Diploma thesis

Microbiological screening of central venous catheters for detection of catheter related bloodstream infections in patients receiving haematopoietic stem cell transplantation

submitted by

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Eidesstattliche Erklärung

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Graz, im Juni 2008 …….....

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Zusammenfassung

Mikrobiologisches Screening zur Detektion von Katheter-assoziierten Bakteriämien/Fungämien bei Patienten im Rahmen einer hämatopoetischen Stammzelltransplantation

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Hintergrund:
Die Gramfärbung/Acridin Orange Leukozyten Cytospin Test (AOLC) und die Differential Time to Positivity Methode (DTP) werden zur Diagnose von Katheter-assoziierten Bakteriämien/Fungämien (CRBSI) bei Patienten mit klinischem Verdacht auf eine CRBSI eingesetzt. Eine Katheterkolonisation mit einem „ cut off level“ von 1000 Organismen/ml Katheterblut wird als Vorläufer einer CRBSI angesehen. Diese prospektive Studie wurde zur Evaluierung des Gramfärbung/AOLC Tests als Screeningmethode zur Detektion von CRBSI im subklinischen Stadium bei Patienten, die eine hämatopoetische Stammzelltransplantation (HSCT) erhalten, durchgeführt.

Methoden:
Zwischen Februar 2007 und Jänner 2008 wurden prospektiv 32 konsekutive HSCT-Patienten mit 36 Katheterepisoden untersucht. Drei mal pro Woche wurde das CRBSI-Screening von routinemäßig verwendeten Lumina der zentralvenösen Katheter mittels Gramfärbung/AOLC Test aus EDTA Blut durchgeführt. Bei klinischem Verdacht auf eine CRBSI wurden vom behandelnden Arzt die derzeitigen Standarduntersuchungen wie Gramfärbung/AOLC Test, die DTP und die Brun Buisson Methode durchgeführt.

Resultate:
618 EDTA Blutproben wurden untersucht. Die Ergebnisse sind in der Tabelle dargestellt. Die Sensitivität und die Spezifität des Screenings mittels Gramfärbung/AOLC Test betrugen 50% und 86%. Der PPV und der NPV lagen bei 50% und 86%.

Tabelle 1

	Gramfärbung/AOLC Screening	
Routine	**positiv**	**negativ**
8 CRBSI*	4/8	4/8
28 keine CRBSI*	4/28	24/28

* nach Durchführung des Screenings innerhalb von 48 Stunden bei klinisch symptomatischen Patienten routinemäßig diagnostiziert

Schlussfolgerung:

Das Screening von routinemäßig aus zentralvenösen Kathetern abgenommenem EDTA-Blut mittels Gramfärbung/AOLC Test stellt ein nützliches Werkzeug zur Erkennung von in der Folge klinisch evidenter CRBSI dar. Aus diesem Grund sollten Patienten mit einem positiven Screeningresultat engmaschig im Hinblick auf die Entwicklung einer klinisch evidenten CRBSI beobachtet werden. Bei Patienten mit einem negativen Gramfärbung/AOLC Resultat ist die Entwicklung einer CRBSI unwahrscheinlich.

Abstract

Microbiological screening for detection of catheter related bloodstream infections in patients receiving haematopoietic stem cell transplantation

Zach J, Zollner-Schwetz I, Zebisch A, Rohn A, Sill H, Strempfl C, Neuhold B, Eberharth W, Krause R.

Background:

The Gram stain/acridine orange leukocyte cytospin (AOLC) test and the differential time to positivity (DTP) method are used for diagnosis of catheter related bloodstream infections (CRBSI) in patients with clinical signs of CRBSI. Catheter colonization with a cut off level of 1000 organisms/ml catheter blood is considered to be a forerunner of CRBSI. This prospective trial is undertaken to evaluate whether the Gram stain/AOLC test could serve as a screening tool to detect CRBSI in a subclinical stage in patients receiving haematopoietic stem cell transplantation (HSCT).

Methods:

From February 2007 to January 2008, 32 consecutive HSCT-patients with 36 catheter episodes were prospectively investigated. Screening for CRBSI was performed by Gram stain/AOLC test three times a week using EDTA blood from routinely used lumina of central venous catheters. If CRBSI was clinically suspected, routine investigation was performed by the Gram stain/AOLC test, DTP and Brun Buisson method.

Results:

618 blood samples were investigated. Results are depicted in the table. The sensitivity and specificity of screening by Gram stain/ AOLC test were 50% and 86%, the PPV and NPV were 50% and 86%.

Table 1

	Gram stain/AOLC Screening	
Routine	**positive**	**negative**
8 CRBSI*	4/8	4/8
28 no CRBSI*	4/28	24/28

* as routinely diagnosed in clinically symptomatic patients within 48 hours after screening.

Conclusions:

Screening of blood drawn from routinely used lumina of central venous catheters by the Gram stain/AOLC test helps to detect patients at risk for clinically evident CRBSI. Therefore patients with positive screening results should be thoroughly observed with regard to development of clinically evident CRBSI. Patients with negative Gram stain/ AOLC test screening results are unlikely to develop CRBSI.

Contents

List of abbreviations

ALL	acute lymphatic leukaemia
AML	acute myeloid leukaemia
AOLC	acridine orange leukocyte cytospin
BSI	bloodstream infection
CDC	Centre for Disease Control and Prevention
CFU	colony forming unit
CML	chronic myeloid leukaemia
CMV	Cytomegalovirus
CRBSI	catheter related bloodstream infection
CVC	central venous catheter
DTP	differential time to positivity
EBV	Epstein Barr Virus
HHV6	human herpes virus type 6
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HSCT	haematopoietic stem cell transplantation
HSV	Herpes simplex virus
ICU	intensive care unit
MALT	mucosa associated lymphoid tissue
MM	multiple myeloma
MRSA	methicillin resistant *Staphylococcus aureus*
MRT	magnetic resonance tomography
NHL	non Hodgkin's lymphoma
NPV	negative predictive value
NST	non-myeloablative stem cell transplantation
PICC	peripherally inserted central catheter
PPV	positive predictive value
SCT	stem cell transplantation
VRE	vancomycin resistant Enterococci

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1 Introduction

In modern medicine central venous catheters (CVCs) play an important role in critically ill patients with regard to administration of fluids, blood products or total parenteral nutrition. Beside the obvious advantages, CVCs bear a great risk to cause several local or systemic infections such as catheter related blood stream infections (CRBSIs), local site infections, septic thrombophlebitis or endocarditis because of their direct link to the patient's bloodstream.

Thirty percent of nosocomial infections in neutropenic patients result from CVCs and therefore they are the most common sources for nosocomial infections ([1,2]). Attributable mortality due to CRBSI in this patient group has been reported up to 25% ([3]). It is impossible to clinically diagnose CRBSIs in neutropenic cancer patients since in most cases typical signs of infection at the insertion site of the CVC like purulent secretion, pain or tenderness are absent ([4]).

Conventional methods for diagnosing CRBSI such as quantitative or semiquantitative catheter-tip cultures require the removal of the CVC. CVCs removed on suspicion of CRBSI prove to be infected in only 20% ([5,6]). Furthermore, the diagnosis is always retrospective. In addition, catheter removal and reinsertion of a new CVC during the neutropenic period exhibit special risk like bleeding, puncture of arterial vessels or pneumothorax.

Former studies have proven that the Gram stain/acridine-orange leukocyte cytospin (AOLC) test as well as the differential time to positivity (DTP) method are able to detect CRBSI without catheter removal ([4,7, 8,9,10]). Recently, it has been shown that the DTP method is highly sensitive and specific for CRBSI, even in neutropenic patients ([4,7]). For the DTP method blood is drawn from the CVC and from a peripheral vein and the difference in the microbial load is measured. The DTP method is based on the difference between the time to positivity of the peripheral-aerobic or anaerobic blood culture bottle and the central-blood culture bottles, which is calculated and expressed in hours. A cut-off limit of > 2 hours is considered to be indicative for CRBSI ([10]). In contrast, the Gram stain/AOLC test is an attractive method for diagnosis of CRBSI because it provides results very quickly.

The Gram stain/AOLC test has a suggested absolute threshold of 10^3 - 10^4 CFU/ml blood resulting from the microbiological cut-off of other diagnostic methods for CRBSI ([8]). Yet it is not known if the Gram stain/AOLC test enables detection of this cut off level before symptoms of CRBSI become manifest.

The aim of the present prospective study was to investigate whether the Gram stain/AOLC test could serve as a screening tool to detect CRBSI in a subclinical stage in patients receiving haematopoietic stem cell transplantation (HSCT).

2 Underlying diseases of included patients

All patients included in the study suffered from a haematological malignancy as the primary disease.

1. Acute myeloid leukaemia
2. Acute lymphatic leukaemia
3. Chronic myeloid leukaemia
4. Hodgkin's disease
5. Non Hodgkin's disease
6. Multiple myeloma

2.1 Acute myeloid leukaemia

Acute myeloid leukaemia (AML) is a malignant clonal neoplasia of certain haematopoietic cells. It is characterized by fast autonomic proliferation of abnormal cells, which originate from the myeloid line of the haematopoietic stem cells. The abnormal cells spread into the bone marrow and interfere with the production of normal blood cells causing progressive bone marrow insufficiency and the symptoms as described bellow.

The average incidence for AML conducts about 2.4/100000/a ([11]). The incidence increases with age and it is the most common acute leukaemia affecting adults. The median age at diagnosis is 63 years and it is more common in men with a male to female ratio of 2.9:1.9 ([12]).

There are two subgroups: de-novo or primary AML, which has no prodromal stage and secondary AML, which is antedated by previous haematological malignancies or therapies.

Several risk factors for AML have been detected ([13]):

1. Bone marrow damage caused by chemicals like benzene, cytostatics (alkylating agents, topoisomerase-II-inhibitor); the highest risk is about 4-6 years after chemotherapy ([12])
2. Exposure to ionizing radiation

3. Genetic syndromes like Down syndrome/ trisomy 21, Klinefelter syndrome/ XXY and variants or Patau-Syndrome/ trisomy 13
4. Myelodysplastic or myeloproliferative syndromes and aplastic anaemia can progress to AML

Risk factors cause specific cytogenetic abnormalties leading to chromosomal translocations or inversions. These translocations or inversions produce fusion proteins, which force the precursor/myeloblast to remain in its immature state and avoid differentiation into a myelocyte. The differentiation can stop in every possible step of the granulopoiesis giving rise to a large number of diverse and heterogeneous diseases. In most cases a variety of genetic events are responsible for the development of AML. The blasts contain atypical karyons, a narrow basophile cytoplasm and in some cases Auer's bodies. According to the World Healthcare Organization's classification more than 20% of the bone marrow has to be affected by blasts and only the immature elements within the circulating blood or bone marrow confirm the diagnosis ([12]).

Most of the patients suffering from AML report a very short history and the early signs are often non- specific often resembling a common cold. The symptoms are caused by an increased number of malignant haematopoietic cells displacing the normal granulopoiesis. General symptoms can be fever, fatigue, absence of appetite and night sweat. A lack of red blood cells can induce shortness of breath, anaemia and paleness and reduced number of platelets produces easy bruising, petechiae with minor trauma, bone pain and joint pain. As a consequence of leukaemic cells, which have reduced to absent immunological function, these patients are more susceptible to bacterial or fungal infection e.g. thrush initiated by *Candida albicans*. Occasionally, other symptoms like enlargement of the spleen, leukaemia cutis, hypertrophical gingivitis or disseminated intravascular coagulation may appear to be the first symptoms. However, some patients show no symptoms, and AML is discovered accidentally during a routine blood test.

2.2 Acute lymphatic leukaemia

Acute lymphatic leukaemia (ALL) is a malignant monoclonal disorder of the bone marrow and affects the lymphoid precursor cells. These immature malignant cells, which are similar to B- or T-precursor cells, can be detected by immunocytologic investigations ([14]). ALL is diagnosed when more than 20% of the bone marrow is affected ([15]). The malignant differentiation to B-precursor cells is the most common type of ALL ([16]). In many cases, immature lymphoblasts are flushed out into the blood circulation and especially affect extramedullary tissues ([11]).

The average incidence for ALL conducts 1/100.000/a and it is more common in children especially in boys than in adults ([11]).

Etiologically three main causes can be considered ([13]):

1. Virus-infections (e.g.: Retrovirus): Integration of viral-oncogenetic chromosomes into haematopoietic stem cells can induce malignant transformation of these
2. Damage of bone marrow by ionizing radiation, benzene and cytostatics
3. Genetic susceptibility factors ([17])

A chromosomal translocation or mutation often results in an abnormal expression of genes (oncogenes) causing neoplastic transformations of haematopoietic stem cells. These malignant lymphoblasts proliferate and displace the normal bone marrow elements causing a decreased production of normal blood cells and the involved symptoms.

ALL patients often report a short history because of the quick begin of haematopoietic insufficiency. Initial symptoms of ALL can be fatique and anaemia, including pallor and cardiac murmur, as a result of displaced erythropoiesis. Fever without any other evident origin and a liability to bacterial infections like tonsillitis and pneumonia or easy bruising or bleeding from wounds and petechiae are caused by leukocytopenia and thrombocytopenia. Furthermore, in 5% leukaemic meningitis can occur affecting the entire neuraxis ([18]).

2.3 Chronic myeloid leukaemia

Chronic myeloid leukaemia (CML) is a myeloproliferative disorder characterized by the increased and uncontrolled growth of granulocytic cells as a result of loss of their capacity to differentiate. CML is typically associated with the Philadelphia chromosome (90% of cases). The peripheral blood cell profile shows a high amount of granulocytic cells, which can be fully functional or immature precursors, including blast cells.

The average incidence of CML is 1.3/100000/a. It occurs more often in men than in women. All age groups can be affected but the incidence is higher in the elderly ([19]). Exposure to ionizing radiation and benzene are the only well known risk factors for CML, which is idiopathic in most cases.

Pathophysiologically, this haematological disease is characterized by a cytogenetic aberration, which is composed of reciprocal translocation between the long arms of chromosome 22 and 9; t (9;22). This translocation relocates the c-abl-proto-oncogen from chromosome 9 to the long arm of chromosome 22 in the BCR region (breaking point region) where the two parts fuse to form the bcr-abl fusion gen. This process is called bcr-rearrangement and the fusion gene generates a protein with a strong tyrosine kinase activity, is continuously activated and has an increased proliferative effect on the cell cycle.

CML displays 3 phases with clinical characteristics and follows a typical course. During this course of disease time new cytogenetic abnormalities in addition to the Philadelphia chromosome are generated:

1. Most of the patients are diagnosed in the initial chronic phase because of a detected leukocytosis and splenomegaly. The differential blood count shows a left shift in the myeloid series of cells. During this phase, patients are usually asymptomatic or they have only mild symptoms like fatigue or night sweat.
2. The accelerated phase is the link between the initial chronic phase and the blast crisis. At this stage the peripheral blast cells add up to 10-30% and basophilic leukocytes are increased ≥ 20%. In contrast erythrocytes and thrombocytes are unresponsively lowered to therapy and fever may occur.

3. Blast crisis is the final phase of CML and behaves like an acute leukaemia with an average survival of 3-6 months. A blast crisis is diagnosed if > 30% myeloblasts or lymphoblasts are present in the bone marrow or the peripheral blood. Sometimes skin or tissue infiltrations, so called chloroma, can also lead the way to the diagnosis.

2.4 Hodgkin's disease

Hodgkin's disease, also known as Hodgkin's lymphoma, is a monoclonal B- cell-lymphoma, characterized by the presence of Hodgkin- Reed- Sternberg- cells, which originate from the lymph nodes' germinal centres. The large, bi-nucleated Hodgkin- Reed- Sternberg- cells amount only 3% of the lymph node's configuration and are mixed with a reactive cell infiltration made up of lymphocytes, monocytes, eosinophils and desmocytes ([20]). In the beginning Hodgkin's disease is confined to single lymph nodes and in the course of illness it emerges as a systemic disease.

The incidence averages 3/100000/a and has a bimodal incidence curve ([21]). The first peak appears at the age of 20 and the second one in those over 80 ([21]).
HIV- and EBV- infections as well as extended immunosuppressions, some kinds of timber preservatives and hair tinting lotions can increase the appearance of Hodgkin's lymphoma but there is no particular etiological reason. Cells, which are latently infected by EBV, can convert into malignant cells via genetic alteration or because of immunosuppression (e.g. HIV-infection) ([20]).

Patients typically present B symptoms according to the Ann Arbor criteria like fever, night sweat and weight loss. In addition, swollen but painless cervical lymph nodes, which can be painful after alcohol consumption, are the most common signs. Hepatosplenomegaly is sometimes present.

2.5 Non- Hodgkin's disease

Non- Hodgkin's lymphoma (NHL) describes another form of malignant clonal haematopoietic cancer, arising from B- or T- lymphocytes and demonstrates different clinical courses of disease and variations of histological patterns.

Because of these variations NHL can be divided into 3 main groups:

1. Indolent or low grade lymphoma
2. Aggressive or intermediate grade lymphoma
3. Highly aggressive or high grade lymphoma

The incidence adds up to 5-10/100000/a increasing with age. NHL is more common in men than in women ([21]). Primary or secondary immune deficiencies are associated with an increased risk for NHL.

The exact aetiology of NHL is unknown but there a several risk factors, which are connected to this disease.

1. Inherited immune deficiencies like the Wiskott- Aldrich- Syndrome, which is a rare X-linked recessive disease characterized by immunodeficiency, thrombocytopenia, eczema and bloody diarrhoea
2. Acquired immune deficiencies among people taking immunosuppressant drugs, cytostatic drugs or patients suffering from autoimmune diseases
3. Exposure to radioactive substances and after therapeutic radiation
4. Viruses and bacteria like Human T- lymphotropic virus type 1 or 2, EBV, HIV and *Helicobacter pylori*.

   Burkitt's lymphomas are associated with Epstein Barr Virus and are endemic in certain areas of Africa.

   MALT- lymphoma of the stomach is often linked to a long lasting infection with *Helicobacter pylori*.

One of the main mutations for forming NHL is a t(14;18)-translocation of the bcl-2 gene, which is located on the fourteenth chromosome and brought forward to the eighteenth chromosome. This genetic mismatch activates oncogenes causing an increased production of BCL-2- proteins. In addition, tumour-suppressor-genes are

inactivated and those cells containing activated oncogenes turn out to have benefits in growth.
Swollen, painless lymph nodes, localized in the axle, the neck, in the submandibular or inquinal region are usually the only symptoms in the beginning of the disease. B-symptoms like loss of weight, night sweat and fever are infrequent in NHL. In 50% of cases the bone marrow is infiltrated by malignant cells displacing the granulopoiesis, erythropoiesis and thrombopoiesis, which explains the possible anaemia, fatigue, petechiae, bleeding and the liability to infections.

2.6 Multiple myeloma

The multiple myeloma (MM), which is also called Kahler's disease or plasma cell myeloma, appears to be a very aggressive B-cell-NHL. It develops out of a single malignant transformed plasma cell (so called B- cell) and spreads diffusely or multilocularly into the bone marrow. These B- cells produce monoclonal immunoglobulins (IgG, IgD, IgA, IgE, IgM) or lambda/ kappa light chains (Bence-Jones- Myeloma), which have no immunological function. An amount of more than 2000 myeloma cells/µl blood represents plasma cell leukaemia, which is rare and has an unfavourable prognosis.

The average incidence of MM is 4/100000/a with a frequency peak at the age of 68 ([22]). It occurs slightly more often in men than in women.

The aetiology is idiopathic but in some cases pesticides or ionizing radiation may be major risk factors for developing a MM. A chromosomal translocation between the immunoglobuline heavy chain gene locus and an oncogene is responsible for the proliferation of the malignant transformed plasma cells originated from postfollicular B-cells and the genomic instability leading to an increased rate of mutations and production of cytokines, especially IL-6 ([23]).

According to the Ossermann's criteria a MM is defined as two out of three criteria:

1. Bone marrow biopsy containing > 15% clonal plasma cells or a nidus of clonal plasma cells within the bone marrow
2. Monoclonal protein in either serum or urine

3. Osteolytical foci within the bone marrow or osteoporosis at simultaneous progeny of plasma cells within the bone marrow

Several other symptoms can confirm a MM:

1. Bone pain: MM cells produce osteoclast-activating-factors. Activated osteoclasts damage especially ribs, the vertebrae, the pelvis, the femur, the humerus and the skull and are best seen in plain radiographs because of their lytic nature or in MRT. The bone damage may lead to spinal cord compressions, pathological fractures and hypercalcemia.
2. The diffuse hypergammaglobulinemia is due to immune deficiency, which is blameable for the most common infections linked to MM like pneumonia initiated by *S. pneumoniae, S. aureus* and *K. pneumoniae* and pyelonephritis due to *E. coli* or other gram-negative organisms. 25% of all MM-patients suffer from relapsing infections as primary symptom ([22]).
3. Normocytic and normochromic anaemia, resulting from the replacement of normal haematopoiesis by infiltrating tumour cells.
4. Renal failure can develop due to hypercalcemia, deposition of amyloid, excretion of Bence- Jones- proteins, hyperuricemia or recurrent pyelonephritis.
5. Neurological symptoms: cerebral circulatory disorders occur as a result of hyper viscosity. Radicular pain may occur because of spinal cord compressions and common signs like weakness, fatigue and confusion due to hypercalcemia.

3 Treatment and treatment-associated complications

In the majority of cases peripheral haematopoietic stem cell transplantation, bone marrow transplantation or the transplantation of umbilical cord blood represent the only curative therapy of haematopoietic malignancies. In case of peripheral stem cell transplantation the donor needs to boost the quantity of leukocytes with daily subcutaneous injections of Granulocyte-colony stimulating factor prior to the transplantation.

This transplantation can be allogeneic meaning that stem cells are collected from a donor, who matches within three or more loci on the HLA gene (HLA-A, HLA-B and HLA-DR) or from cord blood.

Another alternative is an autologous haematopoietic stem cell transplantation (HSCT) consisting of isolation of haematopoietic stem cells from the patient via cell apheresis in the stage of complete remission. The extracted cells are stored in a freezer and are returned to the patient after high dose chemotherapy. Before this transplantation can be performed, the elimination of the patient's malignant cell populations as well as the bone marrow stem cells is achieved through high dose chemotherapy. This method produces no graft versus malignancy effect.

For patients, who are categorized as high-risk because of their co-morbidities, poor organ function or age (older than 55 years), non myeloablative allogeneic stem cell transplantation (NST) or "mini-transplantation" represents another alternative therapy. This treatment uses less intensive immunosuppressive conditioning regimes, which reduces the therapy related morbidity ([24]). As a result of less intensive immunosuppressive conditioning regimes, a mixed donor-host haematopoietic chimerism is enabled ([24]). Afterwards applied donor lymphocyte infusions eradicate the remaining malignant cells using the graft versus tumour effect.

Additionally, patients suffering from haematopoietic malignancies are treated with total body irradiation when complete bone marrow destruction is intended.
The morbidity- and mortality-rate of allogeneic transplantations is significantly higher than the rates of autologous transplantations. ([25])

Side effects can be divided into acute side effects, which occur right after or in-between the period of therapy, and long term consequences affecting the patient's quality of life.

Frequent complications include:

Toxic side effects of the conditioning-therapy consist of nausea, vomiting, mucositis, diarrhoea, haemorrhagic cystitis, cardiomyopathia, veno-occlusive disease of the liver, loss of hair, gonadal insufficiency and secondary malignancies.

Infections are the most common complications and cause of death in patients receiving haematopoietic stem cell transplantation ([26]). The reasons for the increased susceptibility to infections are on the one hand the shut down of the granulopoiesis by chemotherapy and radiation. On the other hand CVCs, oral mucositis, corticoidtherapy and the lack of physiological microbiological flora interfere with the natural defence mechanism. A number of granulocytes lower than 500/µl as well as the period of granulocytopenia is associated with a higher incidence of opportunistic infections ([26]). Extended granulocytopenia (more than 10 days) is associated with an increased risk for systemic fungal infections ([25]). The length of the pancytopenia depends on the quality of the graft and differs from HSCT (9-14 days) and bone marrow transplantation (about 21 days) ([25]).

Typical infectious agents after allogeneic HSCT are (modified from [26]):

Mucositis

- Early phase: herpes simplex virus type 1, α- haemolytic streptococci
- Late phase: herpes simplex virus type 1, *Candida spp.*

Pneumonia

- Early phase: gram-negative aerobe rods, *Aspergillus spp.*
- Late phase: CMV, *Aspergillus spp.*

Sepsis

- Early phase: α-haemolytic streptococci, staphylococci, gram-negative aerobe rods, *Candida spp.*
- Late phase: staphylococci, pneumococci, *Candida spp.*

Although constant advancement of donor- recipient-matching is intended, graft versus host disease (GvHD) remains a major problem of allogeneic HSCT. GvHD is the clinical manifestation of the immunological reaction caused by transfused donor T-lymphocytes, which are different in minor HLA-genes to the patient's. It can be divided into an acute disease, which occurs within the first 3 months after transplantation and affects the skin, gastrointestinal tract (e.g. enteritis) and liver (e.g. hepatitis) and a chronic disease. Acute GvHD is classified in 4 grades depending on the skin manifestation, the bilirubin increase and the amount of diarrhoea. Chronic GvHD appears 100 days after the transplantation took place and damages the connective tissue (e.g. papular exanthema) as well as the above mentioned organs.

Patients, who receive chemotherapy and HSCT, need a vascular access through a CVC. Complications of catheter insertion include pneumothorax as well as arterial and nerve injuries. Long term complications include thrombosis and infections at the insertion site.

An effective therapy never guarantees a lifelong cure of haematopoietic malignancies. The overall survival at 5 years of AML depends on the prognostic group such as favourable, intermediate and adverse, which are defined by the pre-treatment cytogenetics and is 65%, 41% and 14%, respectively ([27,28]). The allogeneic bone marrow transplantation is the only curative option for CML and results in 50 to 70% in recurrence-free survival ([29]). Moreover, the leukaemia-free survival rate at nine years of ALL can be expected to be 32% for patients, who were only treated with chemotherapy, and 34% after an aggressive high dose chemotherapy followed by HLA-identical bone marrow transplantation ([30]).
More than 90% of patients affected by a localized Hodgkin's lymphoma can be cured ([21]). Depending on the diagnosed disease stage and the subtype, 25%-77% of patients with NHL survive five years after therapy ([21]). In cases of MM a high dose chemotherapy and HSCT can merely extend the symptom-free period because there are no curative treatments.

4 Types of commonly used central venous catheters

There are different ways to classify commonly used catheter types.

According to the Centre for Disease Control and Prevention (CDC) a catheter can be characterized by the following criteria ([31]):

1. The type of vessel it is inserted into (e.g. peripheral venous, central venous, or arterial)
2. The catheter's life span (e.g. temporary or short-term [< 30 days] ([7]) vs. permanent or long-term [> 30 days] ([7]))
3. The insertion's site (e.g. subclavian, femoral, internal jugular, peripheral and peripherally inserted central catheter (PICC)
4. Tunnelled vs. non-tunnelled CVC
5. Its physical length (e.g. long vs. short)
6. Certain characteristics of the CVC (e.g. a cuff, impregnation with heparin, antibiotics or antiseptics, and the number of lumina).

Most of the inpatients receive a peripheral venous catheter during their hospital stay, which is usually inserted in veins of forearms or hands and can cause phlebitis and local haematomas with prolonged use.

For intraarterial measurement of blood pressure or blood gas analysis a peripheral arterial catheter is required, which should be preferably placed in the radial artery. Other vessels like the femoral artery, the brachial artery, the dorsal pedic artery or temporal artery should be used only in special situations because of the increased number of complications linked to these anatomic locations. General complications associated with this type of catheter are haematomas caused by erroneous puncture, infections, circulatory disorder and arterio-venous fistula.

Another peripheral catheter is the so called midline catheter, which is inserted via the antecubital fossa into the proximal basilica or cephalic veins and has no entrance to the central veins.

A CVC is a catheter inserted via a wide luminal vein, whose tip is pushed forward to the superior vena cava. Non-tunnelled CVCs are placed percutaneously into central veins like the internal jugular vein, the subclavian vein or the femoral vein.

Long-term central venous catheters were established in 1968, and the design was improved by Broviac et al. in 1973. Hickman et al. further modified the principles.
In case of tunnelled CVCs, a tunnel is formed subcutaneously along the thorax and the catheter is placed through it into the superior vena cava. A Dacron cuff forms the infection barrier. Tunnelled CVCs may remain in place for an extended period of time and are used when long term intravenous access is needed. The main complications of insertion of a CVC are pneumothorax, haemothorax, air embolism, injury of the brachial plexus and by mistake punctured of other blood vessels.

The PICC line was first described in 1975 and is another type of central venous catheters. It is inserted into the superior vena cava via a peripheral vein like the basilic, the cephalic or the brachial vein. Sometimes the implantation occurs to be difficult because of venous valves. Furthermore, the location is more often blemished and as a result of narrow venous lumen, thromboses and thrombophlebitis can appear.

General indications for CVCs are ([32]):

1. Administration of catecholamines or cytostatics
2. Administration of highly caloric and highly osmotic infusions
3. Administration of drugs irrigating vessels
4. Total parenteral nutrition
5. Central venous pressure measurement
6. In cases when a peripheral venous catheter is not possible
7. When a quick blood supply is needed

Totally implantable systems are tunnelled beneath the skin as day surgery procedures, have direct entrance to internal jugular or subclavian vein and possess a subcutaneous port access. Risks can be arterial injuries as well as pneumothorax during the surgery and furthermore infections and thrombosis.

When cell-apharesis or haemodialysis is needed, a dialysis catheter is generally inserted in the superior vena cava via the right internal jugular vein or alternatively via the subclavian vein. This catheter consists of an arterial port, which withdraws blood from the patient, and a venous port that recirculates the patient's blood for

example from the dialysis machine. Long term dialysis catheters contain a Dracon cuff, which is tunnelled beneath the skin 3 to 8 cm, holds the catheter in place and additionally causes a fibrotic reaction that avoids bacterial migration and inoculation via the exit site. Complications of haemodialysis catheters include infections, thrombosis, kinking and unforeseen occurrences associated with surgical placement like misplacement to the brachiocephalic artery or arterial injuries.

A pulmonary artery catheter (= pulmonary catheter, Swan-Ganz-catheter®, flow-directed catheter) usually contains 4 lumina, whose tip is implanted into a branch of the pulmonary artery via the venous system and the right heart. This kind of catheter is required to measure the cardiac output, the pressure within the pulmonary artery, the pulmo-capillary wedge pressure and the central venous pressure of high-risk patients, who are administered a catecholamine-, vasodilative agents- and volume-substitution-therapy. Because of the invasive implantation several complications are associated with pulmonary artery catheters like cardiac arrhythmia, rupture of a pulmonary artery branch, pulmonary infarction, injury of the right heart's valves, thrombosis, infection, intraloop of the catheter and complications associated with the vascular puncture.

In newborns an umbilical catheter can be inserted into either one of the umbilical arteries or the vein under the condition that the umbilical cord stump is still connected to the circulatory system.

5 Catheter related bloodstream infection

CVCs are commonly used in the management of patients receiving intensive care. These catheters allow the administration of blood products, drugs and fluids but also contain the risk of bacterial or fungal infections because of their direct access to the bloodstream.

According to the guidelines of the CDC there are different ways to define a CVC infection in the clinical everyday life ([31]).

1. A significant growth of bacteria or fungi of more than 15 CFU/ml from the catheter tip, subcutaneous segment of the catheter, or catheter hub defines localized catheter colonization.
2. Erythema or indurations within 2 cm of the CVC's exit site without simultaneous bloodstream infection and purulence represents an exit site infection.
3. Clinical exit site infection or tunnel infection: clinical signs of infection like tenderness, erythema, or indurations ≥2 cm from the catheter site along the subcutaneous tract of a tunnelled CVC without a simultaneous bloodstream infection (BSI)
4. A pocket infection is defined as purulent fluid in the subcutaneous pocket of a totally implanted system, which is at risk for spontaneous rupture and drainage or necrosis of the overlaying skin, in the absence of simultaneous BSI.
5. Infusate-related BSI: concordant growth of the same bacteria or fungi from the infusate and the blood culture drawn percutaneously with no other evident source of infection.
6. A CRBSI is defined as bacteremia or fungemia in a patient with a CVC, presenting with clinical symptoms of an infection like fever, chills and hypotension with at least one positive blood culture drawn through a peripheral vein, and no other evident origin for the BSI except the catheter. Moreover, one of the following criteria should be fulfilled: a positive semiquantitative (>15 CFU/catheter segment) or quantitative (>10^3 CFU/catheter segment catheter) culture containing the same organism in species and antibiogram isolated from the catheter segment and peripheral

blood; simultaneous quantitative blood cultures with a ≥5:1 ratio CVC versus peripheral; differential time to positivity (DTP) of CVC blood culture versus peripheral blood culture of >2 hours.

5.1 Epidemiology and effects on the health care system caused by CRBSI

In general the incidence of CRBSI varies with the type of CVC ([33]).

Table 1 Rates of BSI caused by different types of CVCs (modified from [33])

	No. of CRBSI
	per 1000 CVC-days
Catheter-types	**Pooled mean**
Short-term, non-medicated CVC	2.3
Pulmonary-artery catheter	5.5
Non-cuffed haemodialysis catheter	2.8
Cuffed haemodialysis catheter	1.1
Peripherally inserted central catheter	0.4
Long-term tunnelled and cuffed CVC	1.2
Subcutaneous central venous port	0.2

Central venous catheter days = total number of days of exposure to CVCs by all patients in the selected population during the selected time period.
Catheter associated BSI = Number of primary bloodstream infections in patients with central venous catheters >48 hours x 1000/ central venous catheter days.

Recently, a study assessed the occurrence of CRBSI in countries belonging to the European Union compared to non-EU countries. Patients from European countries had a higher incidence of 1.55 episodes/1000 catheters compared to patients from non-EU countries with an incidence of 0.33 episodes/1000 catheters ([34]). The characteristics of CVCs were also analyzed. In 67% non-tunnelled CVCs were the source of infection, which were placed in the internal jugular vein in 44% and 77%

remained in situ for >7 days ([34]). 61% were made out of polyurethane and 67% had multiple lumina ([34]).

The effect of CRBSI on the health care system especially in critically ill and immunocompromised patients ranges from prolonged hospital stays and high healthcare costs to a significant mortality ([35,36]). In 37% of primary BSI in febrile neutropenic cancer patients with short-term non-tunnelled catheters a CRBSI could have been provided evidence as cause ([4]).

A retrospective study by Morano et al. estimated that adult patients suffering from nosocomial bacteremia have a longer median hospital stay compared to a control group (35 vs. 15.5 days) ([37]). Significant differences in the mean total costs of admission, cost of stay, pharmaceutical expenses, costs of microbiological studies, laboratory work-up and radiological studies between patients with nosocomial bacteremia and controls could be demonstrated ([37]). Prolonged hospital stay is responsible for 60% of increased costs due to nosocomial bacteremia, followed by pharmaceutical expenses ([37]).

Kluger and Maki evaluated that about 250000 cases of CRBSI occur in the United States every year ([38]). The mortality rate caused by CRBSI varies from 12% to 25% ([38]). Each episode costs about $25000 (about 17000€) to the health-care system ([38]). Approximately, 6000 cases of CRBSIs are estimated to occur in the United Kingdom every year ([39]). CRBSIs are among the major complication in patients, who need home total parenteral nutrition (0.38 and 0.50 episodes per catheter year) ([40]).

Replacement of CVCs is associated with an 8.7-fold increase of costs compared to the Gram stain/AOLC test (86.08€ vs. 9.87€) ([41]). For this reason a policy of selective removal of devices based on the DTP method or Gram stain/AOLC test reduces costs and should therefore replace catheter removal based on clinical suspicion only([4,7,10,41]).

5.2 Microorganisms causing CRBSI

The microbiological spectrum of CRBSI has changed during the last years and depends on the type of catheter, the size of the hospital and the hospital service/unit ([42]).

Table 2 Most common microorganisms isolated from nosocomial acquired BSIs (modified from [31])

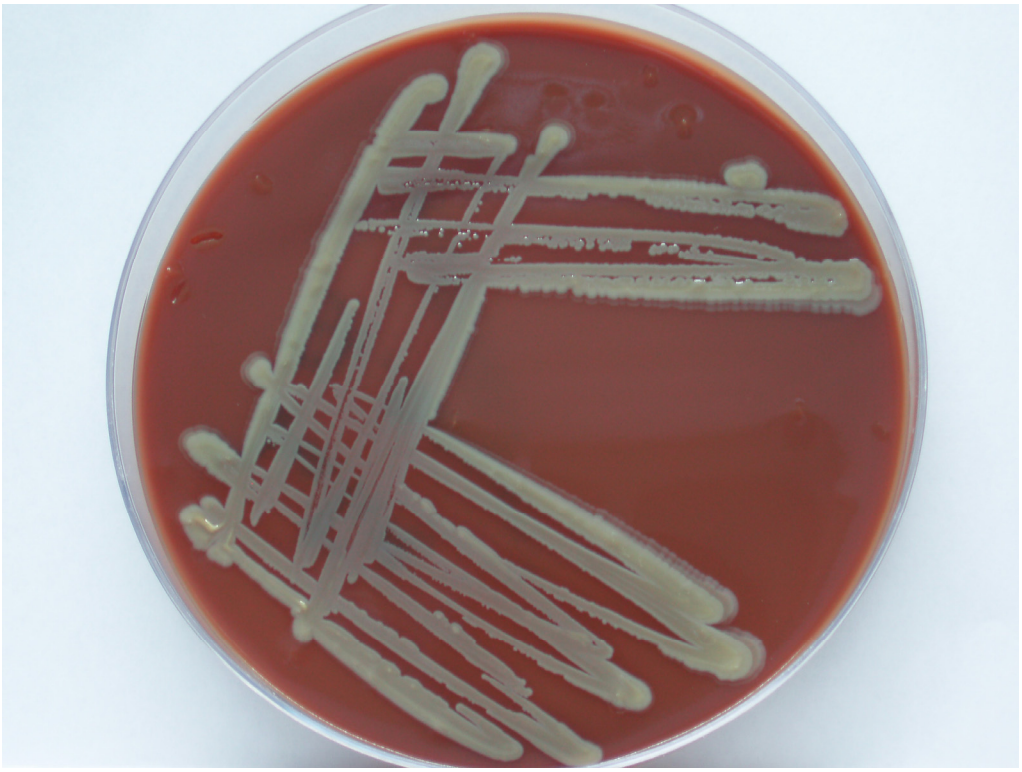
	1986-1989	**1992-1999**
Pathogen	**%**	**%**
Coagulase-negative staphylococci	27	37
Staphylococcus aureus	16	13
Enterococci	8	13
Escherichia coli	6	2
Enterobacter spp.	5	5
Pseudomonas aeruginosa	4	4
Klebsiella pneumoniae	4	3
Candida spp.	8	8

Munoz et al. demonstrated a difference in the epidemiology of microorganisms causing CRBSI between the European Union and non-EU countries. In EU countries coagulase-negative staphylococci were the pathogens isolated most commonly accounting for 39% of CRBSI cases, whereas *Staphylococcus aureus* was predominat in non-EU countries accounting for 13% of CRBSI cases ([34]).

5.2.1 Microorganisms commonly causing CRBSI

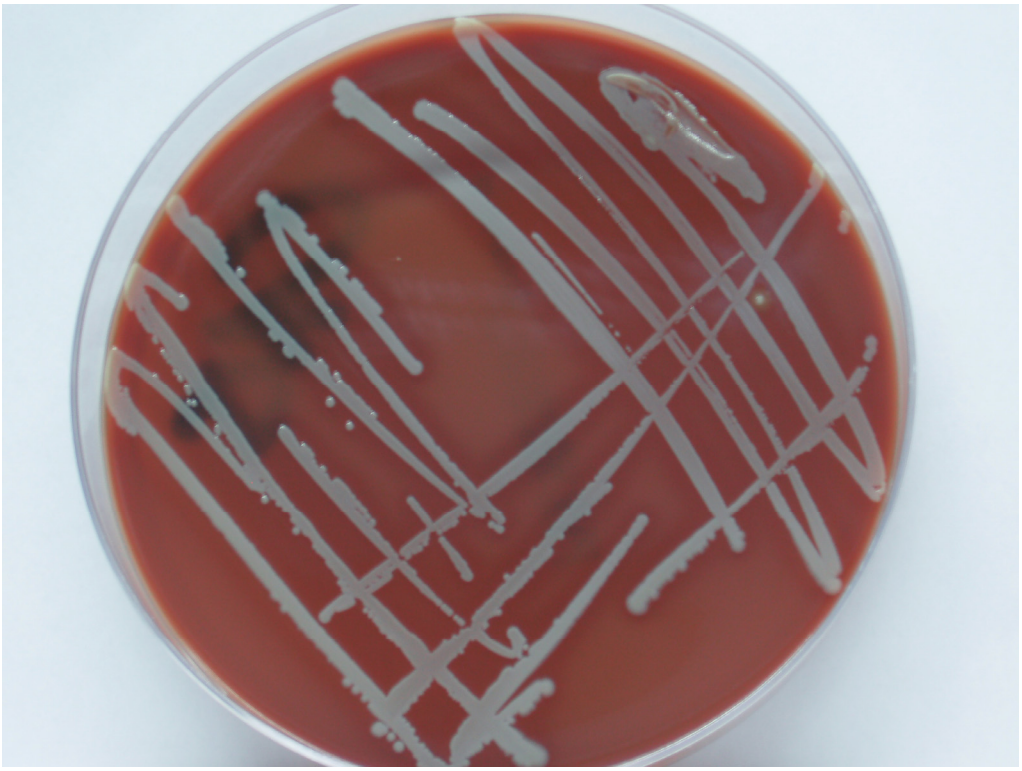
5.2.1.1 *Staphylococcus spp.*

Figure 1 *Staphylococcus aureus* on chocolate agar



Staphylococci are gram-positive, non-motile, non spore-forming, facultative anaerobic bacteria, which occur singly, in pairs, in chains or in clusters and measure 0.5 to 1.5 µm in diameter ([43]). These bacteria are members of the Staphylococcaceae and can be divided into coagulase-positive and coagulase-negative cocci. Coagulase is an enzyme, produced by *Staphylococcus aureus*, which reacts with prothrombin and afterwards converts fibrinogen in fibrin. As a result *Staphylococcus aureus* is coated with fibrin and resistant to opsonization and phagocytosis by the native immune system. Another difference compared to coagulase-negative cocci is the production of several enterotoxins, the toxic shock syndrome toxin-1 (TSST-1) and the Panton-Valentine-leukocidin. The TSST-1 is a member of a superantigen family and has the ability to activate T-cells, tumor necrosis factors and interleukin-1 leading to the clinical picture of fever, rash, hypotension, tissue injury and shock.

Figure 2 Coagulase-negative staphylococci on chocolate agar



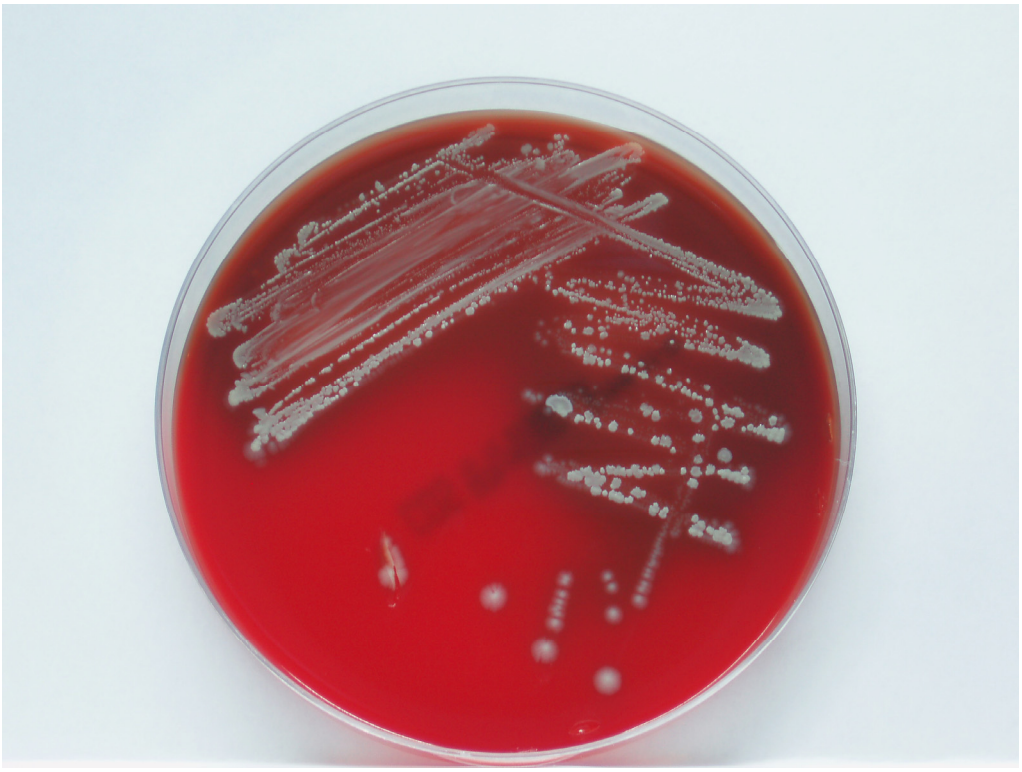
Coagulase-negative staphylococci, especially *Staphylococcus epidermidis* are a major component of the normal microflora of humans and protect themselves against phagocytosis by adsorption of an exopolysaccharide to their surface area, which furthermore promotes the adherence to foreign bodies like catheters made out of polymers. Coagulase-negative staphylococci can be found on the skin, skin glands and mucous membranes and usually have a symbiotic relationship with their hosts. When the natural cutaneous barriers are damaged (i.e. by trauma, devices or needles) they can get access to the bloodstream or tissues and become pathogenic.

Staphylococcus areus is involved especially in skin diseases like furuncles or boils, cellulites, impetigo and postoperative wound infections as well as in more serious infections like bacteremia, pneumonia, osteomyelitis, scaled skin syndrome, toxic shock syndrome and abscesses of the muscle. Coagulase-negative staphylococci are important opportunistic pathogens in infections of the urinary tract, the bloodstream, intravascular catheters, surgical wounds, prosthetic joints and native as well as prosthetic valves.

5.2.2 Microorganisms rarely causing CRBSI

5.2.2.1 *Enterococcus spp.*

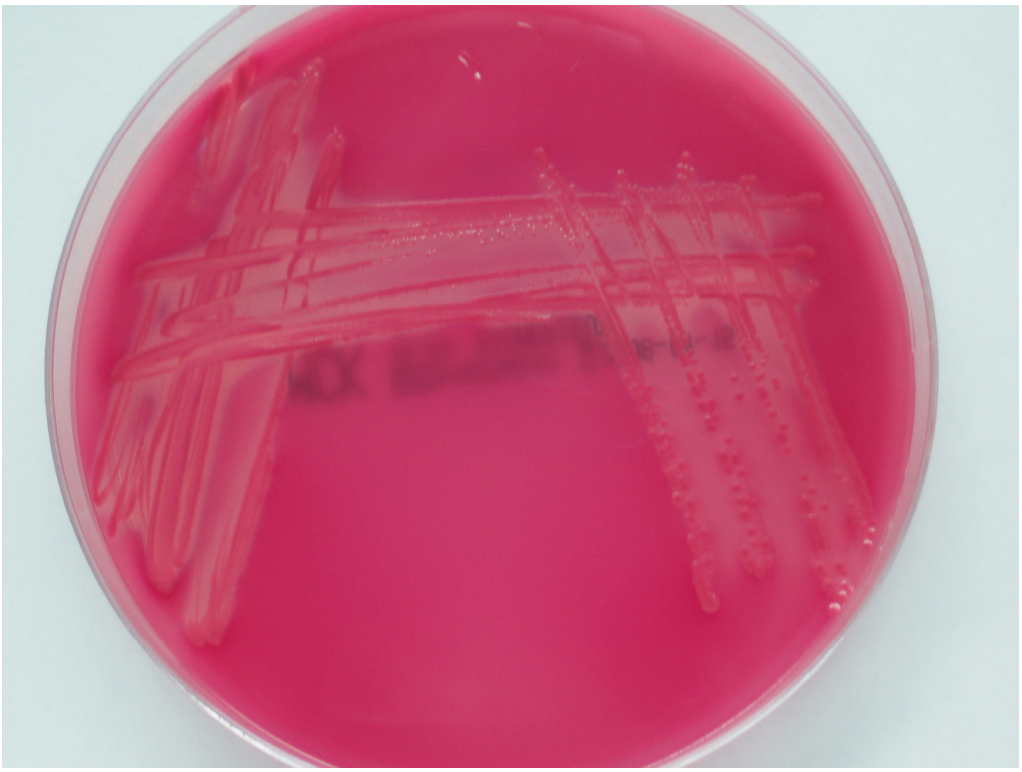
Figure 3 *Enterococcus spp.* on Columbia agar



Enterococci are gram-positive, facultative anaerobic cocci occuring single, in pairs or in chains and ferment glucose to lactic acid as end-product. They interfere with group D antiserum of streptococci and have their optimal growth-temperature at 35°C ([43]). The natural habitats of these bacteria are food, soil, plants and water as well as the gastrointestinal tract and the female genital tract. There are more than 17 species known. *Enterococcus faecalis* as well as *Enterococcus faecium* are associated with infectious diseases in humans. Relapsing urinary tract infections, which are one of the most common infections, are often caused by structural abnormalities. Intraabdominal or pelvic wound infections and bacteremia are associated with surgery, the insertion of CVCs and lack of hand hygiene.

5.2.2.2 *Escherichia coli*

Figure 4 *Escherichia coli* on McConkey agar



Escherichia species are gram-negative, rod shaped, motile or non-motile bacteria belonging to the family of Enterobacteriaceae, range from 0.5 to 2µm in width to 2 to 4µm in length and can be separated into five species ([44]). All of them are non-spore forming, facultative anaerobic and mesophilic. In addition, they produce gas and ferment D-glucose. *Escherichia coli* represents a major part of the intestinal flora of healthy individuals. However, some of its clones can be pathogenic and cause intestinal diseases such as diarrhoea as well as extraintestinal infections like urinary tract- or wound-infections, meningitis, bacteremia or pneumonia especially in immunocompromised patients. Pathogenic and diarrhoeagenic clones of *Escherichia coli* are Shiga toxin- producing *E. coli*, enterotoxigenic *E. coli*, enteropathogenic *E. coli* and enteroinvasive *E. coli*.

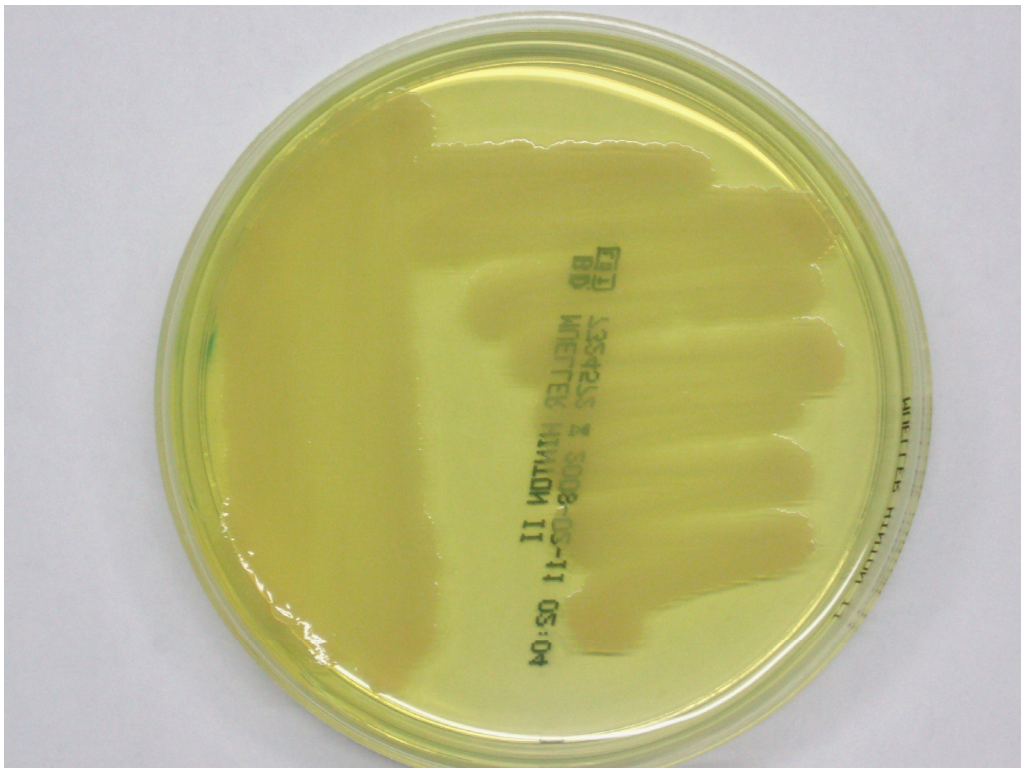
5.2.2.3 *Enterobacter spp.*

Figure 5 *Enterobacter spp.* on McConkey agar

Enterobacter spp. are gram-negative, facultative anaerobic, rod shaped bacteria belonging to the family of Enterobacteriacae. They size from 0.3 to 1.0µm in width to 0.6 to 6.0µm in length and have their optimal growth-temperature between 25 and 37°C ([45]). *Enterobacter spp.* are glucose fermenters and occur throughout the environment. Several subtypes like *E. cloacae* and *E. aerogenes* are well known as nosocomial pathogens. Risk factors for nosocomial *Enterobacter spp.* infections include hospitalization longer than 2 weeks, invasive procedures in the past 72 hours, treatment with antibiotics in the past 30 days, and the presence of CVCs ([46]). The infection originates either exogenously via contaminated hands or medical equipment or endogenously via colonization of the skin, urinary tract or the intestine. The outer membrane of *Enterobacter spp.* is composed of lipopolysaccharides, of which Lipid-A plays the major role as endotoxin in sepsis. Bacteremia, lower respiratory tract infections, endocarditis, central venous system infections or skin and soft tissue infections are some of the diseases caused by pathogenic species of *Enterobacter spp.*.

5.2.2.4 *Pseudomonas aeruginosa*

Figure 6 *Pseudomonas aeruginosa* on Mueller Hinton agar

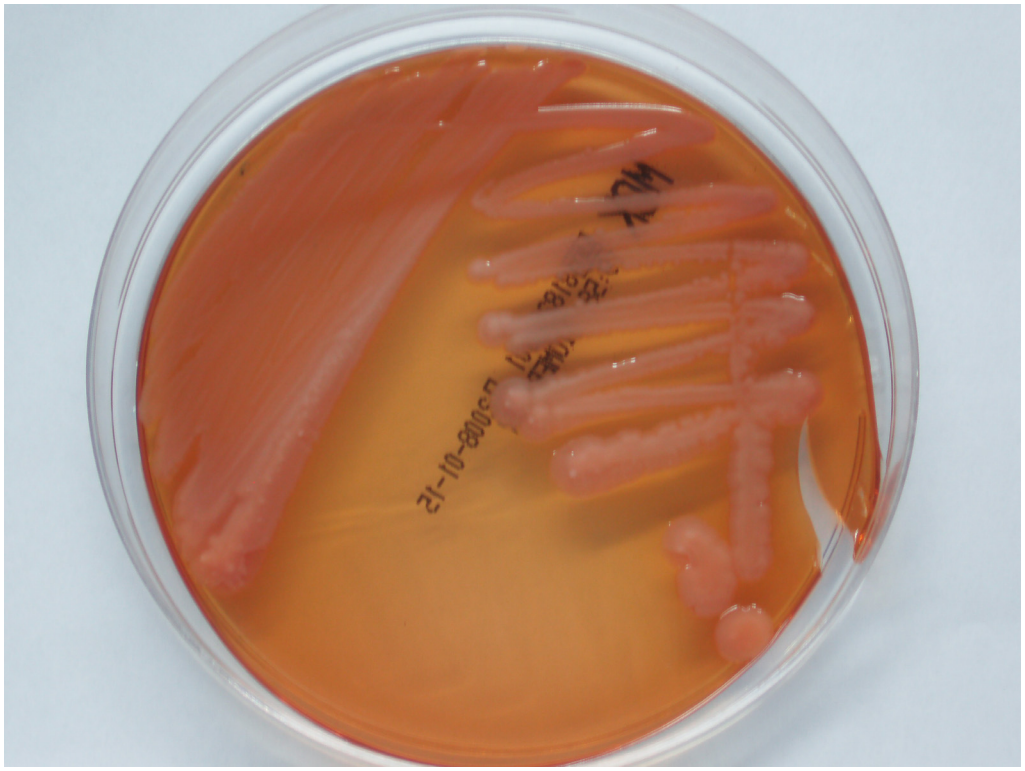


Pseudomonas aeruginosa, the most important opportunistic human pathogen of the genus Pseudomonas, is a non-spore-forming, gram-negative, facultative anaerobic and mesophilic rod and has its optimal growth-temperature between 30 and 37°C ([47]). Its appearance is straight or slightly curved and measures 1.5 to 5 µm in length and 0.5 to 1.0 µm in width ([47]). This bacterium has one flagellum, is oxidase and catalase positive and utilizes glucose. Some *Pseudomonas species* produce a biofilm consisting of exopolysaccharides, protecting *Pseudomonas spp.* from phagocytosis by leukocytes and strengthening the ability for surface-colonisation. Especially inpatients, who received chemotherapy or long-term broad spectrum antibiotic therapy and who have been hospitalized for extended periods, are at risk to develop an infection caused by *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* can be found in certain humid surroundings like hydrous solutions, hot tubes and raw fruits and vegetables. *Pseudomonas aeruginosa* infections spread from superficial skin infections in non-immunocompromised patients like folliculitis or ear canal infections to corneal

ulcers, osteomyelitis, infected valves in intravenous-drug users, pneumonia, especially in intubated intensive care- and cystic fibrosis-patients, nosocomial urinary tract- and CVC-infections in immunocompromised individuals.

5.2.2.5 *Klebsiella pneumoniae*

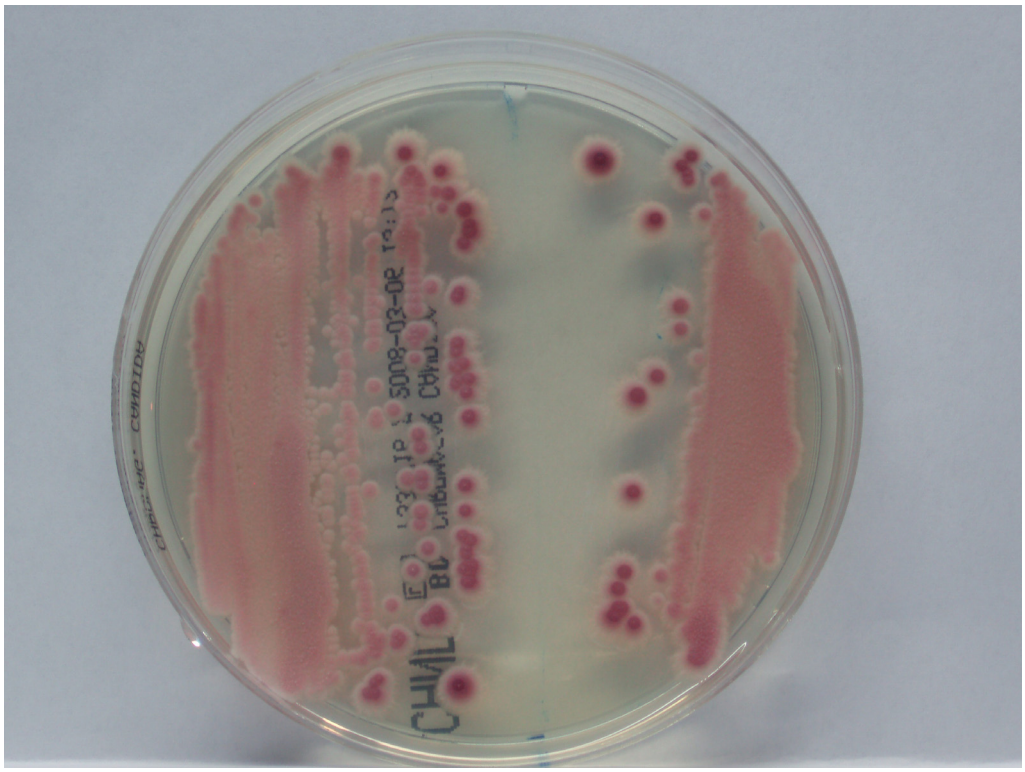
Figure 7 *Klebsiella pneumoniae* on McConkey agar



Klebsiella pneumoniae is a gram-negative, non-motile, non spore-forming, encapsulated and facultative anaerobic rod ([45]). Furthermore, it is mesophilic, lactose fermenting and measures from 0.3 to 1.0 µm in width and 0.6 to 6.0 µm in length ([45]). This bacterium features a prominent acidic polysaccharide capsule, which hinders the phagocytosis by granulocytes and bacterial death by bacterial serum factors. *Klebsiella pneumoniae* is ubiquitous, occurs on human skin, in the nasopharynx and in the intestines. It is the leading infection causing strain of the genus Klebsiella. *Klebsiella pneumoniae* is responsible for several nosocomial acquired infections of the urinary tract, wounds, bloodstream and lungs, especially in patients suffering from alcoholism, diabetes and chronic bronchopulmonary diseases. Its mortality rate can reach up to 50% even with antibiotic-therapy ([48]).

5.2.2.6 *Candida spp.*

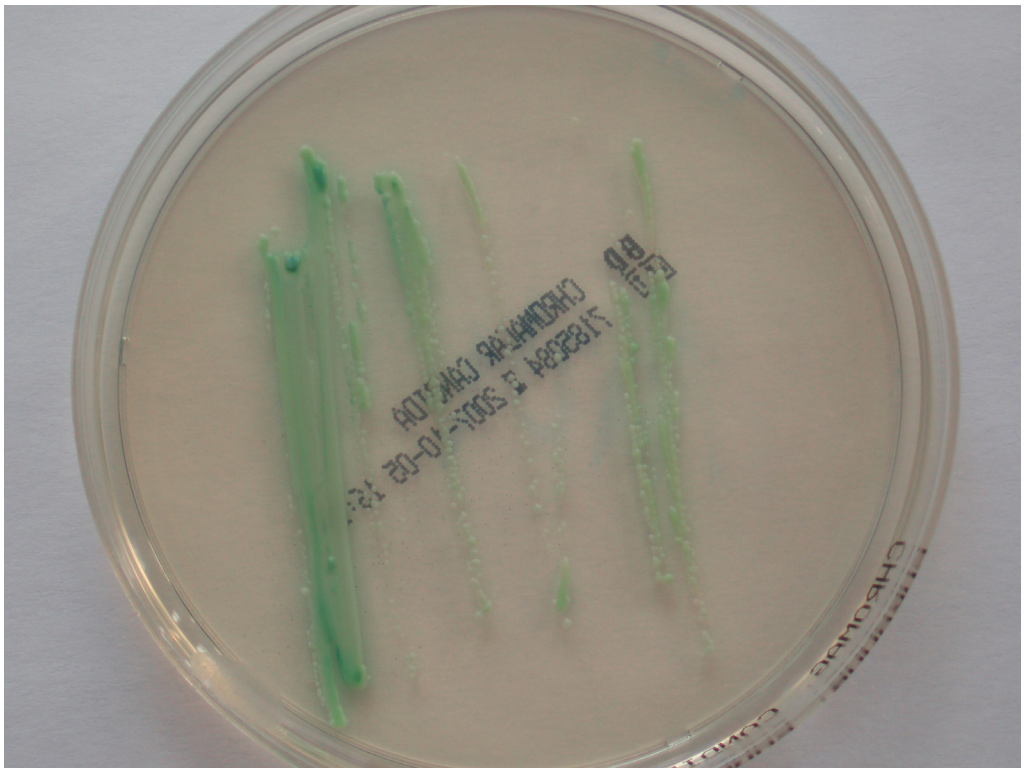
Figure 8 *Candida krusei* on Chrom agar



Candida spp. belong to the family of Cryptococcaceae and are a genus of yeasts, which have a diameter range of about 3 to 4 µm and occur to be ubiquitous in nature ([49]). *Candida spp.* are eukaryotic, unicellular, round to oval shaped yeasts, which multiply by budding of blastoconidia under aerobic conditions at 25 to 30°C and appear as large, round white or creamy colonies on agar plates. These blastoconidia develop out of a mother cell and can be separated from its origin or remain connected to it. These remaining blastoconidia are so called pseudohyphae and measure 5-10 µm ([49]). Common virulence factors are surface molecules secreted as partly proteases and phospholipases. These surface molecules allow the adherence to other surfaces like medical devices. Moreover, *Candida spp.* have the ability to produce true hyphae for spreading. The most important member of *Candida spp.* is *Candida albicans*, which is a causative agent of several opportunistic infections. *Candida albicans* causes three types of infections: mucocutaneous, cutaneous and systemic infections like oral candidiasis, intertriginous candidiasis or candidemia, which is associated with

neutropenia, especially in immunocompromised patients with a central venous access.

Figure 9 *Candida albicans* on Chrom agar



5.3 Risk factors for CRBSI

Risk factors for development of CRBSI are:

1. Underlying diseases like terminal renal failure, medical treatment such as bone marrow transplantations and the acuity of illnesses increase the risk for CRBSI. Multiple mechanical manipulations of the CVC and poor hygiene are also related to an increased risk for CRBSI ([31,50, 51,52]).
2. Critically ill patients, who are prolonged hospitalized, are often colonized with hospital acquired organisms like MRSA or VRE ([31,53]).
3. In the intensive care unit (ICU) setting the incidence of infection is higher than in in-patients of other wards because CVCs remain in situ for extended periods of time and are manipulated multiple times per day for administration of fluids, drugs and blood products. Sometimes CVCs are

inserted in case of emergency under suboptimal conditions of asepticemia at ICUs ([31,52]).

4. The insertion of CVCs into the femoral or internal jugular vein is associated with an increased risk for CRBSI. Administration of total parenteral nutrition through the CVC increases the risk for CRBSI ([52,54,55]).
5. Neutropenia, especially in adult patients suffering from cancer and undergoing high dose chemotherapy, is an independent risk factor for infections of long-indwelling CVCs and sepsis of unknown origin ([51]).
6. Nasal carriage of *Staphylococcus aureus* has been considered as a source for CRBSI ([56]). In a multicenter study 82.2% of the *Staphylococcus aureus* blood isolates were identical to those from the anterior nares ([57]).
7. Polyvinylchloride and polyethylene irregularities enable the adherence and growth of microbial pathogens like coagulase-negative staphylococci and *Pseudomonas aeruginosa*. In addition, polyvinylchloride- and polyethylene-CVCs are more thrombogenic than others made out of Teflon, siliconeelastomer and polyurethane ([58,59]).
8. Multi-lumen CVCs increase the risk for CRBSI compared to single-lumen CVCs ([60]). However, other studies showed that there is no correlation between the number of CVC lumina and incidence of CRBSI ([61]).

5.4 Infectious pathway

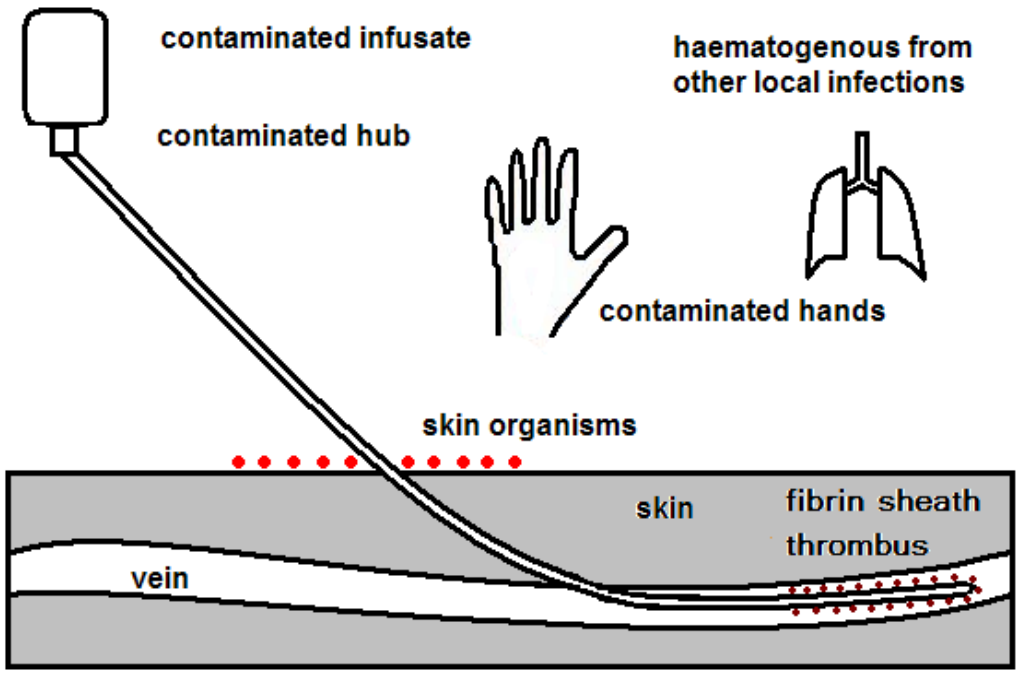
There are two main sources for CRBSI: colonization of the CVC that leads to CRBSI, and less frequently, contamination of fluid administered through the CVC ([62,63]). Rarely CVCs are colonized by microorganisms originating from a distinct infectious focus.

Microorganisms causing CRBSI need to get in contact with the extraluminal or intraluminal surface of the CVC, where they adhere and start to produce a biofilm, which allows persistent infection and haematogenous dissemination ([64]). This biofilm is composed of polysaccharides, fibrin, fibronectin or laminin and prevents phagocytosis and the effect of antibodies.

Microorganisms gain access to the intraluminal or extraluminal surface of the CVC by 1 of the 3 following mechanisms:

1. At the time of insertion or the following days microorganisms belonging to the skin flora invade the percutaneous catheter tract ([65]).
2. The catheter hub becomes contaminated during catheter-insertion over a percutaneous guidewire or when it is manipulated for administration of fluids, drugs and blood products ([66]).
3. Microbial organisms are displaced haematogenous from other local infections and contaminate the CVC ([31]).

Figure 10 potential sources of infection of a percutaneous CVC (modified from [67])



The infectious pathway is slightly different in short-term and long-term CVCs. In short-term CVCs, skin organisms usually migrate from the insertion site into the cutaneous catheter tract. Then they gain access extraluminally and colonize the catheter tip from where they spread ([68,69]). In contrast, long-term CVCs usually become contaminated through the catheter hub or adapters, especially when total parenteral nutrition is needed, and colonize the catheter via the interior lumen ([70,71]).

5.5 Signs and symptoms of CRBSI

The clinical appearance of CRBSI can include signs of local infection, which occur in about 3% ([72]), systemic symptoms, sepsis or a combination of these. The diagnosis of CRBSI in neutropenic patients remains difficult because of the lack of typical clinical signs at the insertion site of the catheter and the occurrence of fever due to non-infectious diseases (i.e. underlying malignant disease, drug related toxic effects) or due to administration of certain drugs like antithymocyte globulin.

Local signs within 2cm of the catheter insertion site describe an exit site infection and are as listed below ([31]):

1. Redness or erythema
2. Swelling
3. Warmth
5. Tenderness or pain
6. Indurations

According to this definition of the CDC, local clinical signs of infections at the subcutaneous part of tunnelled CVCs have to diffuse at least 2 cm into the tunnel without concomitant BSI to be characterized as clinical exit site infection ([31]).

Presence of at least two of the following symptoms indicates a systemic involvement in terms of a systemic inflammatory response syndrome (SIRS) ([73]):

1. Core temperature > 38.0°C or < 36.0°C
2. Tachycardia (heart rate>90/min.)
3. Hypotension (systolic blood pressure<90 mmHg)
4. Tachypnea (breathing rate>20/min.) or a $PaCo_2$ less than 33 mmHg
5. Leukocytosis >12000/µl or leukocytopenia<4000/µl and/or left shift in the myeloid series of cells>10%

Sepsis is considered if an infection is highly suspected or proven and SIRS criteria are met ([74]). Following the definition of a severe sepsis of the European Society of Intensive Care Medicine, a severe sepsis is existent when SIRS criteria and signs of systemic hypoperfusion resulting in end-organ-dysfunctions are present ([75]).

Furthermore, a septic shock is defined by the European Society of Intensive Care Medicine if the following three criteria are met ([75]):

1. Detection of an infectious origin
2. Detection of a SIRS
3. Detection of arterial hypotension despite of appropriate volume replacement

5.6 Diagnosis of CRBSI

There are different types of techniques to verify a CRBSI such as Maki roll or Brun-Buisson method, which require catheter removal. Diagnostic test that do not require catheter removal are the Gram stain/AOLC test or DTP.

Gram/AOLC test ([8,76]): The Gram stain/AOLC is a highly sensitive and specific test for detection of CRBSIs and in case of clinical suspicion of CRBSI an AOLC test can be performed within 30 minutes compared to a common DTP method, which at least takes a few hours. Kite et al. demonstrated that the Gram stain/AOLC test is a quick and simple method to detect CRBSI without catheter removal, with a sensitivity of 96% and a specificity of 92% ([8]). The positive predictive value was 91% and a negative predictive value was 97% ([8]).
Acridine Orange is a nucleic acid selective metachromatic and fluorescent stain. It is cell-permeable, interacts with DNA (green fluorescence) and RNA (red fluorescence) by intercalation or electrostatic attraction and determines the cell cycle ([77]). For the Gram stain/AOLC test a blood sample of 2ml in an EDTA tube is obtained aseptically from the catheter. 1.2 ml of hypotonic formalin saline (1.46 g sodium chloride dissolved in 1l sterile, distilled water and diluted with addition of 100 ml formalin) is added to a 50 µl blood sample. After 2 minutes the lysis is stopped by neutralisation with 2.8 ml of hypertonic saline (1.168g NaCl in 100 ml sterile, distilled water). After centrifugation of the mixture at 352 x g for 5 minutes the supernatant is discarded and the cellular pellet at the bottom of the test tube is homogenised by vortexing for 5 seconds. Then 100 µl of the achieved homogeneous mixture is transferred by a pipette into a cytospin cupule, which contains a microscope slide. To obtain a monolayer of leukocytes and microorganisms on the slide, the cytospin cupule is centrifuged in a cytospin machine for 5 minutes. Afterwards the slide is stained with acridine orange

(concentration 0.001%) and examined with ultraviolet microscopy. The whole slide is scanned for the presence of any microorganisms under a minimum amplification of 100 high-power-fields. A second slide is prepared equally and then stained with Gram stain to identify the detected microorgansims.

Gram stain ([8,78]): For Gram staining, crystal violet is added over the fixed monolayer for 1 minute and then poured off. Next, the sample is covered in iodine solution for another minute and removed again by washing with water. Crystal violet dissociates in aqueous solutions into its positively and negatively charged ions penetrating through the cell wall and cell membrane of both gram-positive and gram-negative cells. The positively charged ions interact with negatively charged components of bacterial cell walls and stain them purple. The negatively charged Iodine interacts with the positively charged ions of crystal violet and creates large complexes of pigments within the inner and outer cell layers. A few drops of decolourizer are added until the solvent is no longer coloured, gently rinsed with a stream of water from a plastic water bottle and then the counterstain is done by basic fuchsin solution for 60 seconds to stain gram-negative bacteria red or pink. Furthermore, the solution is washed off and the slide is dried with absorptive paper. In case of gram-positive bacteria, the decolourizer cannot wash out the large complexes of pigments because of the dehydrate effect of iodine on the multilayer membrane made out of peptidoglycan, which minimizes the intermolecular space. The cell wall of gram-negative bacteria consists of a monolayer peptidoglycan-membrane and an incumbent lipid-membrane. The added decolourizer interacts with the lipids and as a consequence the peptidoglycan-layer is left exposed and the complexes of pigments are rinsed out.

DTP ([9,10]): In patients with signs of CRBSIs a Differential Time to Positivity (DTP) Test is carried out by taking simultaneously aerobic and anaerobic blood culture bottles through the catheter hub and additionally another pair of blood culture bottles from a peripheral vein. Then the blood culture bottles are transported to the local microbiological laboratory and processed immediately by incubating at 37°C in an automatic blood culture detection system for 7 days. The time to positivity for each bottle is recorded. The DTP method measures the difference in the microbial load between blood drawn from the CVC and drawn from a peripheral vein. DTP is considered positive and indicates CRBSI if the blood culture drawn through the

CVC becomes positive at least 120 minutes earlier than the culture drawn from a peripheral vein ([10]). The specificity of this method is 91% and the sensitivity 94% ([10]).

Endoluminal brush sampling ([79]): For the endoluminal brush sampling, which is another in situ method to diagnose a CVC infection, a sterile brush is encapsulated in a polythene sleeve and attached to the hub of the CVC. Then the brush is directed down the catheter lumen to the distal end of the line and encapsulated in a polythene sheath. Afterwards the brush is cut off and sent to the local microbiological laboratory in a sterile container. There one millilitre of phosphate puffered saline is added to the sample, sonicated for one minute at 44KHz and then vortexed for 15 secounds. 100 µl of the suspension are plated over the entire surface of a blood agar plate and incubated under aerobic conditions at 37°C for 24 hours. Next, all visible colonies are counted and the CFU per ml are recalculated. More than 100 colonies per millilitre are suggestive of CRBSI.

Brun-Buisson method ([80]): Removed CVC can be analysed by Brun-Buisson method. After local disinfection of the insertion site, the catheter is removed aseptically with a sterile forceps and the distal 5-6 cm of the catheter tip are cut off and sent to the microbiological laboratory in a sterile tube. One ml of sterile water is added to the catheter tip and vortexed for one minute. 100 µl of the suspension is plated over the surface of a blood agar plate and incubated at 37°C for 48 hours. Colonies are counted to recalculate the CFU/ml and bacteria are identified by routine microbiological procedures. A cut off value of 10^3 CFU/ml is suggestive of CRBSI.

The Maki roll method ([81]): This is a semiquantitative culture method to identify extraluminal CRBSI. After aseptically displacement of the catheter, the distal part of the catheter segment is rolled across blood agar four times and incubated at 37°C for 24 hours. This method can only be a sign of extraluminal catheter infection and is considered as positive at a number of detected colonies greater than or equal to 15 colonies.

5.7 Treatment and prophylaxis of CRBSI

If CRBSI is detected the intravascular line should be removed immediately. In addition, antiinfective therapy should be administered depending on epidemiological data or cultured microorganisms. In case of CRBSI due to CNS removal of the CVC without systemic antibiotic therapy is considered curative in certain cases ([82]).

Table 3 Treatment of CRBSI (modified from [83])

Pathogens	Therapy	Alternative therapy
Coagulase negative staphylococci (in most cases Methicillin resistant)	Vancomycin	Teicoplanin
Staphylococcus areus	Flucloxacillin	Cefazolin
Methicillin resistant *Staphylococcus aureus*	Vancomycin	Teicoplanin or Linezolid
Enterobacter spp., *Klebsiella* spp	Ceftriaxon	Chinolone
Pseudomonas spp.	Ceftazidim	Cefepim
Enterococcus spp.	Ampicillin	Vancomycin
Candida albicans	Fluconazol	Amphotericin B

The treatment duration for uncomplicated CRBSI without hypotension, fever or organ dysfunctions should be at least 10 to 14 days ([84]). In contrast, patients with complicated CRBSI, septic phlebitis or endocarditis should receive appropriate antimicrobial chemotherapy for 4 to 6 weeks ([84]). In case of tunnelled or total implanted CVCs and an infection caused by CNS, an antibiotic therapy without removal of the catheter can be attempted ([85]). For this reason, a systemic antimicrobial therapy and an antibiotic lock therapy, which consists of a mixture of antibiotics and heparin filled in the catheter when it is not used, are combined. Studies have shown that an antibiotic lock therapy can only reduce the non-effective CRBSI-therapy by antibiotic-locks from 57% to 33% ([86]).

The Centre for Disease Control and Prevention recommends different strategies to prevent CRBSIs ([31]). Specific prevention strategies and improved guidelines for the management of intravascular devices can reduce the rate of catheter colonization and associated CRBSI.

1. Quality assurance and continuing education: Reports have shown that well trained staff can decrease the risk for catheter colonization and CRBSI ([87]). In contrast, a reduction of nursing staff below a critical level increases the risk for infection ([88]).
2. For catheter insertion upper extremity sites especially the subclavian site should be preferred to lower extremities, especially the femoral vein. In addition, a meta-analysis of eight studies displayed the reduction of mechanical complications when bedside ultrasound for insertion was used, compared to the standard landmark insertion technique ([89]).
3. Intravascular catheters made of Teflon® or polyurethane are less colonized with microorganisms than CVCs made of polyvinyl chloride. Besides, coagulase-negative staphylococci have an increased affinity for polyvinyl chloride compared to Teflon® ([59]).
4. Skin antisepsis with 2% chlorhexidine preparation: Maki et al. proved that a 2% aequeous chlorhexidine gluconate, used for cutaneous disinfection before insertion of a CVC and for post-insertion site care, compared to 10% povidone-iodine and 70% alcohol, can decrease CRBSI rates (0.5 per 100 catheters vs. 2.3 and 2.6 for alcohol and povidone-iodine) ([90]).
5. Catheter securement devices: Usually sutures protect intravascular catheters against dislocation. However, sutures are points of origin for festering skin suture wounds because of their foreign body pontential. Sutureless devices for securing non-cuffed intravascular devices can lower the rate of suture associated wound infections and minimize the to and fro pistoning of the catheter ([91]), which allows cutaneous microorganisms to invade into the insertion site through capillary action ([92]).
6. In-line filters: Studies have shown that in-line filters can reduce the risk of infusion-related phlebitis by
   - decreasing the infection risk from contaminated infusates or contamination acquired proximal of the filter ([93])

- reducing the risk for phlebitis in patients who are treated with high doses of medication or in those in whom infusion-related infections already caused phlebitis ([93])
- filtering particulate matter that might contaminate intravascular fluids ([93])
- removing endotoxin produced by gram-negative organisms in contaminated infusate ([94])

On the other hand infusion-related infections are rare and dextran, lipids or other solutions can block in-line filters leading to multiple manipulations and decreasing the availability of administered drugs([95]).

7. Antimicrobial/ antiseptic impregnated catheters and cuffs:
    - Chlorhexidine/ Silver sulfadiazine: Nowadays, two generations of these catheter types are available. The first generation catheter is coated with chlorhexidine/ silver sulfadiazine only on the external luminal surface. This antiseptic catheter is less colonized after removal than non-antiseptic catheters and has a 5-fold reduction in the rate of CRBSI ([96]). In contrast, the second generation catheter offers a combination of both antibiotics on the external surface with an increased release and a higher content of chlorhexidine. The internal surface is impregnated with chlorhexidine alone. In vivo and in vitro studies have shown a prolonged antimicrobial activity and a significant efficiency in preventing CRBSI compared to first generation catheter ([97]).
    - Minocycline/ Rifampin: A combination of Minocycline/ Rifampin on the external and internal luminal surface is associated with lower rates of CRBSI and an extended antimicrobial activity compared to first generation chlorhexidine/ sulfadiazine catheters ([98]).
    - Platinum/ Silver: Intravascular catheters are coated with a combination of platinum and silver. They have a broad antimicrobial activity because of its ionic metals. This technology increases the local release of silver ions leading to a lower rate of CRBSI compared to standard polyurethane CVCs (0% vs. 4%) ([99]).
    - Silver cuffs: These kinds of cuffs are attachable to subcutaneous CVCs and released silver ions create an additional chemical barrier inhibiting the deep invasion of cutaneous microorganisms into the

insertion site. The preventive ability of silver cuffs used in short term devices is controversial. Only one study could demonstrate a significant reduction of short term CVC infections with the use of attachable silver cuffs ([100]). Silver cuffs have no benefit with long term devices because they cannot prevent luminal contamination ([101]).

8. Systemic antibiotic prophylaxis: The benefit of oral or parenteral applied antibacterial or antifungal drugs among adults could not be demonstrated yet ([102]). The prophylactic application of vancomycin, which has an effect on the most common CRBSI-causing-microorganisms, holds a great risk for acquiring VRE and therefore outweighs the benefit of using vancomycin prophylaxis ([103]).
9. Antibiotic/ antiseptic ointments: Periodic application of povidone-iodine onto haemodialysis catheter insertion sites in comparison to sterile gauze dressing alone reduces the incidence of exit-site infections (5% vs. 18%), catheter-tip colonization (17% vs. 36%) and BSIs (2% vs. 17%) caused by Staphylococcus aureus ([104]). Mupirocin, which is another antistaphylococcal topical agent, can reduce the risk for CRBSI too (3% vs. 22%) ([105]) but such ointments also have been associated with mupirocin resistance ([106]). Routine use of antibiotic or antiseptic ointments have to be considered very well because beside the opportunity to reduce BSI, side effects like resistances and catheter colonizations with *Candida spp.* can occur ([107]).
10. Antibiotic lock prophylaxis: Antibiotic lock is a local prophylaxis. Antibiotic drugs are instilled into the catheter lumen and remain there for usually 6-12 hours. The positive effect of antibiotic lock prophylaxis seems to be controversial. Rijinders et al. pointed out that the failure to cure CRBSI with a combination of heparin and vancomycin for gram-positive or ceftazidime for gram-negative bacteria could only decrease from 57% to 33% ([86]). Henrickson et al. compared the use of heparin, heparin and vancomycin, heparine, vancomycin and ciprofloxacin as lock-therapies. The prophylactic use of vancomycin-ciprofloxacin lock solution reduced the rate of CRBSI to 0.55 per 1000 catheter-days (0.55 vs 1.72 per 1000 catheter-days under heparin). The rate of CRBSI for vancomycin and heparin lock solution was also significantly reduced (0.37 per 1000 catheter-days) ([108]).

11. Anticoagulants: Catheter thrombosis creates a basis for microbial colonization. Therefore anticoagulation agents like heparin or warfarin are flushed through the catheter to prevent catheter thrombosis. Heparin ([109]) and warfarin ([110]) have been shown to decrease the risk of catheter thrombosis but not the rate of CRBSI. Two trials of prophylactic instillation of urokinase into long-term devices have verified a reduction in thrombosis, premature catheter loss and CRBSI ([111,112]).
12. Replacement of catheter: Short peripheral intravascular catheters should be replaced at 72-96-hour intervals to reduce the risk of phlebitis and CRBSIs. Midline catheters and CVCs have to be changed regarding to a specific indication like local signs of infection or proven CRBSI because no prospective, randomized studies have shown a reduction of CRBSI when routine replacement was undertaken ([113,114]). Pulmonary artery catheters that are left in place longer than 7 days and peripheral artery catheter use longer than 4 days have a higher infection rate than earlier removed catheters ([115]) but no recommendation regarding replacement at scheduled time intervals can be given ([114]).
13. Replacement of administration sets: The optimal interval for routine replacement of administration sets is 72 hours ([116]) except for lipid emulsions and blood products. In case of administered lipid emulsions and blood products more frequent changes of administration sets are necessary because such fluids are conducive to microbial growth and have been identified as independent risk factors for CRBSI ([117]).

6 The study

6.1 Study design

From February 2007 to January 2008, 32 patients at the Division of Haematology, Medical University of Graz, Graz, Austria, receiving HSCT for treatment of a haematological malignancy as primary disease and obtained any kind of central venous access, were prospectively analyzed. For CRBSI-screening by Gram stain/AOLC test we investigated EDTA blood from routinely used lumen of CVCs three times a week. In addition, a chocolate agar plate and an aerobic blood culture bottle for each EDTA blood sample were used as control. In case of clinically suspected CRBSI, routine investigation was undertaken by Gram stain/AOLC test, DTP and Brun Buisson method. Informed consent was obtained from all patients.

6.2 Patients and patient data

To be eligible for the study, patients had to fulfil the following inclusion criteria:

1. Patients had to have a haematological malignancy as the primary disease, such as acute myeloid leukaemia, chronic myeloid leukaemia, acute lymphatic leukaemia, Hodgkin's disease, Non-Hodgkin's disease or multiple myeloma.
2. Patients had to have any kind of central venous access like a non-tunnelled or tunnelled CVC, a total implantable system or haemodialysis catheter.
3. Patients had to receive HSCT for treatment of a haematological malignancy as primary disease.

Information recorded on these patients included:

1. Underlying disease
2. Type of conditioning therapy
3. Type and date of HSCT
4. Graft's number of CD34+ cells
5. Type of CVC and number of catheter lumen

6. Date and site of catheter insertion
7. If necessary date of Port a Cath® tapping
8. Duration of neutropenia and applied total parenteral nutrition
9. Oral decontamination of the gut (yes/no)
10. Antifungal-prophylaxis (azole/ echinocandin/ polyene)
11. Any kind of clinically defined infection or microbiologically defined infection
12. If fever of unknown origin appeared
13. Type of antibiotic/ antifungal therapy that was administered in case of CRBSI.

Patients were evaluated daily for

1. The presence of local signs or symptoms of infection at the catheter insertion site (e.g. erythema, warmth, swelling, tenderness, secretion)
2. Temperature >38°C or <36°C
3. The presence or absence of chills
4. Systolic blood pressure<90 mmHg
5. Heart rate>90 beats/minute
6. Respiratory rate>20/minute
7. Neutropenia
8. Increased CRP

6.3 Material and laboratory methods

6.3.1 Screening methods

EDTA blood samples were drawn three times a week. Blood samples were processed within 8 hours or otherwise stored at 4°C. For screening of CRBSI we used the Gram stain/AOLC test as previously published ([8,76]).

Two 50µl samples of the catheter blood (treated with edetic acid) were each placed into a sterile tube, to which 1.2 ml formalin saline was added. After two minutes 2.8ml hypertonic saline for neutralisation was admixed to each tube followed by centrifugation at 352g for 5 minutes. After decantation of the supernatant, the cellular pellet was homogenised by vortexing for 5 seconds and then 100µl of the suspension were carried over by a pipette to a cytospin cupule comprising a microscope slide. This was centrifuged in a cytocentrifuge for 5 minutes and as a result a monolayer of leukocytes and microorganisms was placed on each microscope slide, which was air dried. One of the slides was then stained with acridine orange (concentration 0.001%) and viewed by ultraviolet microscopy. The other slide was Gram-stained and examined with light microscopy. A minimum of 100 high-power fields were scanned for the presence of any bacterium or fungus within the monolayer of both slides. The presence of bacteria or fungi was considered indicative for CRBSI.

100 µl of the same blood sample were plated over the entire surface of a chocolate agar plate and incubated under aerobic conditions at 37°C for 24 hours as a control. Moreover, one aerobic blood culture bottle was inoculated with remaining 2ml of the same blood sample and incubated at 37°C in an automatic blood culture detection system (BACTEC® 9260 Becton Dickinson, Heidelberg, Germany) for 7 days that enables continuously monitoring of blood cultures by evaluating changes of fluorescence related to microbial growth. Every detected microorganism on the chocolate agar plate as well as in the blood culture bottle was identified by routine microbiological procedures and isolated strains were stored at a temperature of -70°C for further investigations.

6.3.2 Routinely diagnostic methods on the HSCT ward

If a temperature of >38.5°C was measured and a CRBSI was clinically suspected by the treating physician, DTP and Gram stain/AOLC test were performed.

DTP ([9,10]): For identifying the differential time to positivity two blood cultures, an aerobic and an anaerobic sample, need to be drawn through the CVC. Simultaneously, another set of blood culture bottles are obtained from a peripheral vein. Afterwards, blood culture bottles are transported to the local microbiological laboratory and incubated at 37°C in an automatic blood culture detection system (BACTEC® 9260 Becton Dickinson, Heidelberg, Germany) for 7 days. The time to positivity for each bottle is noticed. Afterwards the difference between the time to positivity of the peripheral-aerobic or anaerobic blood culture bottle and the CVC blood culture bottles is calculated and expressed in hours. A cut-off limit of > 2 hours is considered to be indicative for CRBSI ([10]). In case of positive blood culture bottles, blood is Gram-stained and subcultured on chocolate agar and McConkey agar under aerobic conditions at a temperature of 37°C for 24 hours. Detected bacteria are identified by routine microbiological analysis.

Gram stain/AOLC test ([8,76]): At the time of blood culture bottle inoculation an additional serum sample (3ml) is drawn through the central venous access and the peripheral vein. EDTA blood samples collected at night or at weekends are stored at 4°C for further procedure. During regular working hours all samples are transported to the local microbiological laboratory and the Gram stain/AOLC test is performed as described above.

Brun Buisson method ([80]): After local disinfection of the catheter's insertion site with 70% alcohol, the catheter is removed aseptically with a sterile forceps. The distal 5-6 cm of the catheter tip are cut off and sent to the microbiological laboratory in a sterile tube. One ml of sterile water is added to the catheter tip and then the tube is vortexed for one minute. Afterwards, 100µl of the suspension are plated over the surface of a blood agar plate and incubated at 37°C for 48 hours. Grown colonies are counted, the number of CFU/ml is expressed and bacteria are identified by routine microbiological testing.

Figure 11 Laboratory materials (from the left to the right): blood culture bottle, two sterile tubes, EDTA blood sample, formalin saline, hypertonic saline, cytospin cupule comprising a microscope slide

Figure 12 (from the left to the right) Acridine Orange, Gram's Crystal Violet, Gram's Iodine, Gram's decolourizer, Gram's Safranin

6.4 Results

Patients characteristics

From February 2007 to January 2008, 618 Gram stain/ AOLC screening tests from 32 patients (16 males and 16 females; median age, 53 years; range 25 to 69 years) with 36 catheter episodes were investigated. The average observation period was 37 days (range 12 to 104 days). 4 patients died during the observation period but no case was related to CRBSI.

Twenty-five patients had non-tunnelled CVCs (triple-lumen), 6 had tunnelled CVCs (double-lumen), 1 had a totally implanted system (single-lumen), 3 had Perm-Caths® (double-lumen), and 1 had a haemodialysis catheter (double-lumen). Twenty-one CVCs had been placed into the right subclavian vein (18 non-tunnelled CVCs, 3 Hickman® lines) and 11 had been placed into the left subclavian vein (6 non-tunnelled CVCs, 1 haemodialysis catheter, 3 Hickman® lines, 1 Port-A-Cath®). Three Perm-Caths® had been inserted into the right internal jugular vein and 1 non-tunnelled CVC had been inserted into the left internal jugular vein.
Three patients were implanted a second central venous catheter because of accidental displacement of the first CVC by the patient himself (n=1 patient), pressure pain at the insertion site of the first CVC (n=1), on suspicion of CRBSI (n=1) and verified CRBSI (n=1).

Four non-tunnelled CVCs (50%), 1 total implantable systems (12.5%), 1 Perm-Cath® (12.5%) and 2 Hickman®-lines (25%) were infected. Six of these lines had been placed into the right- (5 into the subclavian vein and one into the internal jugular vein) and two into the left side (subclavian vein). Except of one CVC, all CVCs had multiple lumens (87.5%). All patients with CRBSI had only one episode of catheter related bloodstream infection.

Acute myeloid leukaemia (n=14), acute lymphatic leukaemia (n=1), chronic myeloid leukaemia (n=1), Hodgkin's disease (n=1), plasma cell leukaemia (n=1), multiple myeloma (n=8) and different subtype of Non-Hodgkin's disease (n=6) were the underlying malignancies in these patients.

Eight patients received allogeneic grafts, 15 received autologous grafts, 7 underwent NST and 2 received induction-therapies.
The mean duration of neutropenia in patients receiving an allogeneic graft was 21 days and in patients receiving an autologous graft was 7 days. The mean duration of applied total parenteral nutrition in patients receiving an allogeneic graft was 28 days and in patients receiving an autologous graft 11 days. In patients undergoing a NST the mean duration of neutropenia was 19 days and the mean duration of total parenteral nutrition was 37 days. In patients with CRBSI the mean duration of neutropenia was 17 and the mean duration of applied total parenteral nutrition was 18 days.

Antifungal prophylaxis was administered only to patients obtaining allogeneic grafts (Azole to 8 patients) or NST (Azole to 6 patients, Polyene to 1 patient). In 5 cases an oral decontamination of the gut with Amphotericin B plus Trimethoprim/ Sulfamethoxazol was administered.

17 patients showed clinical signs and symptoms of inflammation at the insertion site of the central venous access. Among these, 6 cases of CRBSI (35%) and 2 bloodstream infections without a certain origin (12%) developed. In addition, 2 patients had bacteremia from an unknown focus without clinical signs and symptoms of inflammation at the insertion site of the central venous access.

Clinical presentation:

As depicted in table 4, there was no difference according to the occurrence of SIRS criteria among patients experiencing a CRBSI and patients without a CRBSI. Local signs of infection at the catheter insertion site were more common in CRBSI-cases than in patients without an episode of CRBSI. However, this difference did not reach statistical significance.

Table 4 Clinical features of study-patients

	CRBSI (n=8)	**No CRBSI (n=24)**	**p-value**
SIRS criteria	8 (100%)	24 (100%)	1
Local signs of infection	6 (75%)	11 (46%)	0.15

Microbiology:

The microbes responsible for CRBSI were coagulase-negative staphylococci (n=5 patients; 62.5%), Enterococci (n=2; 25%) and *E. coli* (n=1; 12.5%). Coagulase-negative-staphylococci (n=2), *Staphylococcus aureus* (n=1) and *E.coli* (n=1) were involved in cases of BSI with an unknown origin.
One of 24 routinely done Brun Buisson investigations resulted in catheter-colonization by coagulase-negative staphylococci with a bacterial load of 10^3 CFU/ml.

Antimicrobial therapy:

At the time of inclusion into the study, antibiotics according to the prophylactic regimen were administered to 23 patients (Piperacillin/Tazobactam, Ciprofloxacin, Trimethoprim/Sulfonamid, Trimethoprim/Sulfamethoxazol, Moxifloxacin).

In case of CRBSI, 7 patients were treated with one of the following antimicrobial/antifungal therapies:

1. Amikacin and Cefepim
2. Meropenem and Linezolid
3. Meropenem, Linezolid, Clarithromycin and Caspofungin
4. Amikacin, Cefepim and Metronidazol
5. Meropenem, Linezolid and Metronidazol
6. First Vancomycin, after discontinuing of the first drug another fever episode appeared which was treated with Linezolid
7. Vancomycin and Fusidinacid-Sodiumsalt

In one case CRBSI was diagnosed after removal of the CVC by Brun Buisson method. No symptoms of infections appeared and therefore no specific antibiotic therapy was administered to this patient.

Statistical results:

As displayed in table 5, eight patients experienced a CRBSI (25%). Among these, four could have been detected by the screening-method too. The detection by Gram stain/AOLC screening test was on average 48 hours earlier than by routine investigations. 4 CRBSI-cases were negative in the screening test representing

false negative screening results. In 28 catheter episodes no CRBSI could be detected by routine investigations but among these, 4 episodes were positive in the screening test representing false positive screening results.

The sensitivity and specificity of screening by Gram stain/ AOLC test were 50% and 86%, the PPV and NPV were 50% and 86%.

Table 5 Statistical results

	Gram stain/AOLC Screening	
Routine	**positive**	**negative**
8 CRBSI*	4/8	4/8
28 no CRBSI*	4/28	24/28

* as routinely diagnosed in clinically symptomatic patients within 48h after screening

6.5 Discussion

Bloodstream infections have a mortality rate of 10-20% ([100]) and especially in neutropenic patients they are associated with increased mortality ([2,3]).

The appearance of clinical features like SIRS criteria did not differ between CRBSI cases and patients without an episode of CRBSI. Clinical criteria alone cannot be trusted for diagnosing a CRBSI during neutropenia. For this reason we evaluated whether the Gram stain/AOLC test could serve as a screening tool for detection of CRBSI in a subclinical stage.

We demonstrated that screening of blood drawn from routinely used lumina of CVCs three times a week by the Gram stain/AOLC test can help to detect 50% of patients, whose central venous line is significantly colonized and therefore likely develop a clinically evident CRBSI. Moreover, in the event of negative screening results it was unlikely that patients experienced a CRBSI, as demonstrated by the NPV of 86%. This could exclude the CVC as a source of BSI and enables to focus on further investigations for evaluating other origins of infections. In 4 cases CRBSI was not detected by Gram stain/AOLC test screening tests. These false negative results may have the following reasons. First, randomised analysis of one lumen in multiple-lumen CVCs has a 40% chance of missing a significant catheter colonization that could lead to a clinical evident CRBSI ([118]). Second, routine analysis of suspected CRBSI included the DTP method that might lead to an earlier indication of CRBSI. The colonization has to reach an absolute cut off of 10^3 organisms/ml catheter blood until the Gram stain/AOLC test becomes positive ([8]). The DTP method has a relative cut off level because it monitors the time of microbial growth and compares peripheral and hub-blood cultures ([9,10]). Therefore the DTP method is considered more sensitive compared to the Gram/AOLC test. The Gram/AOLC screening test also showed 4 false positive screening-results. This might be due to antibiotic therapies that suppressed the number of microorganisms within the catheter's lumen after drawing blood samples for the screening tests. Consequently no clinical symptoms of CRBSI subsequently developed.

In comparison to a previous study ([34]) we found less significantly colonized non-tunnelled CVCs (50% vs.67%), a lower incidence of infected CVCs inserted into the internal jugular vein (12.5% vs. 44%), but an increased number of affected multiple-lumen CVCs (87.5% vs. 67%). Furthermore, in our study coagulase-negative staphylococci were the most frequent isolated microorganisms in patients with CRBSI (63%) compared to 37% and 39% described by the CDC ([31]) and Munoz ([34]). However, the smaller study population in our study might influence the distribution of microorganisms.

Yilmaz et al. recently reported that total parenteral nutrition increases the risk for central venous catheter infections ([55]). In accordance with this finding, all of the CRBSI-patients in our study received total parenteral nutrition with a median duration of 14 days.

Previously, studies confirmed neutropenia, especially in adult patients undergoing high dose chemotherapy, to be an independent risk factor for infections due to long-dwelling CVCs ([51]). Since all of our patients with CRBSI had neutropenia (mean duration of 17 days) this risk factor might also contributed to the development of CRBSI in our study patients.

Previous studies reported that performing an AOLC test costs about 9.87€ (dressing pack, syringes, needles etc., nurse/15 minutes, laboratory technician/20 minutes, microbiologist /5 minutes) ([41]). The costs for analysing EDTA blood samples by Gram/AOLC test during the average observation time of 37 days are 157€ for each patient. In our study 8 of 32 patients had a CRBSI. The detection rate of our screening method was 50%. The medical costs of 18500-26500€ for each case of CRBSI ([119,120]) compared to the screening cost of 1256€ to detect one patient at risk for CRBSI (one out of 8 patients in our study) is on average a 18-fold increase of debit for the health care system.

Curriculum Vitae

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Academic and educational studies

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10-2001:	Bachelor of Molecular Biology Karl Franzens University of Graz, Austria
09-1993 - 06-2001:	general qualification for university entrance: "Matura" Certificate, passed with distinction undergraduate institution: private grammar school of Sacré Coeur, Graz, Austria
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Clinical practise during academic studies

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08-2005 - 08-2005:	Clinical elective (CE) Department of Internal Medicine Hospital of BHB, Graz, Austria
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Experience of working

02-2008 - 07-2008:	Clinical research Department of Pulmonology University Clinic of Internal Medicine, Graz, Austria prospective clinical study
10-2002 - 10-2006:	job/ gastronomy Tea and Coffeeshop Heißenberger GesmbH, Graz, Austria
2004 - 2006:	job/ Airport Catering Service Airport, Graz, Austria
07-2002 - 08-2002:	job/ catering service Grindelwald, Switzerland
08-2001 - 09-2001:	job/ catering service Dingle, Ireland

Compulsory optional subjects

Basic Medical English, Medical University of Graz, Austria
Problem based learning, Internal Medicine, BHB Graz, Austria
Clinical exercises in anaesthesia and emergency medicine aid,
University Clinic of Anaesthesia and Intensive Care Medicine, Graz, Austria
Basic Chiropractic Skills, Medical University of Graz, Austria

Languages

German (native)
English (fluent in spoken and written)
French (basics)
Italian (basics)
Latin

Computer skills

MS-Office
medical software

Interests and skills

Hobbies	diving, sailing, golfing, hiking, cooking, yoga

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