton Dickinson (BD) blood culture system or two automated continuous-monitoring blood culture systems (CMBCS) with different growth media in adult patients with acute myeloid leukaemia (AML) and neutropenia (the neutrophil count less than 0.5 x 10^9/l).

Variable	BD		BacT/Alert CMBCS		CMBCSs[1]
Medium	Vacutainer Agar		Standard	FAN	various
Period	*1990–91*[2] (I)		*1992–93*[2] (I)	*1997–2003*[3] (II)	*1992–2006*[4] (III)
Events	n=53 *fevers*		n=73 *fevers*	n=182 *infections*	n=956 *chemotherapy cycles*
LCBI rate	23 %	p= 0.043[5]	40 %	37 %	48%

NOTE: LCBI-rate = the proportion of laboratory-confirmed bloodstream infections of the total number of events.

[1]436 events studied by BacT/Alert and 520 events by Bactec 9240 CMBCS.

[2]A retrospectively collected data (Oulu University Hospital) of patients treated according to the AML 86 -protocol of the Finnish Leukaemia Group

[3]A retrospectively collected data (Oulu University Hospital) in which patients were treated with different chemotherapy regimens.

[4]The register data of the multi centre AML -92 trial by the Finnish Leukaemia Group.

[5] Determined by Pearson Chi-square test.

episode. In 53 episodes (1990–91), a manual blood culture method was used to diagnose LCBI and in 73 episodes (1992–93) BacT/Alert CMBCS was used.

In all, 182 infection episodes (Appendix) occurred in 76 patients included in Part II (Table 10). Thus, 23 patients suffered one infection episode, 20 had two, 19 experienced three with 14 having more than three infection episodes.

A total of 956 chemotherapy cycles (Appendix) in 327 patients of the register data of FLG AML -92 trial (III) were included in the study (Table 10). There were 323 1st cycles, 292 2nd cycles, 219 3rd cycles and 122 4th cycles of the chemotherapy protocol.

5.4 Laboratory-confirmed bloodstream infection (LCBI) rate

5.4.1 The impact of the blood culture system on LCBI rate

After the replacement of the manual blood culture system CMBCS (I) in Oulu University Hospital in 1992, the BacT/Alert CMBCS with standard growth medium was able to detect more BSI – with a LCBI rate of 39.7 % – in febrile neutropenic episodes than the manual blood culture system had previously detected (p = 0.043; Tables 10 and 11).

Table 11. Blood culture findings of febrile neutropenic patients with acute myeloid leukaemia recovered by a manual blood culture system (Becton Dickinson) and BacT/Alert CMBCS with standard growth medium (I).

Category	In 1990–91 manual blood culture system	In 1992–1993 BacT/Alert CMBCS
All febrile episodes		
Blood-culture positive	12 (23 %)	29 (40 %)
Blood-culture negative	41 (77 %)	44 (60 %)
First febrile episode		
Blood-culture positive	10 (28 %)	23 (43 %)
Blood-culture negative	26 (72 %)	31 (57 %)
Other febrile episodes		
Blood-culture positive	2 (12 %)	6 (32 %)
Blood-culture negative	15 (88 %)	13 (68 %)

NOTE: Values are presented as number (percentage) of blood culture positive febrile episodes.
CMBCS = continuous-monitoring blood culture system.

During the years 1997–2003 (II), 90 % of blood cultures of neutropenic AML patients were taken into the BacT/Alert FAN bottles and only 10 % into the standard bottles. Overall, 90 % of the microorganisms detected in the blood cultures were recovered in FAN bottles. Despite the change of the growth medium, the LCBI rate (37.4 %) did not exceed the LCBI rate reached in yhe years 1992–1993 (I) with the standard growth medium (Table 10).

In the nationwide FLG AML -92 trial, an LCBI was diagnosed during 47.7 % of chemotherapy cycles (III). There was no difference in the LCBI rate between institutions using BacT/Alert or Bactec CMBCS ($p = 0.27$).

5.4.2 The associations of the chemotherapy and the phase of the disease with LCBI rate

During the years 1997–2003 (II), 74 (41 %) of the infection episodes (Appendix) occurred during the induction therapy cycles, while 108 (59 %) of the infection episodes took place during the consolidation phase. During the induction therapy cycles, the LCBI rate was 25.7%, while during the consolidation therapy cycles it was elevated to 45.5% (p = 0.008). HD AraC containing chemotherapy regimens – mostly given as a consolidation therepy – had a higher risk for LCBI (odds ratio; OR 2.3; Table 12), while TG-containing regimes – all given as induction therapy

Table 12. The rate of laboratory-confirmed bloodstream infections (LCBI) in adult patients with acute myeloid leukaemia treated with high-dose cytarabine (HD AraC) and thioguanine (TG) -containing chemotherapy regimens (II).

Regimens	LCBI		Odds Ratio	95 % Confidence Interval	*p*-value[1]
	Yes *n* (%)	No *n*			
HD AraC-containing, N= 84	40 (48 %)	44	2.3	1.2 to 4.2	0.009
Non-HD AraC-containing, N= 98	28 (29 %)	70			
TG-containing , N = 51	9 (18 %)	42	0.26	0.12 to 0.58	0.001
Non-TG-containing, N= 131	59 (45 %)	72			

[1]Determined by Wald's test.

with the exception of three ETI treatments – involved a clearly lower risk for LCBI (OR 0.26; Table 12) than the other regimens.

During the nationwide FLG AML -92 trial (years 1992–2006), altogether 456 LCBIs were detected. The overall incidence rate (per 1000 hospital days) for LCBI was 13.2 (95% CI 12.0 to 14.5). There was a great inter-cycle variation in the LCBI rate (from 22.3% to 61.6%). The length of hospital stay, the incidence rate for LCBI per 1000 patient days or per 1000 neutropenia days and the OR for LCBI were clearly higher in cycles II, III and IV than in cycle I (Table 1, III). During the 2nd cycle, the LCBI rate was only slightly higher in the patients who already had achieved CR (64.6%) compared to those who received the second induction course of chemotherapy (53.6%, $p = 0.11$).

5.5 Blood culture isolates

Gram-positive bacteria were the most common blood culture findings. The proportion of gram-negative bacteraemia increased markedly, when the manual blood culture system was replaced by the BacT/Alert CMBCS standard growth medium (Table 13). Although proportionally the number of gram-positive isolates decreased, the absolute number of gram-positive bacteraemias increased. No substantial change was seen in the proportions of microorganism isolated, when the BacT/Alert standard growth medium was almost completely (90 %) replaced by FAN medium (Table 13). Compared to the other chemotherapy courses, no clear excess of gram-positive bacteraemia was detected in association with HD AraC -containing chemotherapy regimens (Table 14).

Table 13. Number of microbes recovered by a manual blood culture system (Becton Dickinson) and by a CMBCS (BacT/Alert) in adult patients with acute myeloid leukaemia.

Blood culture system	Growth medium	Period	LBCI	Gram+	Gram-	Mixed[1]	Yeasts
Becton Dickinson	Vacutainer agar	1990–91 (I)	12	8(67%)	2(17%)	1(8.3%)	1(8.3%)
BacT/Alert	Standard	1992–93 (I)	29	15(52%)	13(45%)	1(3.4%)	0
BacT/Alert	FAN[2]	1997–2003 (II)	68	33(49%)	27(40%)	6(8.8%)	2(2.9%)

NOTE. Values are presented as number (percentage) of blood culture positive febrile episodes. CMBCS = continuous-monitoring blood culture system; LBCI= microbiologically documented bloodstream infection.

[1]Mixed: all gram-positive + gram-negative.

[2]FAN bottles accounted 90 % of the total number of blood culture bottles.

Table 14. The results of blood culture in adult patients with acute myeloid leukaemia after chemotherapy (II).

Chemotherapy	LCBI				
	Gram + bacteraemia	Gram – bacteraemia	Mixed bacteraemia	Fungaemia	Total No
All	33 (49%)	27 (40%)	6 (9%)	2 (3%)	68
HDAraC-containing	21 (53%)	15 (38%)	4 (10%)	0	40
HDAraC-Ida	10	4	2	0	16
Amsa-HDAraC	4	3	0	0	7
Mito-HDAraC	0	1	1	0	2
ME-HDAraC	7	7	1	0	15
Others	12 (43%)	12 (43%)	2 (7%)	2 (7%)	28
MACE	2	1	0	0	3
AI	7	8	1	0	16
IAT	3	1	1	2	7
ETI	0	2	0	0	2

NOTE: Values are presented as number (percentage) of blood culture positive infection episodes.
HD AraC = high-dose cytarabine; LCBI = laboratory-confirmed bloodstream infection.

In the nationwide FLG AML -92 trial, there was a predominance of gram-positive isolates in blood cultures (Table 15). In monomicrobial LCBIs, the proportion of gram-positive microorganisms decreased from cycle I to cycle IV, whereas the change was in opposite direction for the gram-negatives (Table 2, III). The most

Table 15. Blood culture isolates and GI-toxicity of the successive chemotherapy cycles during the Finnish Leukaemia Group AML -92 trial (III), related to the dose of AraC.

Variable	1st cycle (n = 323)	2nd cycle (n = 292)	3rd cycle (n = 219)	4th cycle (n = 122)
Daily dose of AraC	CD; 100 mg/m^2	HD; 3.0 g (2.0 g)/m^2	HD; 2.0 g (1.0 g)/m^2	HD; 6.0 g/m^2
GI toxicity, n (%)	121 (38 %)	88 (30 %)	50 (23 %)	21 (17 %)
OR	1.0	0.65	0.43	0.33
95% CI		0.43 to 0.98	0.27 to 0.70	0.17 to 62
p- value		0.041	< 0.001	< 0.001
Gram-positive bacteria, n (%)	54 (17 %)	131 (45 %)	80 (37 %)	42 (34 %)
OR	1.0	4.1	2.9	2.6
95% CI		2.8 to 5.9	1.9 to 4.3	1.6 to 4.2
p- value		< 0.001	< 0.001	< 0.001
Gram-negative bacteria, n (%)	20 (6.2 %)	77 (26 %)	70 (32 %)	41 (34 %)
OR	1.0	6.8	9.3	9.1
95% CI		3.9 to 12.1	5.1 to 16.9	4.7 to 17.5
p- value		< 0.001	< 0.001	< 0.001
Anaerobic bacteria, n (%)	3 (0.9 %)	4 (1.4 %)	0	0
Fungi, n (%)	1 (0.3 %)	5 (1.7 %)	3 (1.4 %)	3 (2.5 %)

NOTE: Dosage administered in parenthesis indicate patients ≥ 56 years old. CD=conventional dose; HD=high dose. Figures represent chemotherapy cycles with positive blood culture findings. Odds ratio (OR) with 95% confidence interval (95% CI) for World Health Organization (WHO) grade 3 & 4 gastrointestinal toxicity, Gram-positive bacteria and Gram-negative bacteria, compared with cycle I. *p*-values according to the generalized linear mixed model.

common gram-positive microorganisms in monomicrobial LCBIs were CoNSs (30.6 %) and VGS (14.1 %). The most common gram-negative microorganisms were *E. coli* (11.3 %) and *Klebsiella* species (13.1 %). The most common combination in polymicrobial LCBI was gram-positive and gram-negative microorganisms (48.8 %) followed by gram-positive organisms only (Table 3, III). In the 2nd cycle, there were no differences between patients in CR (n = 212) or those with still active leukaemia (n = 80) in terms of the occurrence of LCBI caused by gram-positive cocci, gram-negative rods (Table 16), anaerobes or yeasts. Almost one in five, 19.8 %, of patients in remission exhibited VGS growth in blood culture, compared to 11.2 % ($p = 0.119$) of patients with active leukaemia.

Table 16. Blood culture findings and gastrointestinal (GI) toxicity during the 2nd cycle of chemotherapy (HD AraC – Ida) of the Finnish Leukaemia Group AML -92 trial (III).

Micro-organism	Phase of AML		*p*-value[1]
	Active disease n=80	Remission n=212	
Gram-positive, all	33 (41%)	98 (46%)	0.51
CoNS	20 (25%)	47 (22%)	
VGS	9 (11%)	42 (20%)	0.12
Enterococci	7 (9%)	25 (12%)	
Gram-negative, all	17 (21%)	60 (28 %)	0.24
Klebsiella sp.	6 (8%)	23 (11%)	
Enterobacter sp.	5 (6%)	13 (6%)	
Escherichia coli	3 (4%)	12 (6%)	
GI toxicity[2]	37.3 %	29.1 %	0.19

NOTE: The figures represent the total number of microorganisms recovered and the percentage of WHO grade 3 & 4 GI-toxicity. The percentage of cases with positive blood culture findings are presented in parethesis. Only Gram-positive cocci and Gram-negative bacilli are presented, with three most common species of each. AML = acute myeloid leukaemia; HD AraC – Ida = high-dose cytarabine and idarubicin; CoNS = coagulase-negative staphylococci; VGS = *viridans*-group streptococci.

[1]Determined by Fisher's exact test.

[2]GI toxicity = gastrointestinal toxicity grade 3 & 4 by World Health Organization (WHO).

5.6 Inflammatory response and infection data

In Part I (years 1990–93), there was no difference between the median peak serum CRP values of febrile episodes during the manual blood culture period (169 mg/l with the 25th percentiles 114–222) and the CMBCS period (135 mg/l with 25th percentiles 73–197; $p = 0.107$). In addition, there was no difference in CRP peak value between blood culture positive and blood culture negative episodes neither during the manual blood culture period ($p = 0.26$) or during the CMBCS period ($p = 0.064$).

In Part II (years 1997–2003) in Oulu University Hospital, in addition to 68 LCBIs, 72 focal infections with negative blood culture (Appendix) and 42 FUOs were observed. The median peak CRP value for LCBI, focal infections with negative blood culture and FUO was 190 mg/l (with the 25th percentiles 129–226), 179.5 mg/l (113–250) and 115 mg/l (73–194), respectively ($p = 0.004$, ANOVA). Thus, the mean peak CRP value for FUO was 51.9 mg/l lower than the mean peak CRP value for LCBI ($p = 0.005$) and 48.4 mg/l lower than mean peak CRP value for focal infections with negative blood culture ($p = 0.009$). The median CRP peak values of different chemotherapy cycles are shown in Table 9 (on page 63).

The median CRP peak value of infections occurring during HDAraC-containing courses was 174 mg/l (115.5–239) and 170.5 mg/l (100–229) during the courses without HD AraC ($p = 0.646$). The corresponding values were 173 mg/l (113–228) during TG-containing courses and 173 mg/l (88–237) during courses without TG. The LCBI rate did not differ among those 55 infection episodes with mucosal involvement (34.5%) from those 127 episodes without mucositis (37.8%, p=0.74).

In the FLG AML -92 trial, the median CRP peak value did not differ between the cycles, but the duration of febrile days was longest during the first cycle (Table 1, III). In the 2nd cycle, the median CRP peak value did not differ between those patients with active leukemia and those who had achieved CR. WHO grade IV infections with signs of organ failure, were observed among 22 patients in the 1st cycle (6.7%), 12 patients in the 2nd cycle (4.1%), 13 patients in the 3rd cycle (6.1%) and 9 patients in the 4th cycle (7.4%).

5.7 Gastrointestinal (GI) toxicity

In the FLG AML -92 trial, severe GI toxicity was most common during 1st cycle and decreased significantly in successive cycles (Table 15). In the 2nd cycle, the amounts of Grade 3 & 4 gastrointestinal toxicity (Table 16) did not differ significantly between patients with active leukemia and patients who had achieved remission ($p = 0.194$).

5.8 Mortality

In the FLG AML -92 trial, there were 40 deaths (12.2%), of which 35 (87.5%) were due to infection. 18 (45.0 %) deaths were due to BSI. Overall, 62.9 % of deaths due to infection in general and 66.7 % of deaths due to BSI occurred during the induction courses. The outcome of infections was especially poor during the 2nd induction: in 12.5 % of cycles of there was a death due to infection in general and in 8.8 % cycles a death due to BSI. This was in sharp contrast with the low mortality associated with the consolidation courses of the 2nd cycle (Table 17).

Table 17. Deaths due to infection during the Finnish Leukaemia Group AML -92 trial (III).

Chemotherapy			Deaths due to infection (all infections) No. (%)	Deaths due to bloodstream infection (BSI) No. (%)
Cycle	Type	No.		
1st	induction	323	12 (3.7)	5 (1.5)
2nd	induction	80	10 (12.5)	7 (8.8)
			$p < 0.001$[1]	$p < 0.001$[1]
2nd	consolidation	212	4 (1.9)	1 (0.5)
3rd	consolidation	219	5 (2.3)	3 (1.4)
4th	consolidation	122	4 (3.3)	2 (1.6)

[1]Fisher's exact test was used to analyze the difference between induction and consolidation treatment groups in cycle II.

5.9 The effect of chemotherapeutic agents on microbial growth in *in vitro* -studies

5.9.1 Microbial growth in the absence of chemotherapeutic agents

The microbial control strains multiplied in all cultures with one exception. A strain of *C. glabrata* failed to grow in the standard bottle in a control culture for the test culture with 100 µmol/l Ida, AraC and etoposide and the test had to be repeated. The growth of gram-negative bacteria was the fastest. All the rods resulted in a positive growth signal within 16 h in the standard bottles and within 22 h in the FAN bottles. All the gram-positive cocci resulted in a growth signal within 22 h in the standard bottles and within 53 h in the FAN-bottles. Yeasts strains grew slowest, requiring up to 60 h in standard bottles and up to 80 h in the FAN bottles to evoke a positive growth signal (Tables 1, 2 and 3, IV and Table 1, V).

5.9.2 The effect of the chemotherapeutic agents in the standard bottles

The growth of gram-positive cocci. In comparison with the control culture, doxorubicin had an inhibitory effect on the growth of gram-positive cocci at a concentration of 100 µmol/l (Table 1, IV). No growth could be detected up to a median time of 168 h (min 47, max > 168; $p < 0.01$). Etoposide at 100 µmol/l had also an inhibitory effect on the cocci (Table 1, IV), causing a growth delay

to 70 h (min 16, max > 168; $p < 0.05$). At the lower concentrations tested (1 and 10 µmol/l), however, these chemotherapeutic agents had no effect on the coccal growth (Figures 1 and 2, IV).

Ida, at a 100 µmol/l concentration (Table 1, V) had an inhibitory effect on the growth of gram-positive cocci as compared to controls and to the test cultures in the FAN bottles ($p < 0.05$). Ida had the strongest inhibitory effect on the growth of *S. epidermidis* and two VGS (*Streptococcus mitis* and *Streptococcus oralis*), which failed to grow in the presence of the drug. Inhibition against *Enterococcus faecalis* and *Enterococcus faecium* was less extensive, as their incubation time with Ida was 8.8-fold and 2.4-fold longer than that in the corresponding control culture When Ida was combined with AraC in the presence or absence of etoposide, the same effect as that observed with Ida alone was found: in comparison with the test cultures, the growth of gram-positive cocci was inhibited ($p < 0.05$). In the dilution series (Table 2, V), the growth of streptococci was totally inhibited by an Ida concentration of 25 µmol/l. Growth of enterococci and *S. epidermidis* was delayed in the concentration between 25 µmol/l and 50 µmol/l. *S. epidermidis* was able to grow in a concentration of 100 µmol/l but the growth was not observed until after 58 h.

The growth of gram-negative rods. No growth inhibitory effect on gram-negative rods was observed with the chemotherapeutic agents tested in a 100 µmol/l concentration (Table 2, IV and Table 1, V), except for some inhibition by Ida against *Stenotrophomonas maltophilia* in a standard bottle (Table 1, V). A clear delay in the growth of *S. maltophilia* was detected with an Ida concentration of 250 µmol/l (Table 2, V).

The growth of yeasts. C. glabrata, but no other yeast strains, was sensitive to Ida at a 100 µmol/l concentration (Table 3, IV and Table 1, V). In the dilution series, *C. glabrata* proved to be the most sensitive of the strains tested to the growth inhibitory effects of Ida. The minimum concentration of Ida required to delay the growth of *C. glabrata* was 1 µmol/l and the growth was totally inhibited by a concentration of 10 µmol/l (Table 2, V). When Ida was combined with AraC in the presence or absence of etoposide, the same effect that was observed with Ida alone – i.e. growth failure – was found.

5.9.3 The effect of the chemotherapeutic agents in the FAN-bottles

No growth inhibitory effect of chemotherapeutic agents in any of concentrations used in any of the microorganisms tested was observed in FAN bottles (Tables 1, 2 and 3, IV and Table 1, V); all the growth inhibitory effects of the anti-neoplastic agents were observed in the standard bottles.

6. Discussion

6.1 The study protocol

6.1.1 The clinical studies

Since patients with AML are exposed to intense and long-lasting neutropenia and are at a greater risk of developing a severe infection than other neutropenic patients with haematological malignancies or solid cancers, the population of AML patients was an appropriate group to study the diagnostics of BSI in neutropenic patients. Since most studies in this field have been performed with heterogenous unselected cancer populations, it was decided to perform the clinical part of the present study with a highly uniform patient population. Since, it was possible to access the two AML trials (AML -86 and AML -92) of the FLG, it was possible to select a patient population that was quite homogenous not only with regard to the pathophysiology but also to the treatment delivered. The lack of a confounding factor like antimicrobial prophylaxis was also a favourable feature, considering the fact that the main target of the study was to assess, what is the proportion of BSI in neutropenic fever and the main end-point of the study was LCBI.

The study population was quite small, especially in Parts I and II. Consequently, successive febrile/infection episodes in the same patients had to be included into the study to obtain sufficient power for comparisons. The aforementioned problem was especially important in Part I, where – in addition to being compelled to compare two time-spans with each other instead of direct comparison – several febrile episodes occurring during the same neutropenic episode had to be included in the study and some patients (for example patient No.10 in Table 8, on page 60) played a prominent role in the data. In Part II and especially in Part III, the patient population was larger. The problem of inclusion of successive infection episodes from the same neutropenic period could be avoided in Part II but in Part III, due to nature of the register data, it is possible that in some cases data of several infection episodes have been combined into data of an individual chemotherapy cycle.

Nonetheless, in every clinical part of the present work, several infection espisodes of the same patient had to be included in the study. This is a disadvantage in Parts I and II, whereas in Part III, the successive chemotherapy cycles and infectious events were an integral part of the study protocol. In Part III, a special statistical method was used to diminish the problem of successive events in the

same patient (Brown & Prescott 2006). Although the main purpose of the AML -92 trial was to investigate the outcome of AML in general in patients treated according to the AML 92 -protocol and the infections were not the end-point of the study, the data used in Part III was collected prospectively with a structured way – in several centres by several individuals. Thus, Part III was rather well designed to reveal the relationships between chemotherapy and LCBI.

The widely accepted criteria for severe neutropenia (blood PMN count less than 0.5 x 10^9/l; Bow *et al*. 2006) or granulocytopenia (blood granulocyte count less than 1.0 x 10^9/l; Klastersky *et al*. 1986, EORTC 1993) were used in the study. In the original articles of Part I and II, data about CRP peak value and duration of neutropenia was expressed as mean values, whereas in Part III medians were used. In this respect, using medians is the better approach, because of the heavily right-skewed distributions. The other reason for changing the CRP peak value and neutropenia data to medians was to present data in a uniform way. The peak serum CRP-level over 40 mg/l used as one of the criteria for separating successive infection episodes from each other in Part II can be considered to be a somewhat artificial cut-off level, because tumour-associated fever in some patients may be associated with CRP-levels higher than that cut-off value (Manian 1995).

While the chemotherapy was rather uniform (AML -86 in Part I, AML -92 in the Parts II and III with the exception of a part of the Part II), and the definition of neutropenia remained the same throughout the study period, there were differences in the way in which the problems were approached. In Part I, the event under study was the febrile episode, while in Parts II and III, the event under scrutiny was a broader concept, an infection episode. Fever is the main symptom of severe infections in neutropenic patients, but not all neutropenic patients with bacteraemia or fungaemia are febrile (Garner *et al*. 1996), this being the reason why the concept under scrutiny was expanded. In Part I, the bacteraemia was the end point of the study, whereas in Parts II and III, LCBI was the main objective of interest. The latter difference reflects the wide time span during which this study was being conducted. In 1990s, it was conventional to speak about bacteraemia and sepsis. More recently, with the increasing number of fungaemias, the concept of BSI was introduced. In Part I, actually one candidemia was observed and the statistics were corrected to evaluate the occurrence of LCBI. In Part II, the infection episodes with preceding antibiotic therapy and the episodes with apparent non-infectious causes for fever or elevated CRP were excluded, while in Parts I and III there was no such restriction in inclusion of events. Part II was an introductory study to Part III. In Part II, the main objective was to determine if chemotherapeutic drugs would have

any effect on or associations with the occurrence of LCBI. For that reason, the possible confounding factors were reduced as low as possible. Parts I and II were retrospective studies, in which the author collected the data. The data used in Part III was collected during AML -92 trial by the FLG.

In the original article of Part II, the amount of focal infections with negative blood culture was incorrect. The number of cases was 72, not 71, as presented in the original article. In addition, the figures reported as risk ratios (RR) were actually ORs. There was an attempt to correct these mistakes before the manuscript was published, but unfortunately the article had already gone to press.

6.1.2 The in vitro -studies

The simulated blood culture of the *in vitro* -studies (IV and V) corresponded rather well to the real blood culture taken from a cancer patient, with the exception of the concentrations of the chemotherapeutic agents. The concentrations of the drugs in initial tests were much higher compared to those achievable in patients. The high concentrations were chosen to examine if one of the hypotheses examined in the present study – i.e. do chemotherapeutic agents have a significant growth inhibitory effect sufficient to prevent a positive blood culture in BSI – is sound at all. The intial amount of the microorganisms in the blood culture was about the same (approximately 10 cfu/ml) as has been proposed to exist in BSI (Finegold *et al*. 1969, Kellog *et al*. 2000). The simulated blood cultures were prepared by two highly skilled special laboratory nurses and performed in the same CMBCS cabinet. The time needed by the CMBCS to detect microbial growth was comparable to other studies of the same type (Horvath *et al*. 2004, Flayhart *et al*. 2007). In addition to the microbial strains recovered in Oulu University Hospital, ATCC control strains were also used. However, there is at least one source of uncertainty, i.e. in the correspondence of the *in vitro* -conditions to the *in vivo* -circumstances. In patients, many chemotherapeutic agents are rather rapidly transformed into metabolites, for example Ida to idarubicinol. The transformation of Ida to idarubicinol is mediated by the liver (Robert 1993). It is most probable that this transformation does not occur in a culture of sheep blood but on the other hand, Ida will probably remain unchanged also in a culture of human blood. In most cases, in addition to chemotherapeutic agents, there are also metabolites of chemotherapeutics in the blood of patients. Information about the possible antimicrobial effects of the metabolites of chemotherapeutic agents was not obtained in Parts IV and V. Thus, the *in vitro* blood cultures used in the present study do not completely correspond to blood cultures of neutropenic AML patients.

Compared to previous studies, the present one has clear advantages. The early studies in this field were performed on agar plates (Hamilton-Miller 1984, Bodet *et al*. 1985) whereas the present study was performed in blood culture conditions. Peiris & Oppenheim (1993) used blood culture bottles in their study but the growth medium was a nutrient broth, whereas in the present study, sheep blood was used. In their study Hopfer *et al*. (1983) used blood as a growth medium and also an automated blood culture system but not a CMBCS. In the present study, CMBCS made it possible to determine the incubation time within 10 minutes intervals, thus enabling the detection of also minor influences of the chemotherapeutic agents on the growth of the microorganisms. In their study Nyhlen *et al*. (2002) used Mueller-Hinton broth but they did not simulate blood culture. Their study concentrated on the bactericidal effects of combinations of antimicrobial and antineoplastic agents, while the effects of antimicrobial agents were not investigated in the present study. Kwok *et al*. (2010) performed a meritorious study in which they assessed *in vitro* effects of DNA topoisomerase inhibitors – including also doxorubicin and Ida – on Candida albicans in cultures with broth as a culture medium.

6.2 Blood culture results

6.2.1 Impact of the blood culture system on recovery of isolates from blood culture

There was a clear increase in the amount of detected microbial pathogens in the Haematological Ward of Oulu University Hospital when the manual blood culture system was replaced by the CMBCS (I). In the original article of Part I, there was no difference between the mean peak serum CRP values measured during the blood culture-positive and -negative episodes when the manual blood culture was in use. When CMBCS was used, the mean peak CRP levels of blood culture-positive episodes was higher ($p < 0.02$) than the values of the culture-negative episodes. This finding was interpreted to indicate that the increase in blood culture isolates by the CMBCS was caused by real pathogens. However, when the sole candidemia was included into the data and CRP values were handled as medians, statistical significance in comparing CRP values was lost.

Due to the weaknesses of protocol of Part I study (two time periods were compared with each other in a retrospective analysis and successive events in the same patient were included) and the small patient number, the results of Part I must be interpreted with caution. However, findings of Part I are similar to most of the

other corresponding studies, in which Bact/Alert CMBCS (Rohner *et al*. 1995) and Bactec CMBCS (Cockerill *et al*. 1997) media detected more septicaemia and pathogens than manual systems. Prior data also indicate that the risk of developing infections is not influenced by preceding episodes of infection, if they have been completely eradicated, as was the case in the present study. Thus, the results of Part I most likely indicate the superiority of CMBCS over manual systems in detecting microorganisms also in neutropenic patients.

Weinstein *et al*. (1997) proposed that an issue possibly partly explaining the high rate of BSI observed in some single-centre studies is the fact that all the streptococci and enterococci are interpreted as true pathogens, although these microorganisms may also represent contamination. However, as Weinstein *et al*. also remarked, patients with neutropenia are more likely to have a true BSI episode instead of a contaminant episode, when compared to non-neutropenic patients. In the present study, very intense chemotherapy sufficient to evoke severe AM had been administered to the patients. Thus the enterococci and VGS, which are copiously present in the mouth and the bowel of the patients, had free access to the bloodstream and could have become opportunistic pathogens representing mostly true LCBI. In addition, the patients were profoundly neutropenic, which made them vulnerable to opportunistic pathogens.

In the present study, there was a change in the microbial spectrum: the proportion of gram-negatives increased, when CMBCS replaced the manual system (Table 13, on page 65). The increase of gram-negatives indicates that the elevated blood culture yield was due to recovery of true pathogens and not to contaminant microbial strains. It has been shown that most contaminants recovered from blood cultures are CoNSs (Lee *et al*. 2007), which are derived from the skin. In Part II, strict criteria were used to exclude contaminants from the data and when the manual blood culture system was replaced by the BacT/Alert CMBCS, the quality of obtaining blood cultures did not deteriorate. On the contrary, it was easier to dispense the blood specimen into the BacT/Alert bottles than into the bottles of the manual system. In addition, the importance of disinfection of the skin was emphasized when the CMBCS was taken into use. The LCBI rate of Part II was about the same as it was in study I when the CMBCS was used. This finding further confirms the observation that the CMBCS can detect more microbial pathogens in febrile neutropenic patients than a manual blood culture system.

The very high LCBI-rate in Part III was probably at least partly related to the fact that compared to Parts I and II, a different study protocol was used in Part III: a chemotherapy cycle was used as a unit, not a febrile or an infection episode.

It is most likely that occasionally there were several infections during the same cycle and any of these infections could make the cycle blood culture-positive. The LCBI-rate was not an end-point in Part III. Instead, assessing the relation of the occurrence of LCBI and chemotherapy regimens was the main objective. Still, the elevating trend in the LCBI rate in the present study parallels the rising trend in LCBI rate in other studies (Table 1, on page 29). As described previously (Madani 2000, Velasco *et al*. 2006), the proportion of polymicrobial LCBI was high in this population of neutropenic cancer patients. In view of the fact, that the participating institutions had a predominance of gram-positive LCBI, the large number of CoNS was no surprise (Wisplinghoff *et al*. 2003, Hämäläinen *et al*. 2008, Malagola *et al*. 2008; Table 2, on page 30).

In the *in vitro* studies (IV and V), doxorubicin, Ida and etoposide displayed inhibitory effects on the growth of gram-positive cocci and *C. clabrata*. This is in accordance with earlier studies (Bodet *et al*. 1985, Peiris & Oppenheim 1993) in which doxorubicin also inhibited the growth of aerobic gram-positive cocci. The aforementioned result of the present study has also similarities with results of a study, which was published a little later then Part V. Kwok *et al*. (2010) found that aclarubicin had growth inhibitory activity against *C. albicans* and other anthracyclines – daunorubicin, doxorubicin and Ida – affected the morphology *C. albicans*. In the study of Peiris & Oppenheim (1993), *C. albicans* was sensitive to doxorubicin. In the present study, *C. glabrata* was sensitive to Ida. *C. glabrata* has been a rare finding in blood cultures. This species is difficult to cultivate even in chemotherapeutic-naive situations and its sensitivity to chemotherapeutics may further impair its recovery in patients receiving Ida-containing chemotherapy.

FAN bottles neutralized chemotherapeutic agents and favored the recovery of gram-positive cocci and *C. glabrata*. Nonetheless, the FAN bottles do not seem to increase the recovery of microorganisms in AML patients receiving chemotherapy. This is most likely due to the fact that the growth inhibitory effects of the chemotherapeutic agents were only observed in concentrations exceeding C_{max} in most situations. The lone exception was *C. glabrata*, which due to its relative rarity, could not increase the LCBI rate in the context of FAN bottle use in Part II. On the other hand, in chemotherapeutic-naive situations, the *in vitro* growth of gram-positive cocci and yeasts was slower in FAN than in standard bottles. Thus, the present study indicates that the FAN bottles confer no advantage over standard bottles in patients being treated with chemotherapeutics while earlier studies (McDonald *et al*. 1996) have established their usefulness when blood cultures are obtained during antimicrobial therapy.

6.2.2 *Impact of the chemotherapy and the phase of the disease on recovery of isolates from blood culture*

In the present study, HD AraC was associated with an increased rate of LCBI. The association of HD AraC with an elevated rate of infections is not a new finding. Previously, HD AraC treatment has been shown to be associated with an increased rate of streptococcal infections in general (Cordonnier *et* al. 2003), and also to streptococcal bacteraemia (Kern *et al*. 1990, Bochud *et al*. 1994). In the present study, the increase in the incidence of LCBI associated with HD AraC use was observed when the microbial findings were handled as a totality. In Part II, there was no difference between patients who received a HD AraC containing chemotherapy and those who received non-HD AraC containing regimen in the number of gram-positive infections, streptococcal infections or infections caused by VGS. This lack of difference may reflect the small number of cases.

The inflammatory response, as measured via CRP values, did not differ between HD AraC- and non-HD AraC -containing chemotherapy cycles. This is not suprising because the amount of severe infections did not differ greatly between chemotherapy cycles of AML -92 trial. In Part II, the duration of neutropenia did not differ between HD AraC- and non-HD AraC -containing cycles. The cycles of FLG AML -92 study did differ from each other in the duration of neutropenia. However, the difference in duration of neutropenia was rather small, a mere few days (Table 1, III). It seems that the amount of immunosuppression caused by the various FLG chemotherapy was about the same. Thus, different intensity of chemotherapy courses or different grades of infection are unlikely to explain the dissimilarities in LCBI rate observed between the cycles.

In Parts II and III, TG was associated to a low number of LCBI. This finding may be due to the fact that TG-containing regimens did not contain HD AraC and thus it is probably the lack of HD AraC and not the presence of TG that explains the low LCBI rate in cycles with TG-containing regimens. True, TG has been shown to have antimicrobial properties, for example against *Plasmodium falciparum* (Queen *et al*. 1990), but in Part V even at the highest concentrations, TG did not inhibit the growth of any of the microorganisms studied. In fact, of all the chemotherapeutic agents studied *in vitro*, in concentrations attainable in the plasma of patients, only the anthracyclines were able to exert any clear growth inhibitory effects on microorganisms: Ida prevented the growth of *C. glabrata* and doxorubicin inhibited growth of gram-positive cocci.

The *in vitro* growth inhibitory effect on Ida on *C. glabrata* was minor but is in accordance with the study of Kwok *et al.* (2010), in which another DNA topoisomerase inhibitor – aclarubicin – displayed growth inhibitory activity against another *Candida sp.*, i.e. *C. albicans*. In Part II, the OR for LCBI for regimens containing Ida (n = 132) was 0.37 (95% CI 0.19–0.72). In 37 cases, Ida was given in combination with HD AraC (HDAraC-Ida). HD AraC had a much higher association with LCBI (OR 2.3 ; 95 % CI 1.2–4.2) than Ida. On the other hand, in 51 cases, Ida had been given in combinations with chemotherapeutics containing also TG (44 AIs and 14 ETIs). TG had a lower association with LCBI (OR 0.26; 95% CI 0.12–0.58). It is difficult to interpret the influence of HD AraC and TG on the aforementioned OR calculated for Ida, but it seems clear that growth inhibitory effects caused by of the chemotherapeutic agents studied did not alter LCBI rates in the present study. Nonetheless, it is possible that in some rare cases, microbiological diagnostics of some relatively rare microorganisms, such as *C. glabrata* – which is known to be difficult to culture – may be impaired because of the growth inhibitory effects of chemotherapeutic agents.

Is it possibile that the successive nature of chemotherapy – in addition to antimicrobial treatment – accounts for differing blood culture result during chemotherapy protocols? As could be expected, in FLG AML -92 trial, the incidence of BSIs caused by VGS was high during the 2nd cycle in which HD AraC was administered with Ida. Unexpectedly, the incidence of VGS was lower in the 4th cycle, when the dose of AraC was at its highest (Table 15, on page 66). In 4th cycle, HD AraC was administered with amsacrine. Thus, the combination of chemotherapeutics may also be important for the emergence of BSI caused by VGS. Could the repetitive predisposition to chemotherapy in some way affect microorganisms i.e. possibly changing their virulence or altering other interactions between the microorganisms and the host? As mentioned earlier, DNA topoisomerase inhibitors such as doxorubicin and Ida have been recently shown to have an influence on the growth and morphology of *C. albicans* (Kwok *et al*. 2010). Could corresponding changes occur also in other microorganisms?

There are other reasons for the differing rates of LCBI and differing microorganisms found in successive chemotherapy cycles, which are linked to chemotherapy. It has been shown that the phase of the malignant disease modifies the infection spectrum of patients. In a previous study (Velasco *et al*. 2003), it was found that most of the serious infections in patients with acute leukaemia occurred during the intensification therapy and during relapse, a finding not completely different from the observations of the present study. In Part II, TG given mostly as

induction therapy was associated to a low rate of LCBI. In Part III, patients with active disease in the 1st cycle had less LCBI than patients in the 2nd cycle, during which ICC containing HD AraC was delivered. In the 2nd cycle, all the patients received HD AraC – Ida regimen. The LCBI rate was slightly higher ($p = 0.11$) in those patients who had achieved remission, compared to those who still had active leukaemia. Thus, the phase of the disease may have some major unknown influence on occurrence of LCBI.

From an investigational standpoint, with standardized treatment protocols in use, the effects of successive chemotherapy on microorganisms are a challenging question at least in clinical studies. The phase of the disease requires a certain type of chemotherapy and the change of the phase mandates a change to a different type of chemotherapy. Thus, the disease and the treatment as predisposing factors for LCBI are inseparably intertwined. Only, a situation in which a change to the chemotherapy protocols would be appropriate, might make it possible to separate the influence of the phase of the disease, and thus new information about the influence of the administered chemotherapy could be obtained. On the other hand, this question may be clarified in *in vitro* studies.

6.2.3 Associations between dose of AraC, GI toxicity and recovery of isolates from blood culture

The results of Part III were surprising, when GI toxicity (WHO grade 3 & 4) was taken into account. HD AraC is well-known for its GI toxicity. In the FLG AML -92 trial, GI toxicity was highest during the 1st cycle whereas it would have been predicted to be lowest, because AraC prescribed in the 1st cycle was conventional dose (CD) rather than high dose (HD), as in later cycles. As GI toxicity was highest in the 1st cycle, also the recovery of microorganisms would have been anticipated to have been highest, but instead the lowest LCBI rate was observed in the 1st chemotherapy cycle (Figure 1, III). No exact explanation can be given for this discrepancy between the occurrence of GI toxicity and use of HD AraC, but it is possible that leukemia *per se* at least partly explains these findings. In the 1st cycle, all patients had active disease. Altered intestinal permeability and absorption of sugars have been demonstrated in patients with AML even without treatment (Sundstrom *et al.*1998, Blijlevens *et al.*2004). Another explanation for these surprising findings about GI toxicity may be that measuring toxicity by the WHO method is not an appropriate way to assess GI permeability, which could provide access to the bloodstream for microorganisms in these patients (Bow & Meddings

2006). While measuring GI toxicity, WHO classification takes into account also plasma transaminases, alkaline phosphatase and bilirubin. The elevation of liver enzymes and worsening of liver function may not correlate to increased permeability of GI tract. Nausea and vomiting described in WHO grading may originate purely from the central nervous system. In AML patients if one wishes to measure toxic effects of chemotherapeutics on the GI tract – and also the respiratory tract – this may require a different tool than the grading system of WHO.

The association of chemotherapy-caused AM – especially by HD AraC – with infections has been documented (Slavin *et al*. 1978). It is believed, that HD AraC increases streptococcal bacteremia via its effects on the upper GI tract and the respiratory tract (Kern *et al*. 1990, Bochud *et al*. 1994). On the other hand, a recent study suggested that the impact of mucositis in general as a source of infection in neutropenic patients (at least those caused by gram-positive bacteria) is declining (Cordonnier *et al*. 2003), and in yet another study in patients with acute leukaemia receiving chemotherapy with CD AraC and Ida, increased intestinal permeability correlated with increased rate of LCBI (Bow & Meddings 2006). Thus, the recent data concerning the influence of AM on the occurrence of LCBI is somewhat inconsistent. In the present study, the LCBI rate did not differ between those infection episodes with mucosal involvement from those without mucositis (II), but notwithstanding the GI toxicity findings, the effects of HD AraC on the lower GI-tract seem to be an important risk factor for LCBI, because the occurrence of Gram-negative blood culture isolates correlated with the prescription of HD AraC (Table 15 on page 66).

6.3 Mortality due to infection

In the FLG AML -92 trial, 63 % of deaths due to infection occurred in patients receiving induction treatment. As in Part II, there was the lowest amount of LCBI during the 1st chemotherapy cycle. In addition, the highest amount of GI toxicity and also the highest amount of mortality due to infection was observed during the 1st chemotherapy cycle. Thus, despite the lower LCBI rate, the outcome of infections is worse among AML patients with active disease than among those who have achieved CR.

There is nothing new in the finding that patients with active leukemia have an increased risk of death (Velasco *et al*. 2003). However, it is a slightly odd finding that the mortality due to infection of patients with active disease was higher than of those in CR, when the inflammatory response measured by CRP was not different between the different cycles or between patients with active disease and remission

in the 2nd cycle. Furthermore, there was no difference in grade IV infections between different chemotherapy cycles. Instead, there did seem to be an association between WHO grade 3 & 4 GI toxicity and mortality due to infection. The present study does not reveal whether this is a causative association, or if the increased GI toxicity simply reflects active leukemia, which in some other way weakens the ability of the patient to resist infections.

6.4 Fever of unknown origin – what is it?

Although with the CMBCSs, the LCBI rate of febrile neutropenic cancer patients has risen to 40 % and even beyond that value (Table 1, on page 29), fever without any obvious infectious cause still often responds to antimicrobial therapy (Harter *et al*. 2006). In Part II, the proportion of FUO of infection episodes was 23 %. Peak serum CRP valus were lower in FUOs than in LCBIs or in focal infections with negative blood culture. This indicates that while most of cases of FUO may be infections, all of those infections are not severe. At least a part of these putative infections must be focal without bacteraemia or fungaemia. As stated earlier, when there is a lack of neutrophils in blood, pneumonias may be invisible in X-ray (Valdivieso *et al*. 1977). Correspondingly, also other focal infections may be difficult to identify in patients with neutropenia.

However, there can still be some rise in the LCBI rate of febrile neutropenic cancer patients. Will this rise be documented by CMBCSs or by the novel techniques such as real time PCR? This depends on the identity of these microorganisms that nowadays are not found. The use of RCR techniques is based on the awareness of the microorganisms that are usually found in blood cultures of neutropenic cancer patients. Furthermore, PCR suffers from the disadvantage that it detects also dead pathogens and parts of pathogens, which weakens the potential for its results to be directly applied in the treatment of infections.

It is possible that the microorganisms missed in blood cultures of febrile neutropenic patients are species that do not usually cause infection. The sensitivity of blood cultures to detect slow-growing and fastidious organisms can be poor. Blood cultures may overlook even important causes of community-acquired pneumonia that are fastidious to culture, such as *Legionella pneumophilia*, *Clamydia pneumoniae* and *Mycoplasma pneumoniae* (Sočan *et al*. 1999). In particular, some yeast species are difficult to culture (Kirby *et al*. 2009). Recently, new potential pathogens have been described, such as those found in food products (Yamazaki *et al*. 2009). Thus, an improvement in culture techniques may lead to a rise in the LCBI rate.

Microorganisms that are missed in blood cultures could be species that have not even been characterized yet. It should be borne in mind that the GI tract provides a great potential for the delivery of opportunistic pathogens. Of the 1200 GI microorganisms described so far, only about 12 % are recovered by application of both molecular and cultivation-based approaches (Zoetendal *et al*. 2008). A significant intersubject variability has been discovered in stool and mucosa community composition (Eckburg *et al*. 2005). It has been estimated that the GI microbial diversity consists of up to 1000 microbial species per individual and more than 5000 species in total (Zoetendal *et al*. 2008).

In summary, the answers to the question about the origin of FUO may require advances in basic microbiological research in addition to the development of better diagnostic techniques. One could speculate that these techniques should be able to detect parts of microbes and toxins produced by microbes. However, it is possible that all of the pathogens multiplying in blood will never be recovered. It also must be kept in mind that BSI represents only part of the severe infections of neutropenic patients. There will probably always exist the need to administer empiric antimicrobial therapy, when a neutropenic patient becomes febrile.

6.5 Future prospects

In the present study, the 2nd cycle of the FLG AML -92 protocol appeared to have a pivotal role. Before that time the patients had received the same CD AraC containing chemotherapy treatment and during the 2nd cycle, all the patients received the same HD AraC containing chemotherapy regimen. However, at that point, two groups had been developed: those who had achieved CR and those with still active leukaemia. Despite the marked difference between the patients with active leukaemia in the 1st cycle and patients in CR in 3rd and 4th cycles in LCBI rate, no marked differences could be demonstrated in the 2nd cycle (Table 16, on page 67). This lack of difference between the groups probably at least partly reflects the quite small case number in 2nd cycle (80 cases vs. 212 cases). If the number of patients would have been greater, the difference in amount of VGS might have been more significant between patients with remission than those with active disease and also the difference in GI toxicity measured by WHO classification might have been more marked between the two patient groups. These results indicate that HD AraC is not the sole major risk factor for VGS infection in neutropenic infection but the phase of the leukaemia also has an effect. These results also indicate that patients with active leukaemia are more prone than patients in remission to suffer

GI symptoms and have elevated liver enzymes, but the knowledge about other aspects of GI toxicity of chemotherapeutic agents – such as increased permeability of GI tract – might be more useful than GI toxicity graded by WHO classification, in assessing infection risks associated to chemotherapy.

Thus, Part III possibly was not powerful enough to detect differences in 2nd cycle. With a more powerful study it might be possible to assess the relations between phase of the malignant disease, LCBI rate, outcome of infections and GI toxicity in 2nd cycle with a homogenous patient population receiving the same kind of chemotherapy. Comparing successive chemotherapy cycles (1st, 2nd, 3rd and 4th) with each other may cause a bias. As shown by Kwok *et al*. (2010), chemotherapy – as well as antimicrobials – causes alterations at least in some microorganisms. Considering the relative rareness of AML and the labour-intensivity of its treatment, designing a more powerful study than the present one is not easy but it might be possible in the context of multi-centre treatment trials.

Future studies should probably use intestinal permeability as a marker of GI toxicity caused by chemotherapy of ALM as proposed by Bow & Meddings (2006). In particular, the intestinal permeability of patients receiving the 2nd chemotherapy cycle for AML should be investigated, to reveal possible differences between the patients who have achieved CR and those with still active disease. In addition to absorption of sugars (Blijlevens *et al*. 2004), putative structural changes in the intestinal mucosal barrier need to be studied. Not only ulcers (Slavin *et al*. 1978), but even relatively minor changes, for example in the paracellular pathway of the intestinal epithelium (Turner 2009), may be important. Further studies could assess the differences between GI changes related to active leukaemia and those caused by HD AraC regarding barrier function and immune defence. As is widely known, intestinal mucosal surfaces are crucial sites of innate and adaptive immune regulation and immune activation is often related to increased mucosal permeability (Turner 2009). Altered intestinal permeability has been demonstrated in patients with AML without treatment (Blijlevens *et al*. 2004). The intestinal immune function of patients with active AML may also be disrupted.

What about serious infections? Most of deaths due to infection occurred in patients receiving induction treatment (CD AraC in 323 cases, HD AraC in 80 cases) active leukaemia and high GI toxicity graded by the WHO classification. Unfortunately, the register data of FLG AML -92 trial was not sufficiently complete to include data about focal infections (for example, invasive candidiasis) and their association to LCBI, outcome of infections and GI toxicity.

There have been continual changes in the field of neutropenic infections. To some extent, the changes have been induced by the therapy given to the patients, partly for some other reasons. It is also possible that emerging new treatments for AML will give rise to new problems and at least some of the questions discussed in this chapter may become irrelevant. However, it seems that chemotherapy regimes based on HD-AraC and anthracyclines will still occupy a central role in treatment of AML for many years to come.

7. Conclusions

1. The development of automated continuous-monitoring blood culture systems has improved the diagnostics of neutropenic infections and as a result, the proportion of laboratory-confirmed bloodstream infection (LCBI) of febrile episodes has increased since the early 1990s.

2. Some chemotherapeutic agents modulate the occurrence of infections. In particular, administration of high-dose cytarabine (HD AraC) predisposes patients to bloodstream infections (BSI).

3. The propensity of alimentary mucositis -producing chemotherapeutic agents such as HD AraC to promote infections in patients with acute myeloid leukaemia (AML) may be dependent on the other chemotherapeutic agents prescribed with this drug. As a predictor of outcome of infections in neutropenic patients, active leukaemia appears to be more important than the chemotherapeutic regimen given.

4. Some chemotherapeutic agents were found to inhibit the growth of microorganisms, but the effects on microorganisms observed in these *in vitro* studies are most likely to be clinically unimportant. Nonetheless, the detection in blood cultures of some difficult-to-culture microbial strains – *C. glabrata* for example – may be impaired by the growth inhibitory effects of chemotherapeutics. Overall, chemotherapeutics affect the LCBI rate mostly in ways other than via growth inhibition. Alterations in physiological barrier functions are the most likely mechanisms.

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Appendix

Definitions in the clinical data

The following definitions were used:

A cycle of chemotherapy:
The time span from the beginning of a chemotherapy course to the beginning of the next course.

Neutropenia:
Neutropenia was considered to begin when bood PMN count dropped below 0.5 x 10^9/l (Bow *et al*. 2006) and to end when PMN count rose above 0.5 x 10^9/l. In cases without consecutive PMN counts, the neutropenia episode was considered to be over when the leucocyte count reached or exceeded 1.0 x 10^9/l (EORTC 1983). If a patient was neutropenic when the induction treatment began, the neuropenia was considered to begin when the induction treatment began.

A febrile episode (I):
The patient was considered febrile, if axillary temperature was 38.5 °C or higher once, or axillary temperature was 38 to 38.5°C at least two h (Giamarellou *et al*. 2000, Velasco *et al*. 2003). Febrile episodes were regarded as separate instances if the axillary temperature was less than 37.5°C > three days with clearly decreasing CRP values between periods.

An infection episode (II, III):
In study II the definition of an infection episode was based on clinical judgement: an infection episode was considered to be present when at least 2 blood cultures (two set of blood culture bottles) were obtained during a decline of blood PMN count or neutropenia and the clinician prescribed antibiotic medication for neutropenic infection. The infection was considered to have resolved when the patient's body temperature remained below 37.5 C° for three consecutive days, or the clinician considered the resolution to be sufficient to start a new chemotherapy course in spite of the subfebrile temperature, broad-spectrum bacterial antibiotics had been withdrawn for at least three consecutive days, and the serum CRP level was below 40 mg/l. In study III, infections were reported according to the protocol of the AML -92 trial by the FLG (Koistinen *et al*. 2007).

Laboratory-confirmed bloodstream infection (I to III):
Laboratory-confirmed bloodstream infection (LCBI) means a febrile episode or an infection episode with clinically significant bacterial or fungal growth in blood culture. In study I, to be considered a true pathogen, the common skin contaminants had to be recovered in at least two blood cultures taken at different times or from different veins. In study II, the CDC criteria (Garner *et al*. 1996) were followed: the growth of a common skin contaminant (such as CoNS, *Bacillus* and *Propionibacter sp*., etc.) in blood culture was considered to indicate bacteraemia if the same bacterial strain was detected in at least two blood cultures taken at different times or from different veins, and one of the following symptoms was documented: fever over 38°C, chills or hypotension. As a modification to CDC criteria, a common skin contaminant detected in only one blood culture was considered the cause of bacteraemia if the same bacterium was detected in a peripheral blood culture and in a removed central catheter. In study III, the judgement considering true pathogens and contaminants was done independently in each participating hospital.

LCBI rate:
In studies I and II, LCBI rate means the proportion of laboratory-confirmed BSI of the total number of febrile/infectious episodes (%). In study III, LCBI rate means the proportion of chemotherapy cycles with LCBI of all chemotherapy cycles.

Focal infection with negative blood culture (II):
Focal infection with negative blood culture means that no bacteraemia or fungaemia was detected but a focal infection described in the CDC classification was identified (Garner *et al.* 1996).

Fever of unknown origin (II):
Fever of unknown origin (FUO) means an episode of fever without bacteraemia, fungaemia or focal infection, which disappeared along with a decrease of the CRP value when the patient was started on broad-spectrum antibacterial or antifungal therapy (Durack & Street 1991).

Definitions in *in vitro* -studies

The following definitions were used:

Test culture:
Test culture means a culture performed in a BacT/Alert® blood culture bottle containing growth matrix (broth and sheep blood), a microbial strain and a chemotherapeutic agent or their mixture.

Control culture:
Control culture means a culture performed in a BacT/Alert® blood culture bottle containing growth matrix (broth and sheep blood), a microbial strain and physiologic saline instead of chemotherapeutic agents.

Positive culture:
Positive culture means that BacT/Alert Cabinet® gave a positive signal indicating microbial growth in a blood culture bottle and the result was confirmed by detecting microbial growth in a subculture on an agar plate.

Negative culture:
Negative culture means that BacT/Alert Cabinet® did not give a positive signal indicating microbial growth during the maximal incubation time and the subculture did not reveal microbial growth.

Incubation time:
Incubation time means the time span from the inoculation of the microbe into the blood culture bottle to the signal by BacT/Alert Cabinet® indicating microbial growth.

Growth inhibition:
Growth inhibition means the inhibition in microbial growth caused by a chemotherapeutic agent, manifesting in an increase of incubation time or in a negative culture.

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