DISSERTATION

Biphasic peptide nucleic acid fluorescence in situ hybridization and acridine orange leucocyte cytospin staining for anticipative diagnosis of central venous catheter related bloodstream infections

submitted by

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Declaration

I hereby declare that this dissertation is my own original work and that I have fully acknowledged by name all of those individuals and organizations that have contributed to the research for this dissertation. Due acknowledgement has been made in the text to all other material used. Throughout this dissertation and in all related publications I followed the guidelines of “Good Scientific Practice”.

Graz, 20.11.2014

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1. Abbreviations and Definitions

AOLC: Acridine Orange Leucocyte Cytospin

CFU: Colony Forming Units

CNS: Coagulase Negative Staphylococci

CRBSI: Catheter Related Bloodstream Infection

CRP: C-reactive Protein

CVC: Central Venous Catheter

DTP: Differential-Time-To-Positivity

FISH: Fluorescence in-situ Hybridization

GvHD: Graft versus Host Disease

HD: Hämodialyse/ hemodialysis

HO: Hämatoonkologie/ hematooncology

HSCT: Hematopoietic Stem Cell Transplantation

KAST: Katheteraustrittsstelle

MRSA: Methicillin-resistant *Staphylococcus aureus*

NPV: Negative Predictive Value

PNA FISH: Peptide Nucleic Acid Fluorescence in situ Hybridisation

PPV: Positive Predictive Value

rep-PCR: repetitive-sequence-based Polymerase Chain Reaction

rRNA: ribosomal Ribonucleic acid

TTP: Time-To-Positivity

ZVK: Zentralvenenkatheter

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4. Abstract in German

Hintergrund:

Aktuell werden nur bei Verdacht auf eine Katheter-assoziierte Infektion (CRBSI) diagnostische Schritte eingeleitet. Diese prospektive Studie soll den Einsatz eines regelmäßigen Screenings bei Hämodialyse- (HD) und hämatoonkologischen (HO) Patienten mit zentral-venösem Katheter (ZVK) *in situ* zur frühzeitigen Diagnose einer CRBSI evaluieren.

Methoden:

Insgesamt wurden 237 Patienten (55 HD, 182 HO) mit 402 (60 HD, 342 HO) Katheterperioden untersucht. Drei Mal pro Woche wurden 3 mL EDTA Blut von beiden Katheterschenkeln der HD Patienten und täglich vom distalen Schenkel der HO Patienten gesammelt. Als Screeningtests wurde der universale PNA FISH Test und die AOLC Färbung durchgeführt. Eine quantitative Kultur auf Schokoladen-Agar diente als Kontrolle. Bei klinischem Verdacht auf eine CRBSI, wurden diagnostische Maßnahmen unabhängig von den Ergebnissen des Screenings angeordnet. Die behandelten Ärzte waren hinsichtlich der Screening-Ergebnisse verblindet.

Resultate:

Insgesamt wurden 19 CRBSIs (2 HD, 17 HO) mit Routine-Tests detektiert, resultierend in einer CRBSI-Rate von 1.9/1000 Kathetertagen (HD 0.5/1000, HO 2.6/1000). Die 2 HD CRBSIs konnten im Screening durch kontinuierlich positive PNA FISH- und AOLC Ergebnisse 7 und 8 Tage früher detektiert werden, bevor die Routinetests positiv wurden. Bei den HO Patienten konnten 2 CRBSIs mittels positiven universalen PNA FISH Test und 5 durch positive AOLC Färbung 2 Tage früher diagnostiziert werden. Die Sensitivität und Spezifität des universalen PNA FISH Tests waren 100% HD/12% HO und 95% HD/98% HO, der PPW und NPW

waren 40% HD/22% HO und 100% HD/95% HO. Die Sensitivität und Spezifität der AOLC Färbung waren 100% HD/29% HO und 96% HD/97% HO, der PPW und NPW waren 50% HD/33% HO und 100% HD/97% HO.

Konklusion:

Screening auf CRBSI in HD Patienten scheint sinnvoll, jedoch auf Grund der geringen CRBSI-Rate sind noch weitere Studien notwendig. In HO Patienten scheint das Screening auf CRBSI nicht sinnvoll und kosteneffizient zu sein. Gründe für falsch negative Ergebnisse inkludieren einen CRBSI Ursprung in einem anderen nicht-gescreenten Katheterschenkel. Gründe für falsch positive Ergebnisse inkludieren eine Katheterkolonisation ohne hämatogene Streuung.

5. Abstract in English

Background and objectives:

Currently, routine diagnostics for detection of catheter-related bloodstream infection (CRBSI) is performed only in patients with clinical signs of infection. This prospective trial was undertaken to evaluate a possible value of screening for CRBSI in hemodialysis (HD) and hematooncological (HO) patients with central venous catheters (CVCs) *in situ*.

Methods:

In this study, 237 patients (55 HD, 182 HO) with 402 (60 HD, 342 HO) catheter periods were investigated. Three times per week EDTA blood was drawn from both lumina in HD patients and daily from the distal catheter lumen in HO patients prior to connection to hemodialysis (HD) or routine blood sampling (HO). Screening for CRBSI was performed with the universal PNA FISH test and compared to the AOLC stain. Additionally, blood samples were cultured quantitatively on chocolate agar. If CRBSI was clinically suspected, routine investigations were performed. Attending physicians were blinded to the screening results.

Results:

Twenty CRBSI cases (2 HD; 17 HO) were detected by routine investigations resulting in a CRBSI rate of 1.9/1000 catheter days (HD 0.5/1000; HO 2.6/1000). In both of the HD CRBSI patients, infection could be anticipated 7 and 8 days before routine diagnosis by positive universal PNA FISH test and AOLC stain. In the HO patients, 2 CRBSIs could be anticipated by positive universal PNA FISH test and 5 by AOLC stain 2 days prior to routine diagnosis. Ten patients showed positive screening results but no clinical signs of CRBSI. The sensitivity and specificity of the universal PNA FISH test were 100% HD/12% HO and 95%

HD/98% HO, the PPV and NPV were 40% HD/22% HO and 100% HD/95% HO, respectively. The sensitivity and specificity of the AOLC screening were 100% HD/29% HO and 96% HD/97% HO, the PPV and NPV were 50% HD/33% HO and 100% HD/97% HO, respectively.

Conclusion:

Screening for CRBSI in HD patients seems to be useful but due to the small number of CRBSIs, further studies are needed. In HO patients, screening for CRBSI does not appear to be a useful and cost-efficient tool. Reasons for false negative results might include origin of CRBSIs from other lumina not sampled for screening. False positive results might origin from catheter colonization without subsequent spread of microorganisms into the peripheral bloodstream.

6. Introduction

Central venous access is widely used to administer intravenous fluids, medications, blood products, parenteral nutrition, to monitor hemodynamic status, and to provide renal replacement therapy (*e.g.* hemodialysis and -filtration). Despite the advantages of central venous access, central venous catheters (CVCs) also carry a high risk for local or systemic infections including catheter related bloodstream infections (CRBSI), possibly with subsequent endocarditis or other metastatic infections (1-3).

Diagnosis of CRBSIs remains challenging as systemic clinical signs are unspecific and local signs of infection are often absent. Currently measures for diagnosis of CRBSI are performed when CRBSI is clinically suspected. Thus, patients may actually suffer from CRBSI and are at risk to concurrently suffer from or develop complications like endocarditis or septic embolism when diagnostic procedures for the detection of CRBSI are introduced. Therefore the hypothesis arises if CRBSI can be anticipated in a subclinical stage of disease, enabling earlier establishment of therapeutic measures, which may prevent clinically evident CRBSI resulting in a reduction of morbidity, mortality and costs. This study was conducted to evaluate the prognostic value of the universal peptide nucleic acid fluorescence in situ hybridization (PNA FISH) test and the AOLC stain regarding anticipation of CRBSI in hemodialysis and hematooncological patients with CVCs *in situ*.

6.1. Central Venous Catheters (CVCs)

Usually peripheral access is attempted first, but if this is not possible due to *e.g.* poor peripheral venous status or if larger amounts of fluids have to be infused, certain medications are administered or renal replacement therapies are applied, CVCs are widely used (4). Compared to peripheral catheters, CVCs are much longer and reach far deeper into the major veins of the body to provide a secure and durable intravenous access (5).

6.1.1. Indications

Multiple indications for implantation of CVC exist: peripheral veins are inaccessible, infusion of potent vasoactive drugs or irritating solutions, administration of parenteral nutrition, providing hemodialysis/-filtration, or hemodynamic monitoring (4).

Despite recommendations favoring arteriovenous fistulae, and efforts such as the 'Fistula First Initiative', CVCs are still increasingly used for hemodialysis, although they carry a high risk for local or systemic infections (6-8).

With the exception of totally implanted central venous access (*e.g.* Port-a-caths) no administration of systemic antimicrobial prophylaxis is recommended prior to CVC insertion to prevent catheter colonization (3).

6.1.2. Contraindications

Because CVCs may be lifesaving, there are no absolute contraindications. Coagulopathies (with an increased risk of hemorrhage), morbid obesity (more difficult and dangerous implantation), cutaneous burn or infection on the chest wall (different locations for implantation possible), or venous thrombosis on the planned catheterization site are relative contraindications for implantation of CVC and may lead to a more complicated placement procedure (4).

6.1.3. Classification

CVCs can be classified according to different aspects (3):

1) Site of insertion (*e.g.* subclavian, femoral, internal jugular)
2) Pathway from skin to vessel (*e.g.* tunneled, non-tunneled)
3) Physical length (*e.g.* short, long)
4) Special characteristics of the catheter (*e.g.* cuff, impregnation, number of lumen)

Long-term CVCs are usually tunneled to provide a barrier against microorganism, implanted in a surgical setting to form the tunnel, and are cuffed to stay in place and be less likely to the be pulled out accidentally (Figure 1) (2). Non-tunneled CVCs are usually for short-term use (Figure 2).

Figure 1: Picture of Medcomp Splith Cath® (Medcomp® Harleysville, PA) long-term hemodialysis catheter.

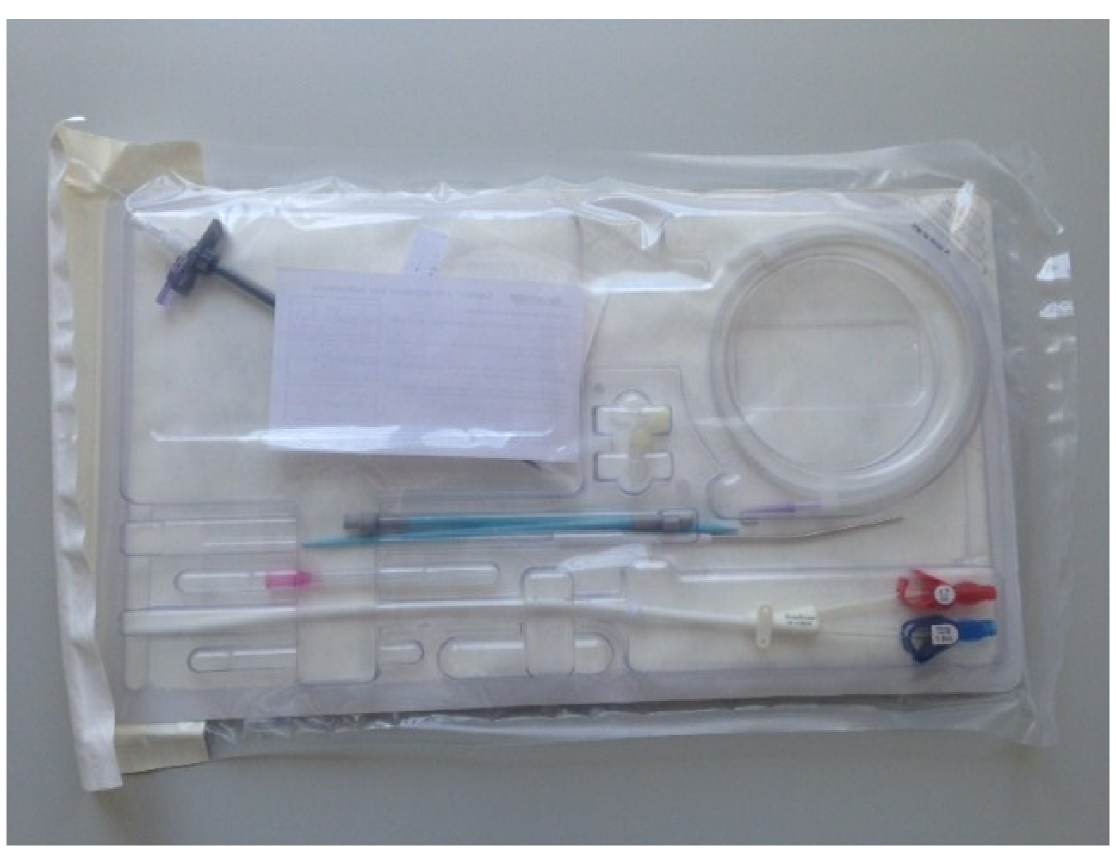
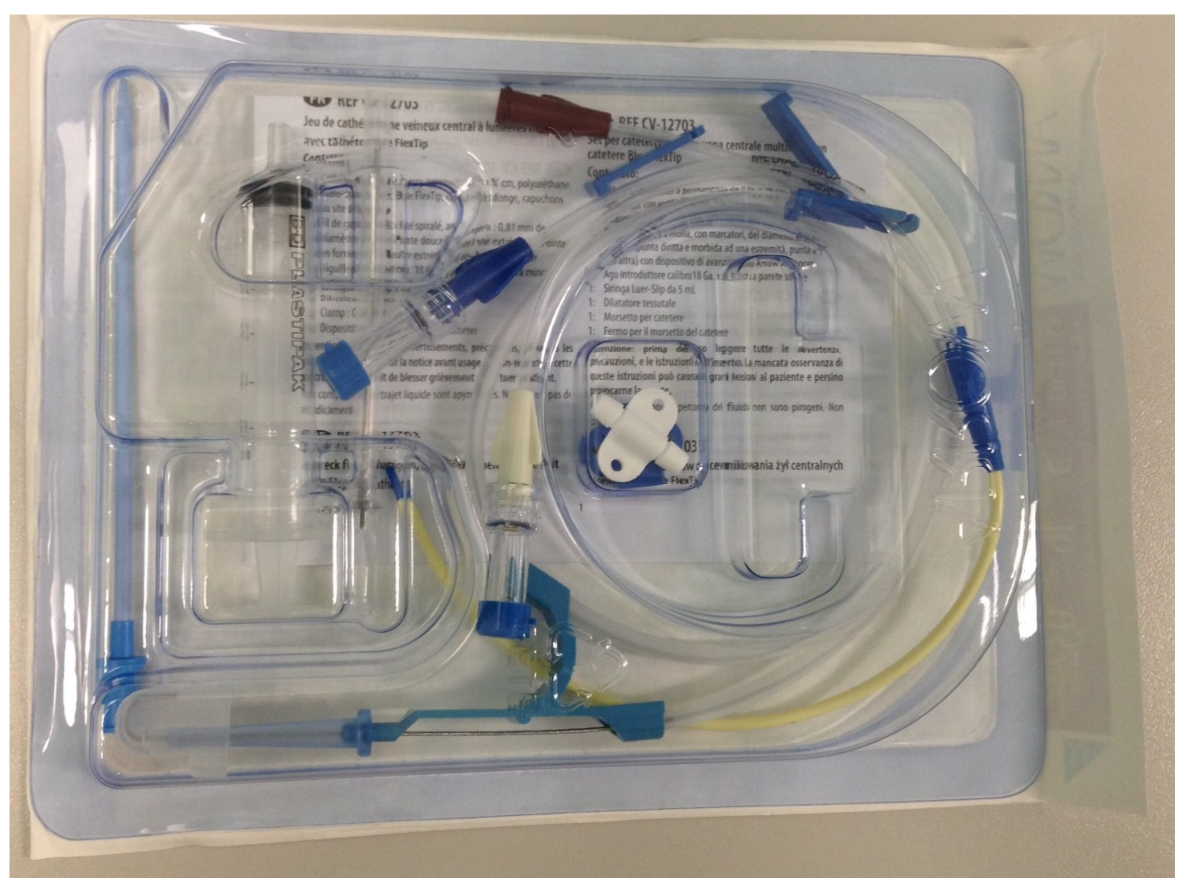


Figure 2: Picture of the triple-lumen Arrow international catheter® (Arrow-Howes; Arrow International, Reading, Pa.) for short-term non-tunneled central venous access.



6.1.4. Special Types of CVCs

Certain CVCs are coated or impregnated with antimicrobial or antiseptic agents. These devices can decrease the risk for CRBSI and potentially decrease costs associated with treating CRBSIs, despite the higher costs of an antimicrobial/antiseptic impregnated catheter (9). According to the literature, catheters with chlorhexidine/silver sulfadiazine and minocycline/rifampin coating or impregnation prevent CRBSIs (10,11), whereas the results of studies using catheters impregnated with platinum/silver showed reduced efficacies (12-14). Anaphylaxis with the use of these chlorhexidine/silver sulfadiazine catheters has been observed in rare cases (15-17). According to the literature, several prospective clinical studies have shown that the risk for development of resistance due to these CVCs seems to be low (18).

6.1.5. Materials

CVCs can be made of different materials like Teflon®, silicone elastomer, polyurethane, polyvinyl chloride, or polyethylene. It has been shown that devices made of Teflon®, silicone elastomer, or polyurethane showed lower adherence of microorganisms than other materials including polyvinyl chloride or polyethylene (19,20).

Some catheter materials have surfaces with irregularities leading to enhanced microbial adherence of certain species including *Candida albicans* (20). Additionally, certain catheter materials demonstrated higher thrombogenic potential than others, a possible predisposition for CVC colonization and CRBSI (21).

6.1.6. Implantation

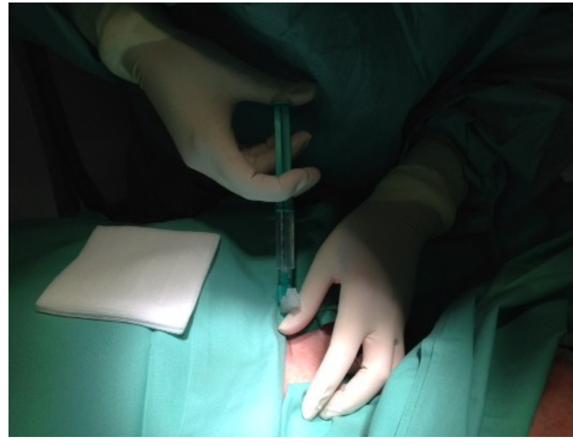
The modified Seldinger technique is actually used as the standard approach for implantation of CVCs. After local anesthesia, subclavian venous catheterization is performed under aseptic conditions, patient's local skin is disinfected, and full sterile barrier precautions are used including full-length sterile drapes, gowns, caps, masks, and gloves. The patient is placed in the supine position, with the head and shoulder in the neutral position or turned to the contralateral site according to the insertion site (4).

For CVC implantation in the subclavian vein, the skin is punctured at 2–3 cm below the clavicle, along the mid-clavicular line, toward the upper border of the suprasternal notch; the puncture needle is advanced until the right subclavian vein is punctured. Puncture of the vein is signaled by a rapid flush of venous blood into the barrel of the syringe filled with saline. If the vein is not punctured before reaching the predetermined depth of needle insertion, the needle is withdrawn slowly along the same pathway. Often a flush of venous blood will occur during the withdrawal. In this case the needle might have penetrated the anterior and posterior vein wall. Then the guidewire is set in place and cautiously the needle is fully withdrawn. The subcutaneous tissue is dilated with a scalpel or a dilatator, the catheter is advanced over the guidewire into the right position and the guidewire

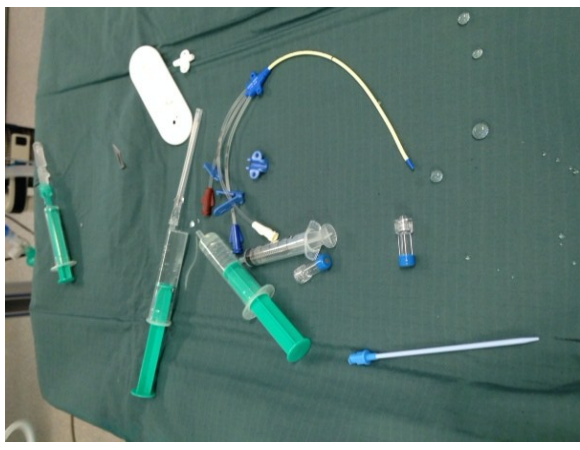
fully removed. If the catheter is properly positioned (confirmed by *e.g.* c-arm x-ray unit), blood should be easily aspirated and injected from and into each of the catheter lumen. Finally the catheter is secured to the skin with sutures or staples, and a sterile dressing is applied (4).

A chest radiograph is obtained to check for proper position and potential complications such as pneumothorax. The patient should be monitored for erythema, pain, swelling, wound dehiscence, or ulceration after implantation (Figure 3) (4).

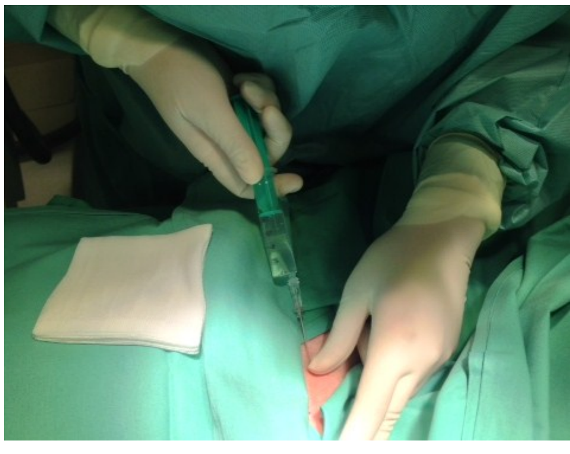
Figure 3: Implantation of a non-tunneled short-term CVC.



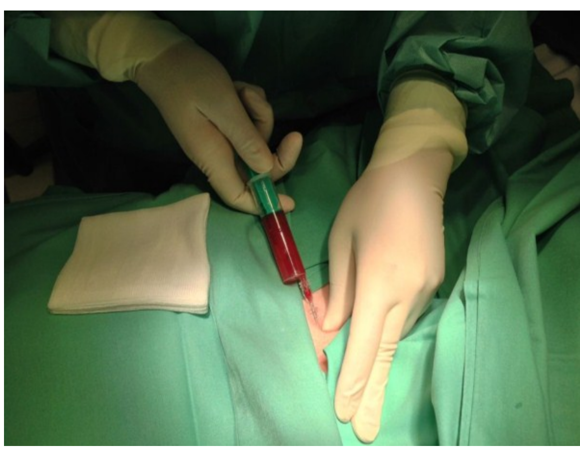
(A) Under aseptic condition, full sterile barrier and after disinfection of the patient's skin, the local anesthesia is injected.



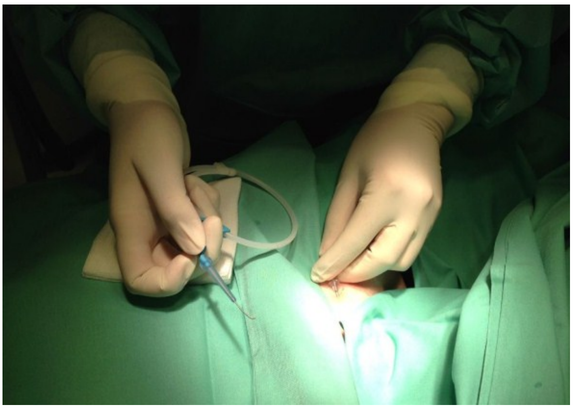
(B) Different parts of the CVC implantation kit on an aseptic table.



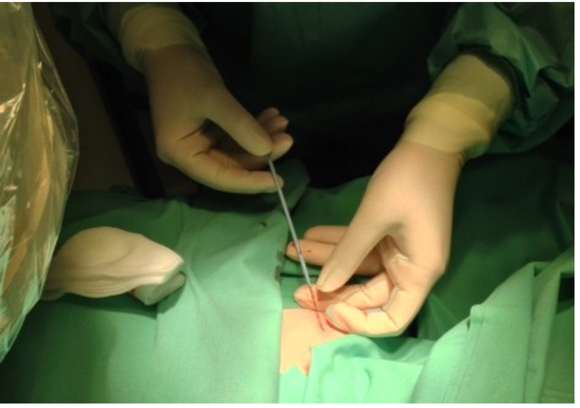
(C) Skin puncture along the mid-clavicular line. The needle is advanced until the subclavian vein is punctured.



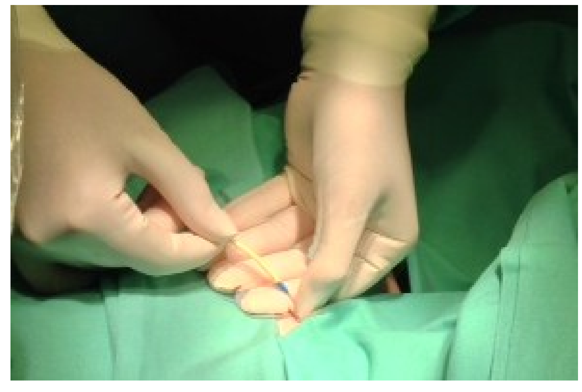
(D) Puncture of the subclavian vein is signaled by a rapid flush of venous blood in the barrel of the syringe.



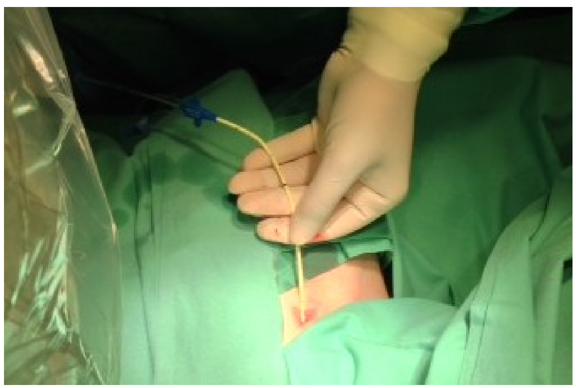
(E) The guidewire is set in place.



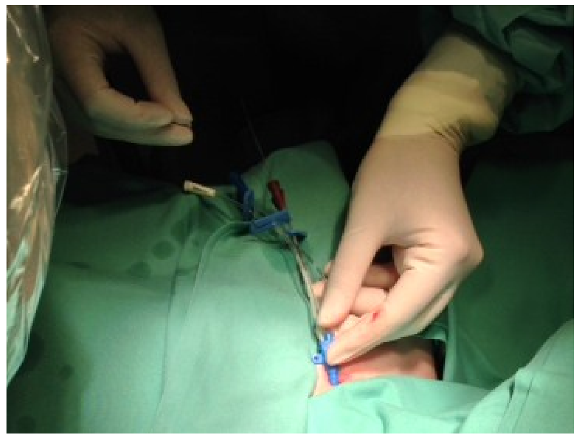
(F) Subcutaneous tissue is dilated with a dilatator.



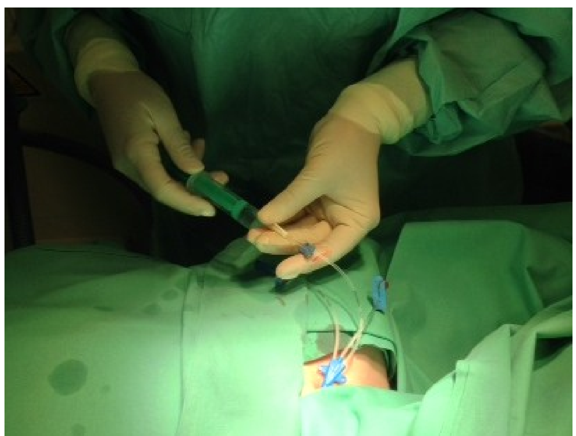
(G) Catheter is advanced over the guidewire.



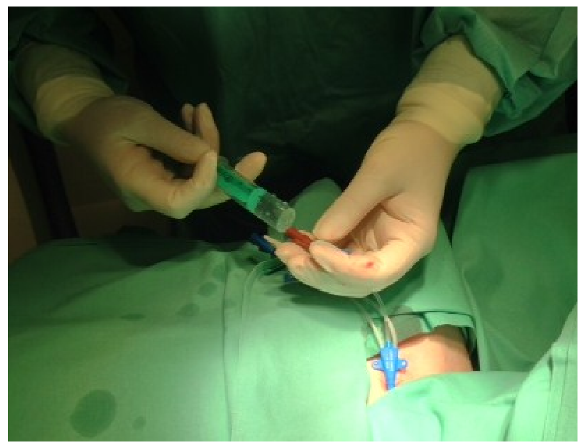
(H) Catheter is positioned correctly.



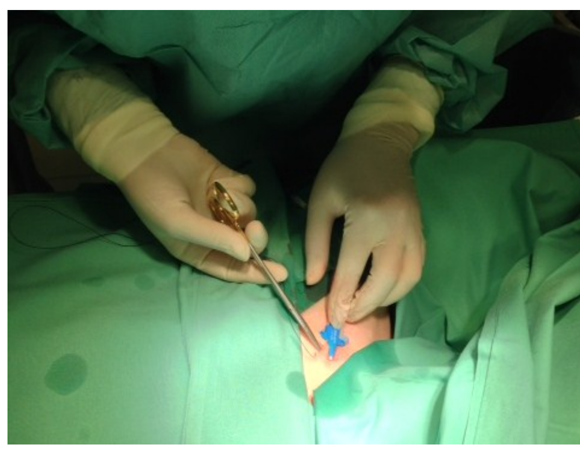
(I) Cautious removal of the guidewire.



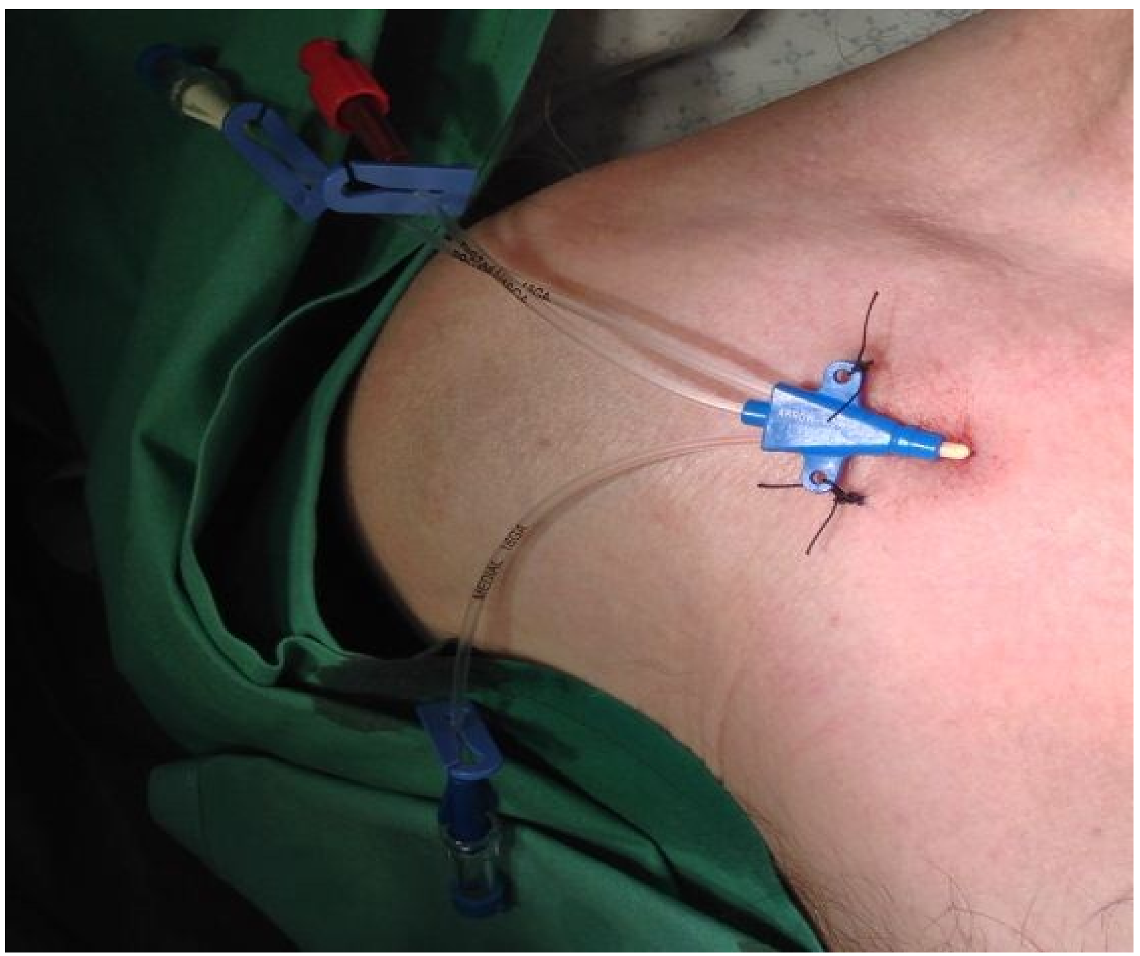
(J) All catheter lumen are checked for correct aspiration.



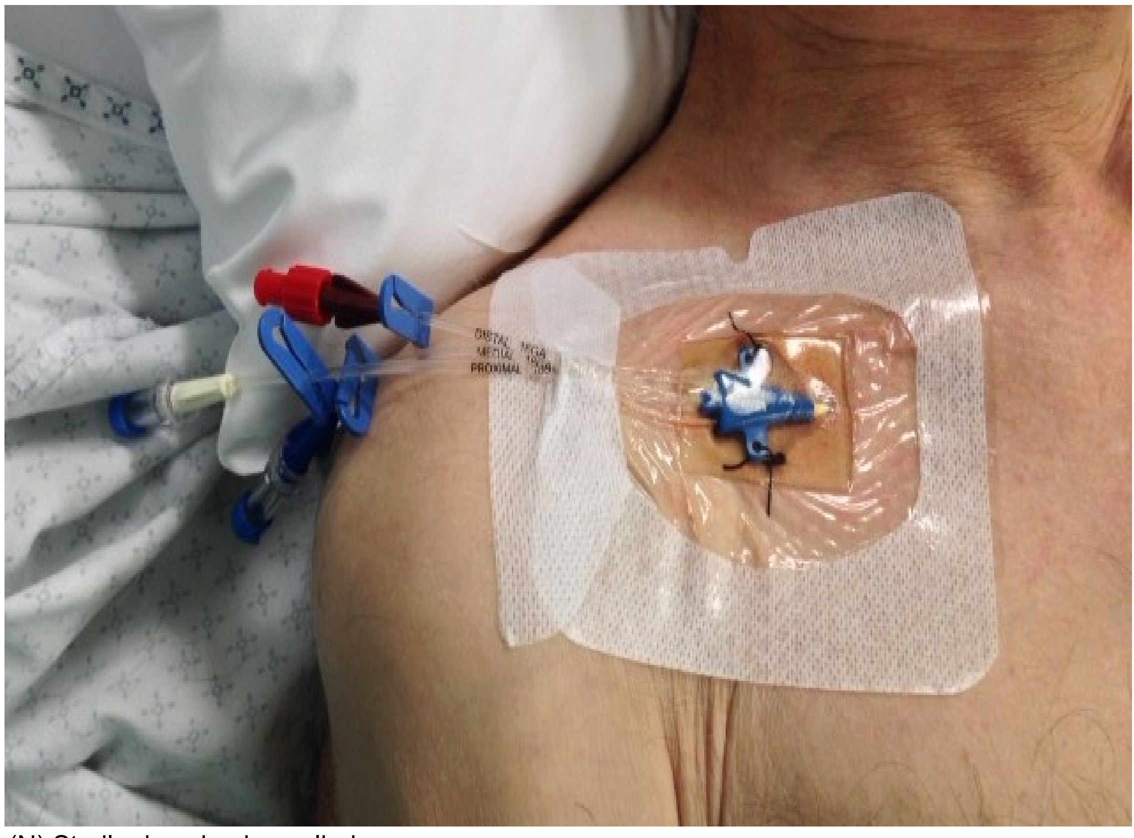
(K) And all catheter lumen are also checked for correct injection.



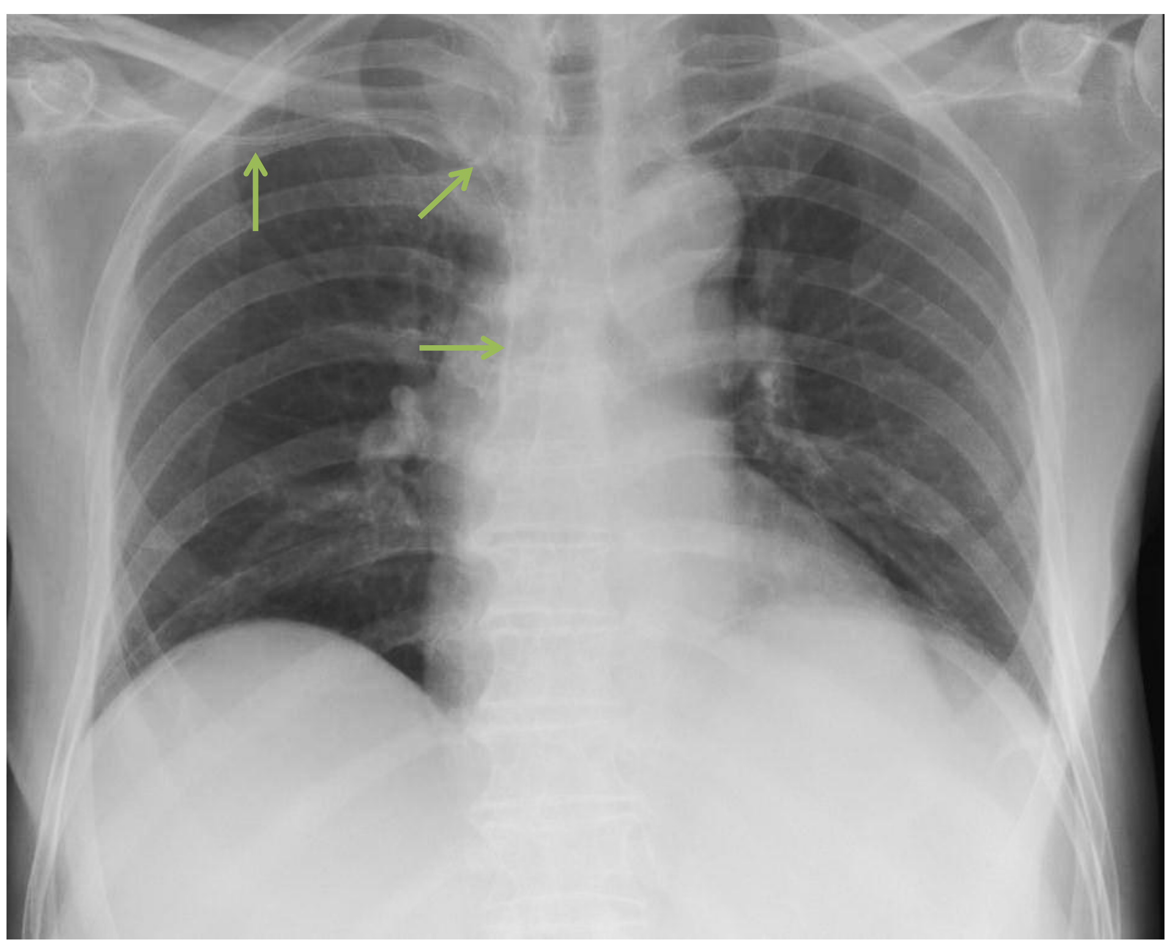
(L) The catheter is secured with two skin sutures.



(M) Implanted 3-lumen non-tunneled short-term catheter secured with skin sutures.



(N) Sterile dressing is applied.



(O) Finally chest radiograph is made to confirm proper position (marked with green arrows) and to check for potential complications such as pneumothorax.

6.1.7. Complications

In 15% of CVC implantation complications occur including mechanical complications (e.g. subclavian artery puncture, subclavian vein laceration, subclavian vein stenosis, hemothorax, air embolism, hematoma, or pneumothorax), venous thrombosis, or CRBSI (3,4).

6.2. Subcutaneous Venous Access Devices or “Ports”

Subcutaneous venous access devices or “ports” are regularly used in the care of patients with chronic diseases. A port is a totally subcutaneous implanted central venous access, which is accessed by transcutaneous puncture using special port needles. A port provides reliable access to central veins for blood withdrawal and administration of medication (6).

6.2.1. Indication

The main indication for port implantation is administration of chemotherapy or transfusion on a weekly or monthly basis in a patient with extremely poor peripheral venous status. The main advantage of a port in contrast to a CVC is the possibility for patients to have normal daily life activities including taking a bath, swimming, and exercise. Additionally, an approximately 10-fold decreased risk for infection compared to CVCs has been observed (6,22).

6.2.2. Contraindications

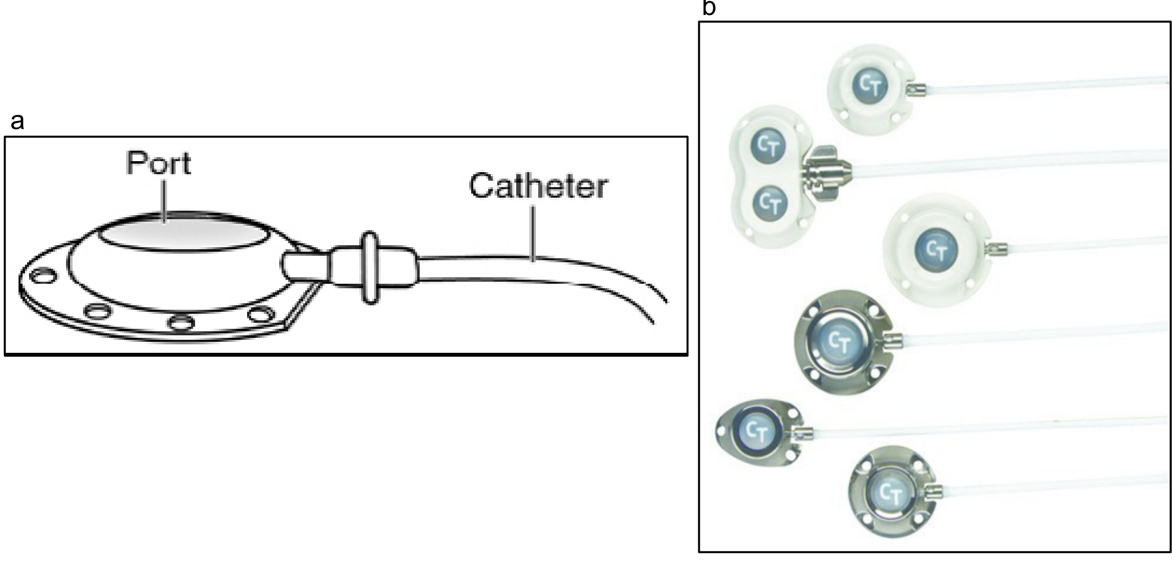
Contraindications for port placement are: 1) severe uncorrectable coagulopathies (leading to extensive ecchymosis); 2) positive blood cultures or uncontrolled sepsis (must be treated prior to the implantation of the port due to potential colonization of the port); 3) conditions in which the port cannot be positioned subcutaneously in the chest wall, *e.g.* burns, trauma, or neoplasm. In this case an alternative position can be used like the arm or the abdomen; 4) cystic fibrosis patients frequently require chest percussive therapy (so an alternate position should be used); and 5) cachectic and malnourished patients (the subcutaneous port will not heal in or quickly erode through the fragile skin layer) (6).

6.2.3. Materials

The latest ports are light, radiolucent, magnetic resonance imaging-compatible, power-injectable (i.e. for high pressure and flow used for power contrast injection, *e.g.* during contrast-enhanced CT) (23). The newest used materials are either cathoflex (polyurethane) or silicone (polysiloxanes). Cathoflex has the advantage of greater wall strength for a greater inner lumen; whereas silicone is softer and more comfortable for the patient (6).

Ports are available as single or double-chamber ports, with or without Groshong valve (2). The decision for or against certain models depends on the indication.

Figure 4: Schematic design and picture of totally implanted ports.



(a) The left schematic design shows the structure of a subcutaneous totally implantable port used for central venous access. (24)
(b) On the right side, several commercially available ports with different materials, sizes, shapes, and numbers of chambers are displayed. (25)

Fig. 5: Schematic design of use and function of a totally implanted port.

The schematic design shows the use and function of a totally implanted port. An access needle is used to puncture the port via the septum for aspiration and/or injection of fluids. The port is connected to the catheter, placed in the *Vena subclavia* for central venous access. (26)

6.2.4. Implantation

Implantation of the port is performed under aseptic conditions, full sterile barrier precautions, and after disinfection of the patient’s local skin. A moderate intravenous sedation is applied to the patient. After the subclavian vein is punctured ultrasound-guided, a port pocket and tunnel is surgically created for insertion and connection of the port to the catheter. The function of the port is controlled and it’s position checked by a c-armed x-ray unit. When aspiration and injection is verified, the port is locked with anticoagulants and finally the port pocket is closed with sutures and a sterile dressing.

Immediately after implantation the port can be used but the patient has to be monitored for erythema, pain, swelling, wound dehiscence, or ulceration.

Figure 6: Position of a totally implanted port on the human thorax.

Thorax x-ray shows a double chamber port on the right side of the thorax.

There is no consensus concerning antibiotic prophylaxis prior to port placement (6). However, in high-risk or immunocompromised individuals the application of 1g intravenous cefazolin is recommended. In case of penicillin allergy equivalent doses of clindamycin or vancomycin can be used (27). Ports should be flushed after each use or on a monthly basis with anticoagulants or saline if the port is inactive (6).

6.2.5. Complications

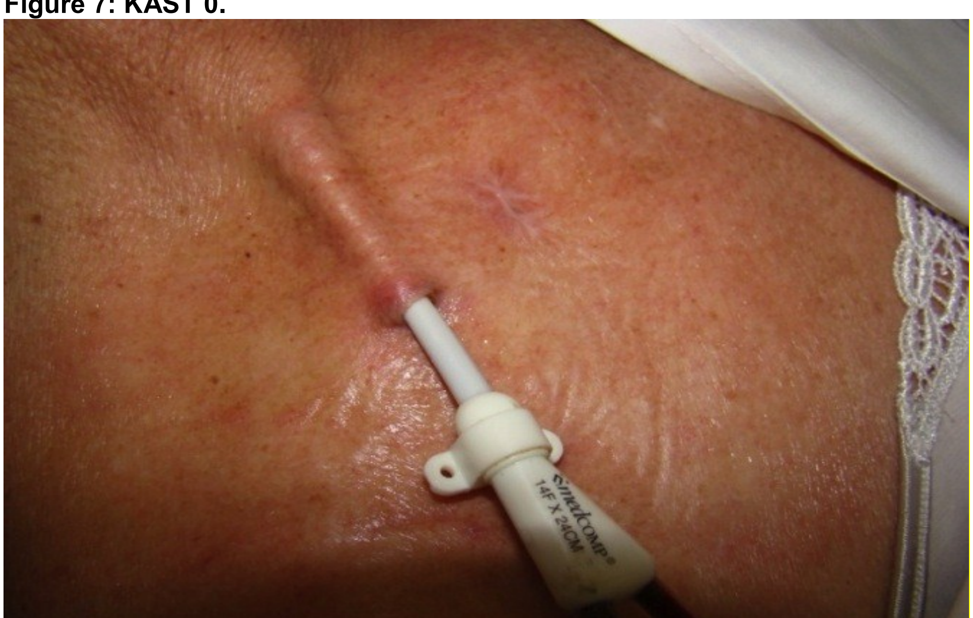
Complications are rare: 1.3% periprocedural (*e.g.* venous thrombosis, hemorrhages, arterial puncture, cardiac arrhythmia); 3.3% early (within the first 30 days) but the majority are late onset complications (>30 days) in 9.4% such as CRBSI, catheter-induced venous thrombosis, and catheter migration (28).

6.3. Local infectious catheter complications

Local catheter infections are defined as an exit site infection, tunnel infection, or pocket infection and are characterized according the presence of induration, erythema, warmth, pain, and tenderness at/or around the catheter exit site. An association between local catheter infection and CRBSI has been identified, but they can also occur independently (2,29,30).

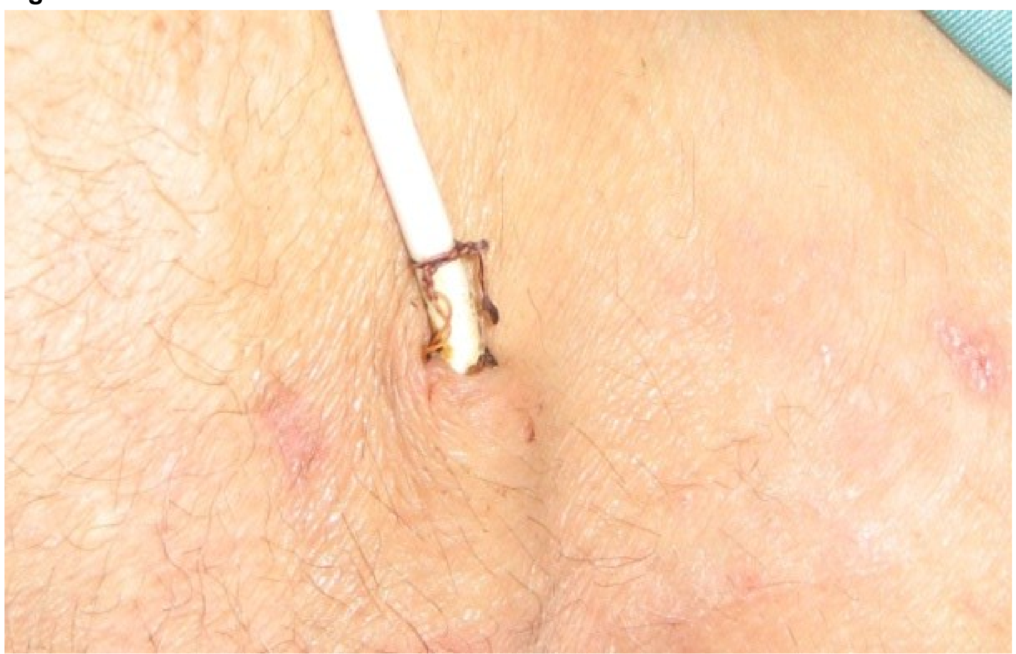
According to the Guidelines of the “Gefäßzugang zur Hämodialyse – Empfehlungen der Arbeitsgruppe Pflege (GHEAP)”, a classification of the condition of the catheter insertion site was developed and adapted for our hospital, the Medical University of Graz. This classification is called “KAST” for “Katheteraustrittstelle” and ranges from 0 (normal catheter insertion site) to 5 (local infection of the catheter insertion site including tunnel infection). Detailed description and pictures of KAST 0-5 are presented below (Figure 7-12) (31,32).

Figure 7: KAST 0.



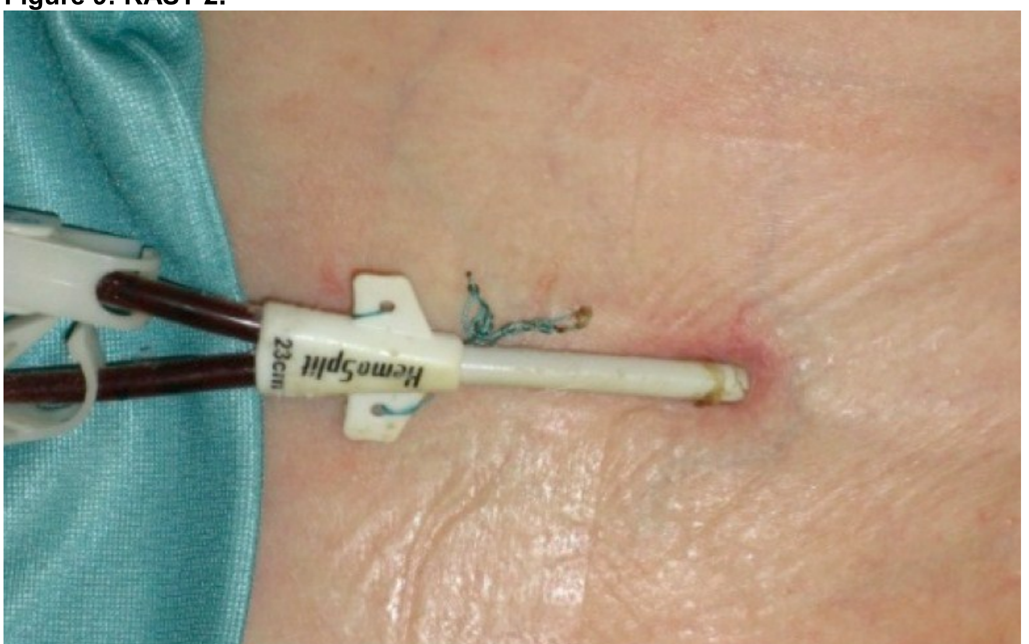
Picture of a normal inconspicuous CVC insertion site, KAST 0 (provided by Dr. Gernot Schilcher).

Figure 8: KAST1.



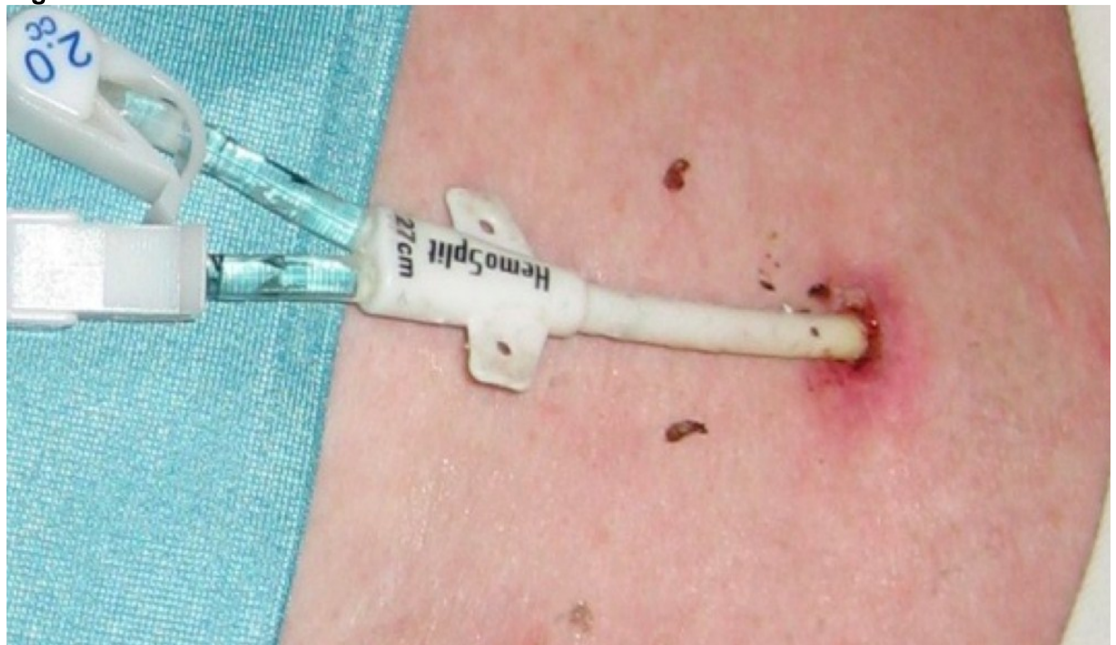
Inconspicuous condition of the skin at the catheter insertion site, mild redness < 0.5 cm, no secretion. If necessary the dry incrustation may be removed, KAST 1 (provided by Dr. Gernot Schilcher).

Figure 9: KAST 2.



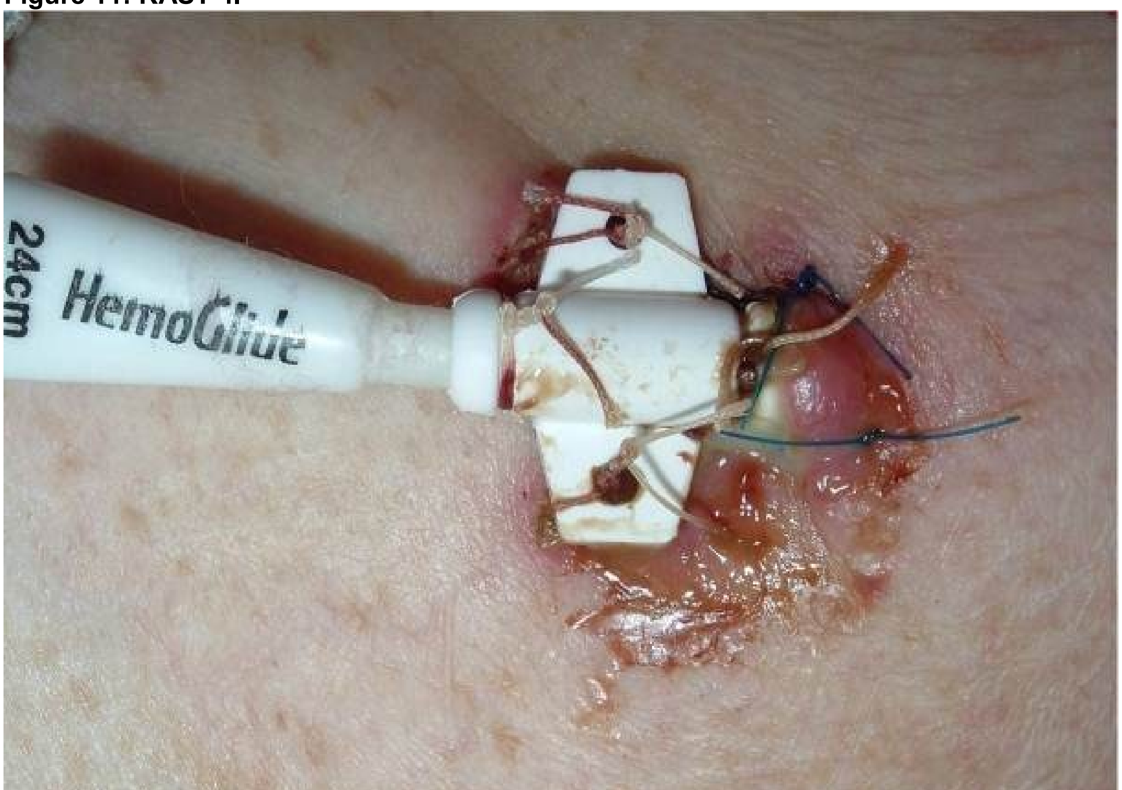
Moderate redness > 0.5 cm, mild clear secretion, no purulence, no pain or tenderness on palpation at the catheter insertion site, KAST 2 (provided by Dr. Gernot Schilcher).

Figure 10: KAST 3.



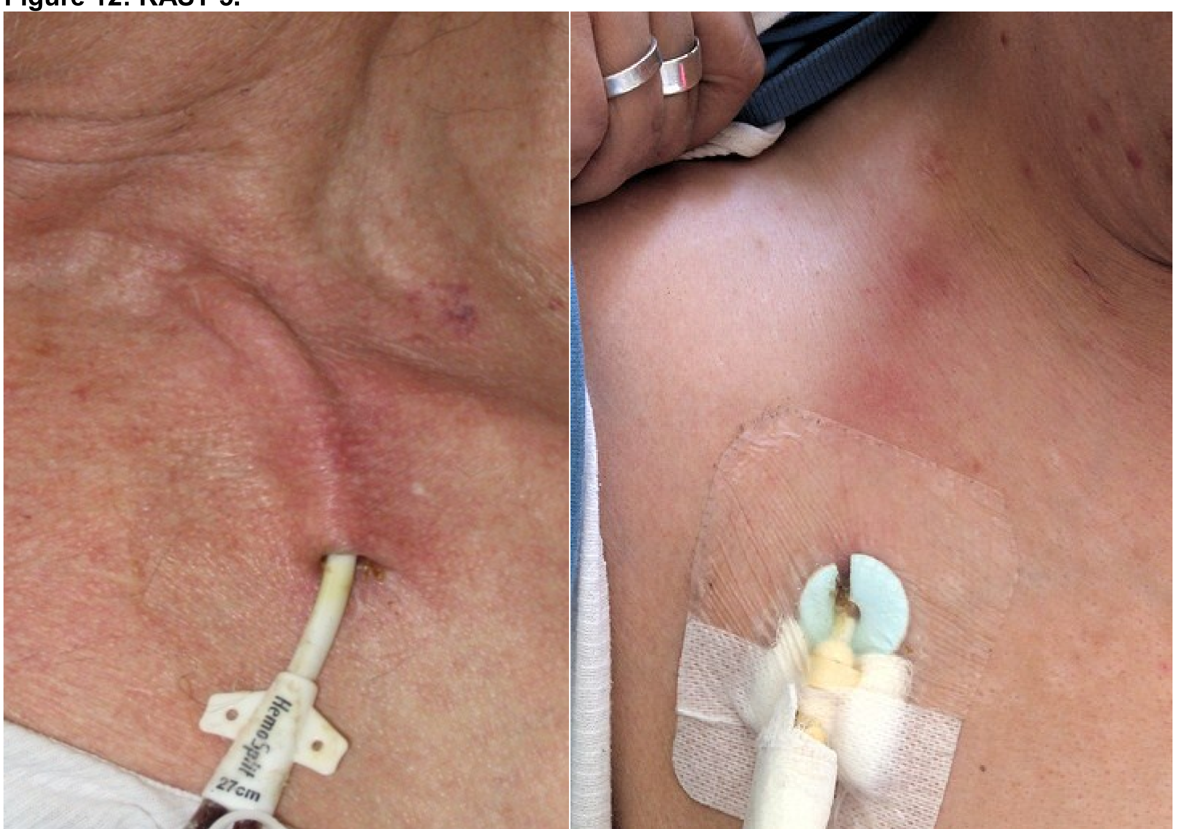
Considerable redness > 0.5 cm, considerable secretion with incrustation, and possible purulence, KAST 3 (provided by Dr. Gernot Schilcher).

Figure 11: KAST 4.



Considerable redness > 0.5 cm, florid purulence, in most cases pain and/or tenderness on palpation, KAST 4 (provided by Dr. Gernot Schilcher).

Figure 12: KAST 5.



Combination of KAST 4 (considerable redness > 0.5 cm, florid purulence, pain and/or tenderness on palpation) with redness along the catheter tunnel, KAST 5 (provided by Dr. Gernot Schilcher).

This KAST-classification is routinely used in our hemodialysis ward for a standardized evaluation of the catheter insertion site. KAST is evaluated prior to each hemodialysis and in case of catheter dressing changes. Results are included in the hemodialysis monitoring database. With the help of this KAST-classification a regular objective evaluation of the CVC insertion site is available for each member of the nursing staff and attending physician for each patient with CVC *in situ*. Changes of KAST can quickly be recognized for a rapid adaptation of therapeutic strategies as well as CVC care.

6.4. Central venous catheter related bloodstream infection (CRBSI)

6.4.1. Definition

CRBSI is a clinical definition for identifying the catheter as the source for a bloodstream infection and requires specific laboratory testing (*e.g.* Differential-time-to-Positivity) (3). Still, diagnosis remains challenging as local signs of infection like purulent secretion, pain, or tenderness are often absent and systemic signs like fever, chills, and hypotension are unspecific (33-36).

The incidence of CRBSI depends on various factors like the type of catheter, frequency of catheter manipulation (*e.g.* administration of fluids, drugs, and blood products; drawing of blood samples), and patient-related factors (*e.g.* underlying disease, neutropenia) (3).

6.4.2. Epidemiology

An estimated number of 250000 CRBSIs occur annually in the USA with an attributable mortality ranging from 12% to 25% and added costs of up to 56000 USD per case, resulting in 14 billion USD for CRBSIs in the USA (2,3,19,22,37-40).

Detailed epidemiological data for Austria is missing. According to the numbers from the USA and calculated on the total population of Austria of 8.4 million, an estimated number of 6690 CRBSIs occur in Austria *per anno*.

6.4.3. Pathogenesis

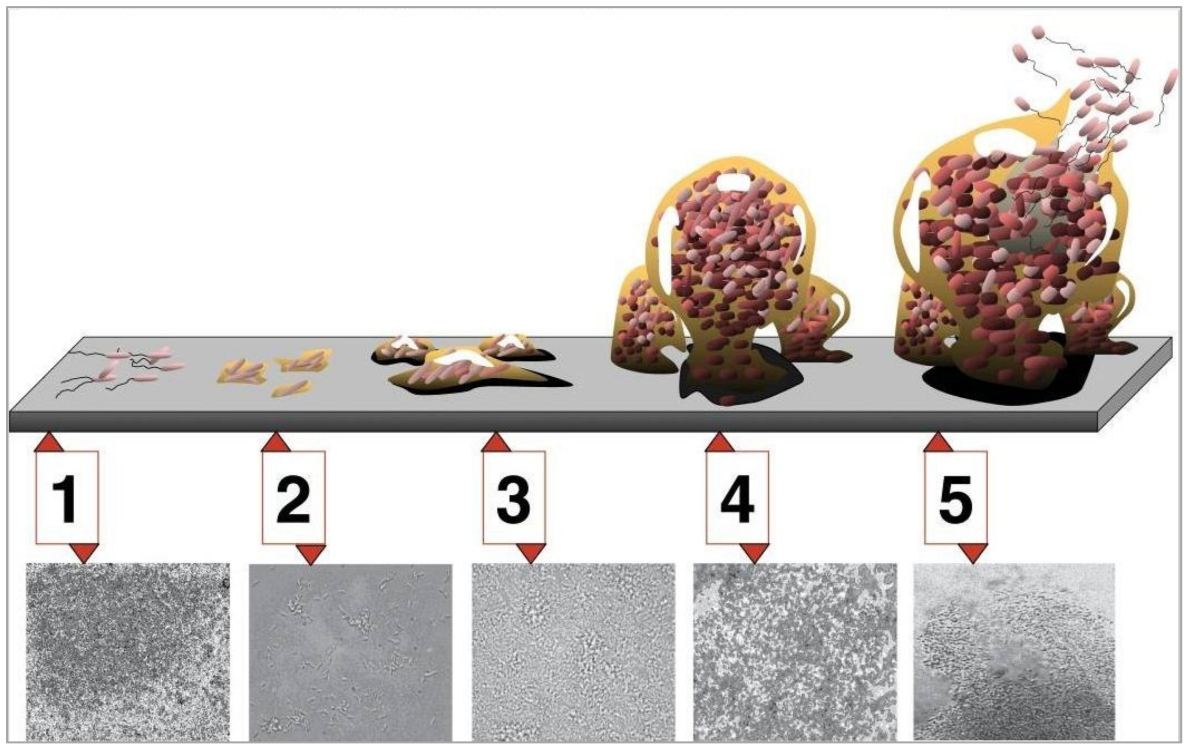
Four different routes for the development of CRBSI are described: 1) migration of skin organisms at the insertion site into the cutaneous catheter tract with colonization of the catheter tip (3,41,42); 2) contamination of the catheter hub leading to an intraluminal colonization, especially in long-term catheters (43), 3) hematogenous seeding from another focus of infection and rarely, 4) infusate contamination (44).

The pathogenesis is a dynamic process: from adherence to colonization and finally to the development of CRBSI. Detailed pictures of this dynamic process of biofilm development are available in Figure 13, page 35. This interaction primary depends on 4 main factors: 1) microbial factors / intrinsic virulence factors, 2) host factors, 3) catheter material, and 4) iatrogenic factors (3,45).

Microorganisms have different strategies to attach to a catheter and also to evade the patient's immune system and antimicrobial agents. The hydrophobic characteristics facilitate the microorganism to adhere to the CVC surface. Additionally, some pathogens can produce extracellular "slime" or exopolysaccharides to form a microbial biofilm. This biofilm protects the microorganism and supports more resistance to various microbial agents.

A thrombin sheath is formed by various host-derived proteins on the intra- and extraluminal site of the CVC including fibrin and fibronectin which become receptors for various microorganisms including *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Candida albicans* (3,20,45,46). By expression of clumping factors, *Staphylococcus aureus* can adhere to the protein adhesins (3,47,48).

Figure 13: Development of biofilm.

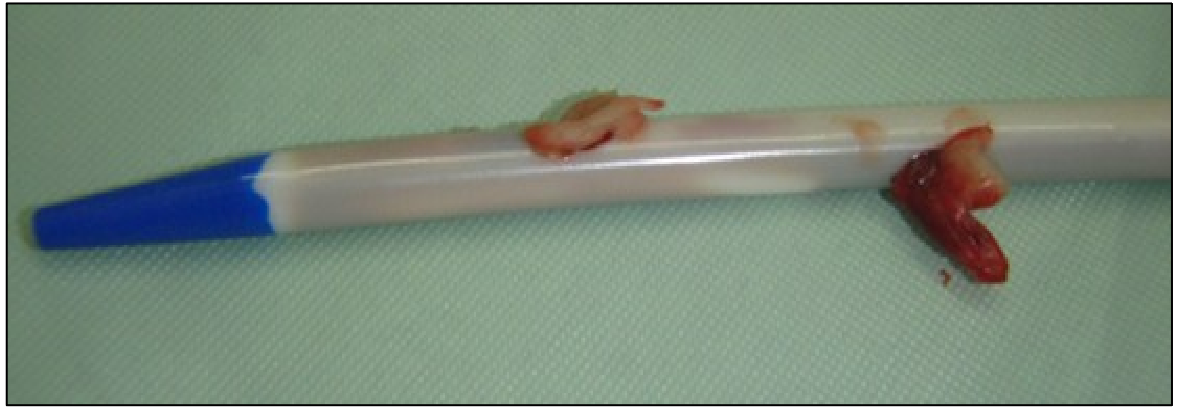


Stage 1: Initial attachment; Stage 2: Irreversible attachment; Stage 3: Maturation I, Stage 4: Maturation II, Stage 5: Dispersion. Each stage of development in the figure is paired with a photomicrograph of a developing *Pseudomonas aeruginosa* biofilm. All photomicrographs are shown to the same scale. (Image Credit: D. Davis) (49).

The physical characteristics of the CVC material also promote CRBSI including hydrophobicity, irregularities, defects, surface charges, as well as thrombogenicity (3,21,45). Currently the most effective method of preventing a CRBSI is the use of a CVC coated with antimicrobial agents (45).

Finally iatrogenic factors like total parenteral nutrition, long-term hemodialysis, blood products, and lipid emulsions may contribute to the development of CRBSI (45,50). It is known that the presence of dextrose-containing fluids markedly enhances the adherence of *Candida* spp., leading to an increased proportion of CRBSIs caused by fungal pathogens among patients receiving parenteral nutrition (3,50).

Figure 14: Picture of removed CVC responsible for CRBSI.



Distal segment of a removed CVC used for hemodialysis from a patient with CRBSI caused by *Staphylococcus aureus*. Thrombogenic formations appear through sideholes of the CVC tip (provided by Dr. Robert Krause).

6.4.4. Pathogens

The most commonly reported determined pathogens causing CVCs are coagulase-negative staphylococci (CNS) with about 44% of all CRBSI-cases, followed by *Staphylococcus aureus*, *Enterobacteriaceae*, enterococci, and *Candida* spp. (3,51,52).

Over the last years, a trend to an increase of CRBSI caused by multidrug-resistant pathogens including methicillin-resistant *Staphylococcus aureus (MRSA)*, vancomycin-resistant *Enterococcus faecium* and fluconazole-resistant *Candida* spp. has been reported (53).

6.4.5. Diagnostic methods

Conventional methods for diagnosing CRBSI require removal of the CVC for quantitative or semi-quantitative catheter-tip cultures (1,54). Unfortunately, only 15% of CVCs, removed from patients with clinically suspected CRBSI, proved to be infected. Therefore about 85% of suspected CVCs were removed unnecessarily (2). If central venous access is still needed, the implantation of a new CVC increases personnel and material costs, and puts patients at risk for mispunctures and injury to vessels and organs (*e.g.* pneumothorax) (2).

6.4.5.1. CVC *ex vivo* examination

6.4.6.1.1. Semi-quantitative roll-catheter-tip cultures from Maki (extraluminal examination)

The semi-quantitative culture technique from Maki is used for identifying infections due to intravenous catheters by rolling the catheter segment across blood agar.

This technique distinguishes relevant colonization (greater than or equal to 15 colony forming units (CFU)/mL) from contamination, whereas relevant colonization was associated with localized inflammation or systemic infection (54).

Rolling the whole length of the catheter tip across an agar culture plate is not as technically easy as it might seem since placing the catheter tip (= distal 5-6 cm of the CVC) on the agar plate may be difficult, making the recovery of bacteria adherent to the external surface random. The second major disadvantage of this diagnostic test is, that only extraluminal microorganisms are cultured and no information about microorganism of the intraluminal site is available (1-3,54).

6.4.5.1.2. Brun-Buisson Technic (intra- und extraluminal)

After removal of the CVC, the CVC tip is transferred into a sterile dry (plastic) tube, 1 mL of sterile water is dripped on the catheter and the tube is further vortexed for 1 min. One hundred µL of this suspension are cultured on a chocolate agar, incubated at 37°C and examined daily for 2 days (4,55).

Growth of 10^3 CFU/mL or more is defined as a positive result and < 10^3 CFU/mL as a contamination or colonization not indicative for CRBSI. The cut-off concentration of 10^3 CFU/mL had 97.5% sensitivity and 88% specificity. This quantitative tip culture is simple and can be used in the routine laboratory (5,55).

6.4.5.2. CVC *in situ* examination

Unfortunately, only 15% of CVCs removed from patients with clinically suspected CRBSI proved to be infected. Therefore about 85% of suspected CVCs were removed unnecessarily calling for diagnostic procedures with the CVC *in situ* (2,4).

6.4.5.2.1. Simultaneous quantitative blood cultures

For this diagnostic test, blood has to be drawn simultaneously from the CVC and the peripheral vein. Blood samples of 10 mL are placed in an isolator tube (Isolator 10, Wampole, Granbury, NJ, USA) for quantitative culture by lysis centrifugation (2,3,56).

A colony count of five-fold or greater from blood culture drawn through the CVC versus the peripheral vein is indicative for CRBSI (2,4,57).

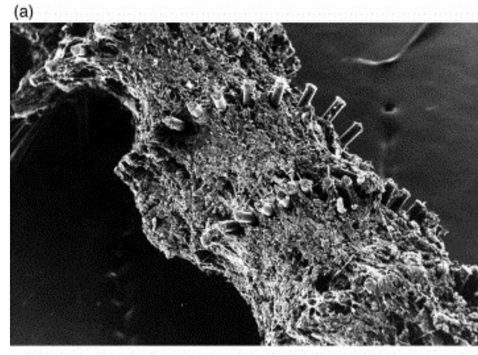
This procedure was found to be the most accurate test for diagnosis of CRBSI in a meta-analysis of studies of diagnostic tests (pooled sensitivity and specificity for short-term catheters 75% and 97%, and for long-term catheters 93% and 100%, respectively), when compared to the gold standard involving quantitative catheter culture methods. However, the use of the simultaneous quantitative blood culture technique has been limited because it is labour intensive and expensive (2,3,57).

6.4.5.2.2. Endoluminal brush

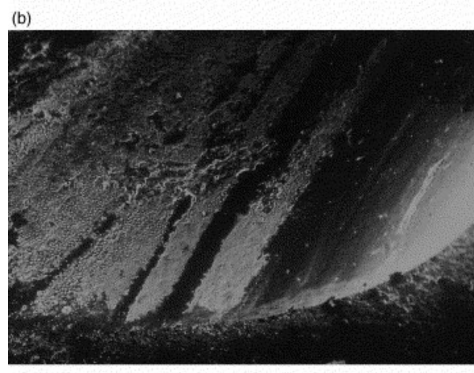
A tapered nylon brush on a steel wire is passed through the catheter hub and lumen, withdrawn, and immediately placed in a buffered container. This container undergoes sonication and vortexing after which the solution is cultured onto blood agar plates. Counts of greater than 100 CFU/mL are deemed positive (2,58).

This technique is based on the fact that bacteria adhere to the fibrin sheath on the inner surface of the CVC and fibrin becomes enmeshed in the brush's bristles (Figure 15). Kite and colleagues reported sensitivity for the test of 95% and specificity of 84% (9,58). However, this method has been criticized as being impractical and risky. Reported side effects include arrhythmias, embolization, and bacteremia related to the disruption of the existing biofilm (10,11,57).

Figure 15: Scanning electron micrographs of endoluminal brushes and CVC lumens pre- and post-brushing (59).



(a) Endoluminal brush laden with microbial laden biofilm after CVC brushing.



(b) CVC lumen surface showing striations on biofilm caused by brush bristles.

(c) Unbrushed section of double lumen CVC showing endoluminal biofilm.

6.4.5.2.3. Differential Time to Positivity (DTP)

For this method simultaneously one pair of central (aerobe and anaerobe blood culture bottle) and one pair of peripheral blood cultures is drawn from patients with suspected CRBSI. The time to positivity (TTP) is recorded with an automatic device for detection of blood culture positivity (*e.g.* BACTEC® or BACTALERT®). An earlier positivity of 2 h of the central blood culture compared with peripheral blood culture and growing of the same isolate is indicative for CRBSI. This cut-off DTP of 120 min showed 94% sensitivity and 91% specificity for diagnosis of CRBSI, 94% positive predictive value (PPV) and 91% negative predictive value (NPV). This diagnostic procedure is simple, effective, and cost-efficient.

Blood culture results with TTP exceeding 3 days should be interpreted cautiously. A DTP of less than 120 min does not exclude the possibility of colonization of CVC and therefore the authors suggest that if no other focus for infection can be found paired blood cultures should be repeated (12-14,60).

Unfortunately drawing of blood cultures peripherally is not always possible. For adequate results drawing of blood cultures has to be performed simultaneously and labeling of the blood culture bottles must be accurate (central/ peripheral).

6.4.5.2.4. Gram/Acridine Orange Leucocyte Cytospin (AOLC) stain

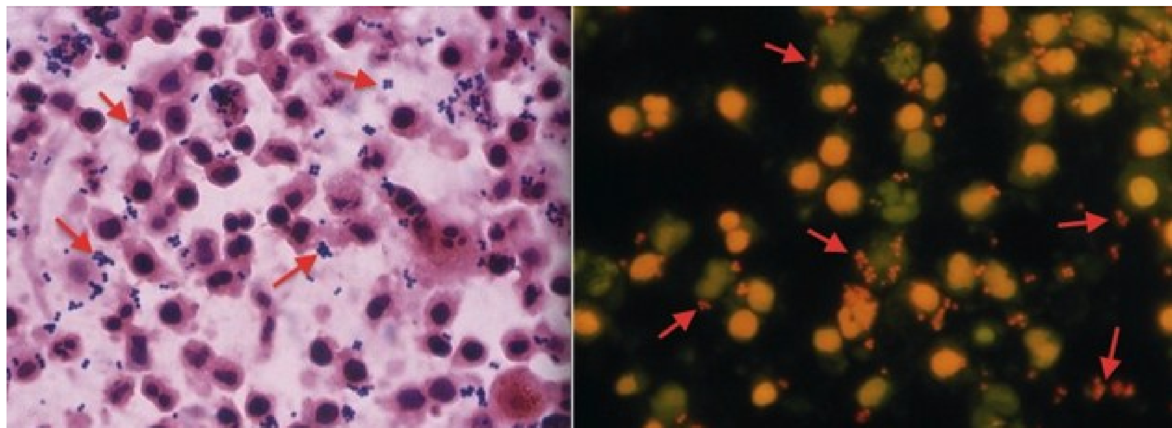
For this diagnostic procedure, blood collected in an ethylenediaminetetraacetic acid (EDTA) blood sample tube is drawn from (ideally every) catheter lumen of a CVC using the first portion of blood.

Within the laboratory, 50 µL EDTA blood are lysed with 1.2 mL of hypotonic formalin saline (1.46 g sodium chloride dissolved in 1 L sterile, distilled water and diluted with 100 mL formalin). After 2 min, the solution is neutralized with 2.8 mL hypertonic saline (1.168 g NaCl in 100 mL sterile, distilled water). Then the mixture is centrifuged at 352 g for 5 min and the supernatant discarded. The cellular pellet at the bottom of the test tube is then homogenized by vortexing for five seconds and transferred by pipette to a cytospin cupule containing a microscope slide. This is centrifuged in a cytospin machine for 5 min to create a monolayer of leucocytes

and microorganisms on the slide. The slide is then stained with acridine orange (concentration 0.001%) and examined with ultraviolet microscopy.

At least 100 high-power fields are examined and the presence of any microorganism within the cellular monolayer is considered as a positive result (50-53). Previous studies reported a detection limit of 10^3-10^4 CFU/mL blood (15-17,61-64).

Figure 16: Microscopic picture of a Gram stain (left) and the corresponding AOLC stain (right).



The left picture shows a Gram stain of a blood culture positive with *Staphylococcus aureus* (arrow). On the right side the microscopic picture of the corresponding AOLC stain from the same EDTA blood sample. *Staphylococcus aureus* appears orange under the ultraviolet microscope (arrow) (Provided by Dr. Robert Krause).

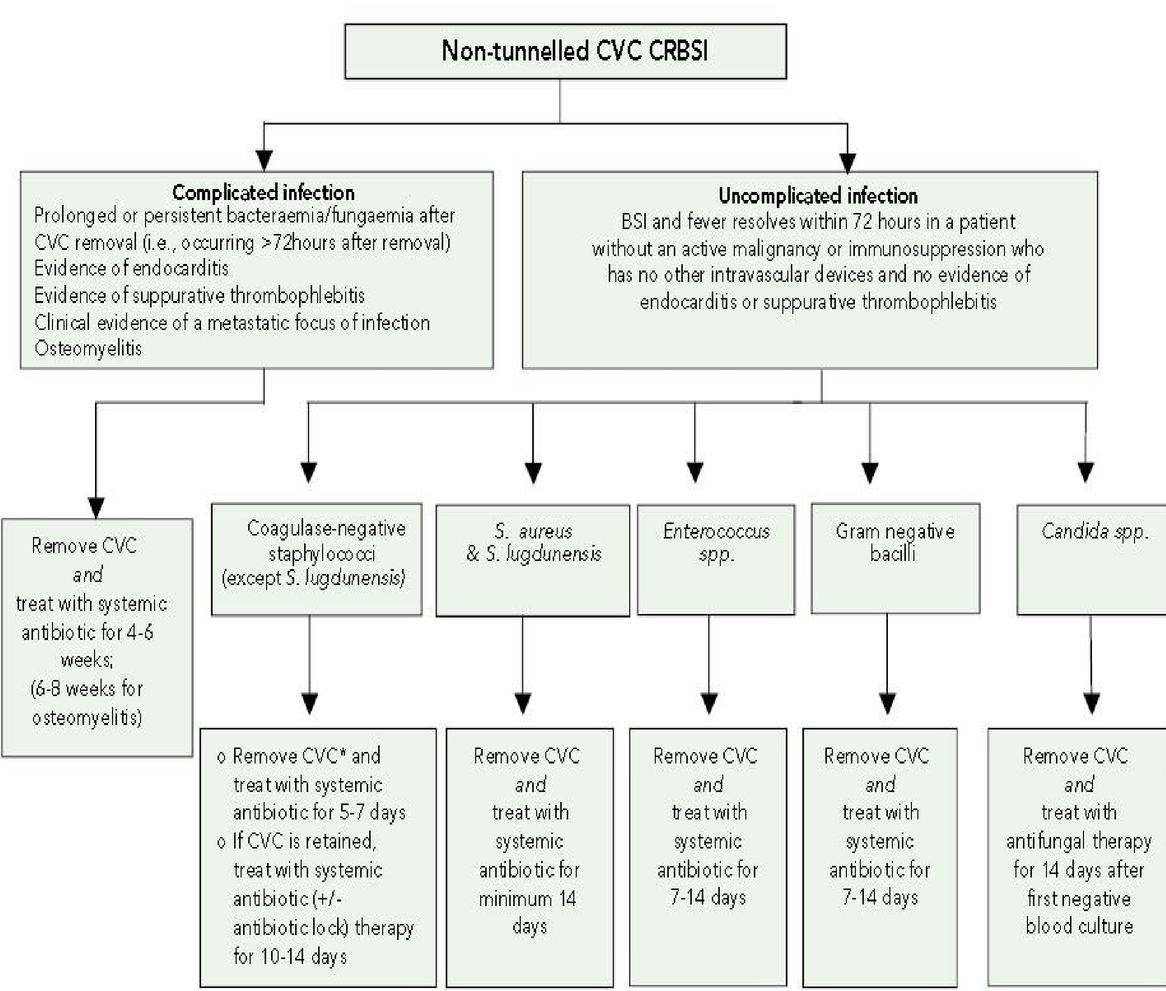
6.4.6. Current difficulties and drawback in CRBSI diagnostics

Currently, diagnostic tests for CRBSI are performed in patients with clinically suspected CRBSI (i.e. presenting with unspecific signs such as fever, hypotension, chills, leukocytosis and no other obvious focus of infection). In true cases of CRBSI the patient is already suffering from a systemic infectious complication and possibly further, potentially life-threatening sequelae like septic dissemination of microorganisms or endocarditis. Since CRBSIs are not entirely preventable, even with strict hygienic measures, earlier in vivo diagnosis of CRBSI is desirable to avoid CRBSI-associated morbidity and mortality.

6.4.7. Management

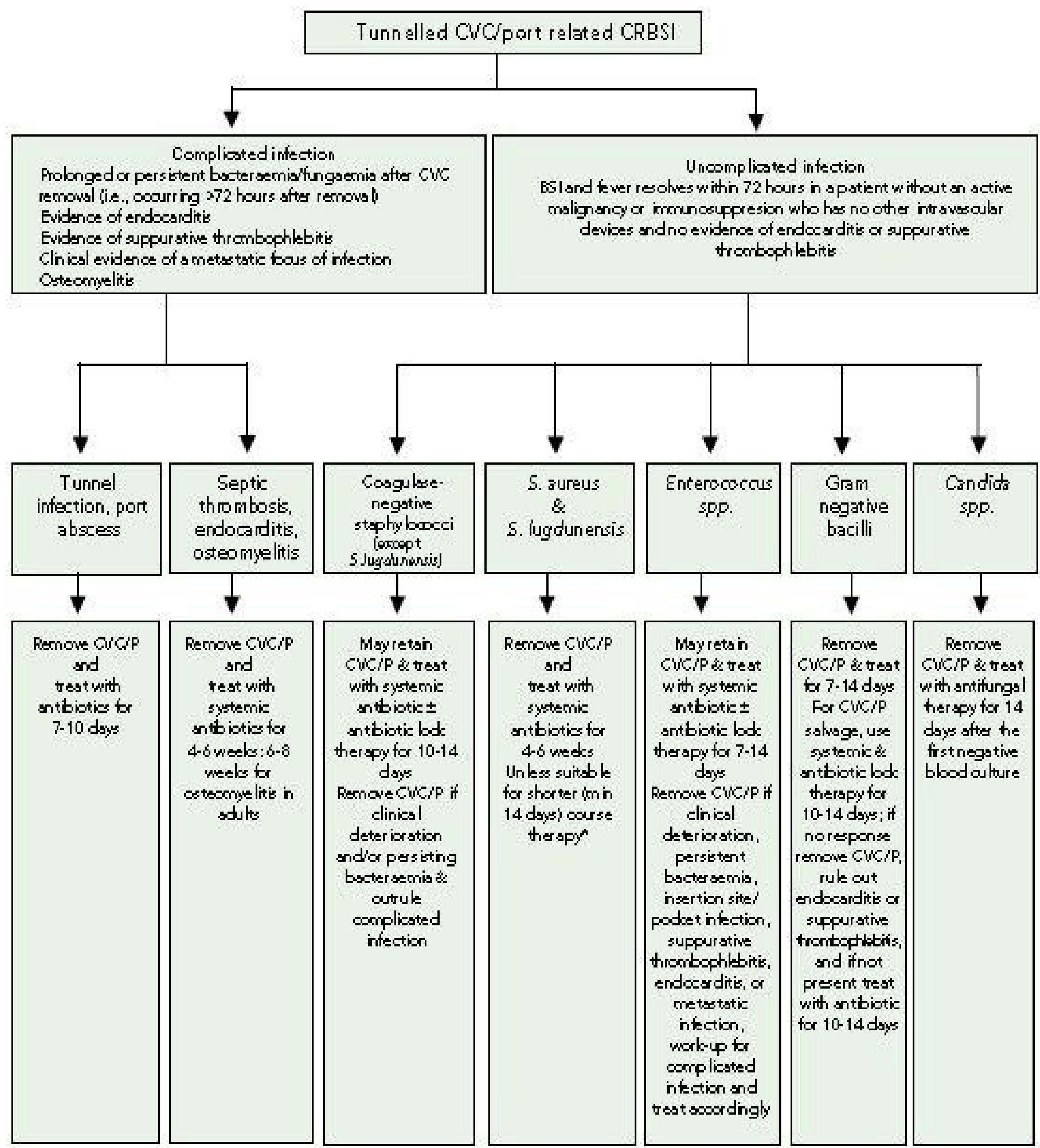
How to manage a CRBSI depends on multiple factors: 1) is a CVC removal possible or should it be retained, 2) the type of antimicrobial therapy (systemic therapy or concomitant antibiotic catheter lock solution), and 3) duration of the therapy. But the most important factor is 4) the type of organism causing the CRBSI. Recently, a useful flowchart for management of CRBSI has been published (1,18).

Figure 17: Flowchart for the management of CRBSI of non-tunneled CVC (65)



Detailed explanation of management of CRBSI of non-tunneled CVC. Management should be planned depending on the type of implanted CVC, whether the infection is complicated or uncomplicated and the causative pathogen determined.

Figure 18: Flowchart for the management of tunneled CVC/ port related CRBSI (65).



* Patients can be considered for a shorter duration of antimicrobial therapy (i.e., a minimum of 14 days therapy) if the infected tunnelled CVC / port is removed and

- Fever and bacteraemia resolve within 72 hours of initiating appropriate antimicrobial therapy.
- The patient has no prosthetic intravascular device (e.g., pacemaker, recently placed vascular graft).
- There is no evidence of endocarditis or suppurative thrombophlebitis on TOE and ultrasound, respectively.
- There is no evidence of metastatic infection on physical exam and sign/symptom-directed diagnostic tests.
- The patient is not diabetic, not immunosuppressed (i.e., not receiving systemic steroids, neutropaenia, or other immunosuppressive drugs such as those used for transplantation).

Detailed explanation of management of tunneled CVC/ port related CRBSI. Management should be planned depending on complicated or uncomplicated infection and the causative pathogen determined.

If CRBSI is caused by Staphylococcus aureus, CVC should be removed and antibiotic therapy started based on the susceptibility pattern for a more rapid response to antibiotic therapy and lower relapse rate (2,19,20,66,67).

Usually Gram-negative bacilli emerge from a non-catheter-related source (*e.g.* nosocomial urinary tract infections). However, CRBSIs caused by *Klebsiella pneumoniae*, *Enterobacter* spp., *Pseudomonas* spp., *Acinetobacter* spp., and *Stenotrophomonas* spp. have also been reported (2,20,68,69). In this cases the CVC should be removed and an appropriate targeted antibiotic therapy started for 14 days (2,21).

Candida spp. can also cause CRBSI. In these cases the CVC should be removed and administration of fluconazole or echinocandin therapy should be performed until 2 weeks after the first negative blood culture. Limited data is available about the efficacy of antifungal catheter lock solutions (2,4,29).

6.4.8. Prevention

Preventive strategies are useful to avoid CRBSI. Major avoidable risk factors have been identified for infectious complications and should therefore be reduced to a minimum.

New technologies for prevention like attachable cuffs, novel catheter hubs that were engineered to reduce the risk of hub contamination, and catheters with special anti-infective coatings (detailed information on page 18 of this manuscript) are also available. The potential of these new technologies to reduce the risk for CRBSI strongly varies and should be further researched (4,70).

The recommendations for prevention are (3,4):

1) Check the indication for implantation of the CVC or port and clearly weigh the risk and benefit for each patient.

2) Insertion of the catheter should only be performed by physicians with special training and after supervision by experienced physicians. If available, implantation of the CVC should be ultrasound-guided to reduce the number of mispunctures.

3) A maximum sterile barrier is highly recommended. Personal involved in CVC insertion is advised to wear sterile gowns, caps, masks, and gloves with sterile full-length drapes on the operating field. The insertion site should be cleaned with 2% chlorhexidine (in 70% isopropanole) instead of 10% povidone-iodine or 70% alcohol. If 2% chlorhexidine is unavailable Octenidine can be used.

4) The subclavian vein is the preferred location rather than the jugular or femoral vein due to the density of patient’s local skin flora, one of the major risk factors for CRBSI. Central venous access via the jugular or femoral vein showed a higher risk for colonization and/or CRBSI.

5) Catheter exchange over a guidewire is not recommended.

6) “Defatting” of the insertion site with acetone should not be performed routinely.

7) Application of topical antibiotic creams or ointments is not recommended, since they promote fungal infections and increase antimicrobial resistance. Application of antiseptic dressings (*e.g.* chlorhexidine) is recommended.

8) Transparent polyurethane film dressings have shown more reliability and permit continuous visual inspection of the site.

9) Reduce manipulation on the catheter to as little as possible.

10) Use the CVC or port with the minimum number of lumina or port chambers essential for the patient’s management.

11) Routinely performed replacement of the CVC or port is not recommended and should not be implemented in the routine.

12) Adequate nurse-to-patient ratio is highly recommended, especially in the intensive care unit (3,4,70).

6.4.9. Catheter Care

The most effective hygienic measure is hand hygiene. Before and after every manipulation of the catheter including palpating, inserting, replacing, accessing, repairing, or dressing, hand hygiene has to be performed (3,6).

For the dressing regimen, gauze and transparent dressings are available and can be chosen as preferred. No difference for CRBSI rate has been shown. If blood is oozing from the catheter insertion site, sterile gauze dressing should be selected and regularly replaced. If the catheter insertion site is inconspicuous, a transparent dressing might be preferable for a continuous visual control of the catheter insertion site, but there is no recommendation from the guidelines (3,6,22).

Regarding the change of dressing, if the catheter insertion site is inconspicuous and the patient shows no signs or symptoms indicating an infection, replacement of the dressing depends on the dressing used (*e.g.* every second day if gauze dressing is used, every 7 days with transparent dressing) (3,6,22).

No recommendation can be made regarding the frequency of needle replacement to access implanted ports (3,6).

6.5. Aim of the Study

The aim of this prospective study was to evaluate whether screening using either the new universal PNA FISH test or the AOLC stain could help to predict CRBSIs in non-symptomatic hemodialysis and hematooncological patients with CVC *in situ*. Results were compared to those obtained with the routine procedures, initiated for symptomatic patients by the attending blinded physicians.

Results obtained from the screening were evaluated regarding different aspects. The following questions were investigated:

1) Is routine screening useful for prediction of CRBSI?

2) Is the bacterial count indicative for differentiation between contamination, colonization, and/or infection?

3) Should screening be performed by using universal PNA FISH test, AOLC stain, or both assays?

4) How often should the screening be performed?

5) Should each CVC lumen and port chamber be screened?

7. Material and Methods

7.1. Study Design

For evaluation of the predictive value of the universal PNA FISH test and the AOLC stain, two patient groups with risk for CRBSI were included. This prospective study was performed in two phases. In the first phase, patients with tunneled long-term CVCs used for hemodialysis were included. In the second phase, hematooncological patients with non-tunneled, short-term CVCs and long-term totally implanted ports were investigated.

Results obtained from the universal PNA FISH test and the AOLC stain were compared to those obtained from the routine procedures including DTP, blood culture, and/or routine Gram/AOLC stain. For comparison, the attending physicians of the hemodialysis, the hematooncological ward, and hematopoietic stem cell transplantation (HSCT) ward were blinded to screening results of their individual patients. If CRBSI was suspected from the attending physician, routine diagnostic measures were initiated including blood cultures for DTP and/or routine Gram/AOLC stain. If a patient had clinical signs and symptoms indicating CRBSI but adequate standard diagnostic measures had not been initiated to investigate suspected CRBSI, the attending physician has been informed about the positive screening result(s). This procedure was demanded from the local Ethics Committee due to the results of our recent study (6,63).

Additionally, all patients were monitored for signs of infection by viewing computerized databases and patient’s charts.

The study was approved by the Ethics Committee of the Medical University of Graz, Austria, and all patients provided written informed consent. The study has been registered at clinicaltrials.gov (clinicaltrials.gov identifier: NCT01481038).

7.2. Study patients

7.2.1. Hemodialysis patients

In this study, hemodialysis outpatients from the Department of Nephrology, Medical University of Graz, were enrolled. Inclusion criteria were CVC *in situ*, CVC active and used for hemodialysis, and over the age of 18. Recruitment of study patients was performed at our hemodialysis unit between September 2011 and February 2012. All patients had tunneled double lumen Medcomp Split Cath® III CVCs for long-term use. Some patients were able to maintain the hemodialysis CVC for more than one year without malfunction, thrombotic, or infectious events. Blood samples for screening were collected from both catheter lumina prior to each hemodialysis three times a week. In the meantime, the CVC was mainly not used, resulting in a low number of catheter manipulations (use of the CVC 3 times a week for about 4 hours). If clinically indicated, intravenous administration of antibiotics, electrolytes, and other medications was performed before/during/after hemodialysis. Between each of the hemodialysis visits, the CVC was locked with trisodium citrate 4% or heparin 5000 IU/mL. The attending physician chose the type of lock solution independently from the screening study. No antimicrobial, antibiotic or antifungal catheter lock solution was instilled routinely prior to or during the study period.

For the PNA FISH test and the AOLC stain, the first portion of blood, that routinely had to be removed prior to each hemodialysis, was collected in 3 mL EDTA blood tubes (Vacuette, Greiner Bio-One, Kremsmünster, Austria). The specimen contained the aspirated catheter lock anticoagulant. Blood specimens were collected from both catheter lumina (arterial and venous). Usually, hemodialysis was performed three times a week, thus three screenings per week including the collection of 6 EDTA blood tubes per week were performed.

Samples were immediately processed during laboratory working hours. As recently reported, EDTA blood samples collected at night or during the weekend were stored at 4°C to preserve bacterial/fungal load, and both the universal PNA

FISH test and the AOLC stain were performed subsequently during regular working hours (27,64).

Additionally, patients were monitored for signs of bacteremia including core temperature of >38 °C or <36 °C, tachycardia >90/min, tachypnea >20/min, and transient hypotension; and for clinical signs of local inflammation at the catheter insertion site. Patient’s routine laboratory blood results including leucocytes and C-reactive protein (CRP) and microbiological results including routine blood cultures, routine AOLC stains and routine Brun-Buisson tests were also checked. Furthermore, patients were investigated for CRBSIs if they presented with emergencies between routine treatments.

7.2.2. Hematooncological patients

In the second study phase, the screening was continued at the Department of Hematology, Medical University of Graz. Between March 2012 and December 2013 adult patients were prospectively recruited from the HSCT ward. Inclusion criteria were CVC/port *in situ*, active use of the CVC/port, and over the age of 18. All patients were admitted to the hospital for HSCT or treatment for complications after HSCT.

Additionally, patients with CVC/port *in situ* admitted to the general hematological ward from February until December 2013 were included in our study. These hematological patients were admitted to the hospital for various reasons but were not admitted to the HSCT ward. Reasons for admission included suspicion of a hematological disease, staging or disease-specific treatment, chemotherapy, complications of chemotherapy, stem cell apheresis, allogeneic HSCT, and treatment of Graft versus Host disease (GvHD). Disease-specific treatment or chemotherapy was the main reason for admission and also for implantation of a CVC/port in these patients.

All hematooncological patients had double, triple, or 5-lumen CVCs (Arrow International®) without antimicrobial/antiseptic impregnation or Smiths Medical Port-a-Cath® with Gripper® access needles. Decisions for CVC or port implantation and the number of lumen/chambers of the implanted CVC/ports were made depending on the indication for implantation independently from this study.

In the entire hematooncological study group, the catheter manipulation rate was much higher compared to the hemodialysis group due to the amount of necessary administration of fluids, chemotherapy, antibiotics, antifungals, parenteral nutrition, and blood products, and additionally due to routine drawing of blood samples. Usually, CVCs were active during the whole day and were occasionally locked during the night with 0.9% saline. Due to simultaneous infusions, ramps of stopcocks are used for therapy in patients admitted to the HSCT. The mean catheter manipulation rate was 22.2 CVC manipulations per day, which can be further divided into manipulation of the CVC itself (mean 8/day) and manipulation of ramp of stopcocks (mean 14.2/day). The number of catheter manipulation per day clearly depends on the patient’s condition and resulting administration of

therapy. No antibiotic/antiseptic lock solutions were instilled. In some cases, the CVC was active for over 24 hours (*e.g.* planned HSCT during night time due to late delivery of stem cells by plane).

In the HSCT ward, blood was collected in a 3 mL EDTA blood tube from one dedicated (distal) catheter lumen using the first portion of blood (containing the aspirated 0.9% saline and blood) that routinely had to be removed prior to the scheduled routine blood sampling (daily screening). From patients admitted to the general hematological ward, blood specimens were also collected prior to every scheduled blood drawing for routine laboratory testing from one catheter lumen. The used CVC lumen was noted on the EDTA tube whenever a study sample was collected and included in the data collection sheet. In contrast to the hemodialysis patients, blood samples for screening tests were drawn from one dedicated lumen. Due to ethical reasons, blood sampling of all lumina in addition to the one lumen routinely used was not feasible in hematological patients. If every lumen had been screened, the additional collection of blood would have been not performable because most of the hematooncological patients showing anemia, leucopenia, and/or thrombopenia.

Samples were immediately processed during laboratory working hours. As recently reported, EDTA blood specimens collected at night or during the weekend were stored at 4°C to preserve bacterial/fungal load, and both the universal PNA FISH test and the AOLC stain were performed subsequently during regular working hours (6,64).

Additionally, patients were monitored for signs of bacteremia (see above) and signs of local inflammation at the catheter insertion site whenever a change of CVC dressing was performed or any kind of adverse event with the CVC occurred (*e.g.* CVC suture became loose, or bleeding from the CVC insertion site). Furthermore, patients were investigated for CRBSIs if they presented with emergencies at the emergency department.

The group of hematooncological patients is a heterogeneous one, especially concerning the state of the immune system. There are patients without adequate function of the immune system (absolute neutropenia, immunosuppression after

HSCT), and relatively immune-competent patients (e.g., patients admitted to the hospital for stem cell mobilization followed by apheresis).

7.3. Screening tests

7.3.1. PNA FISH-Test

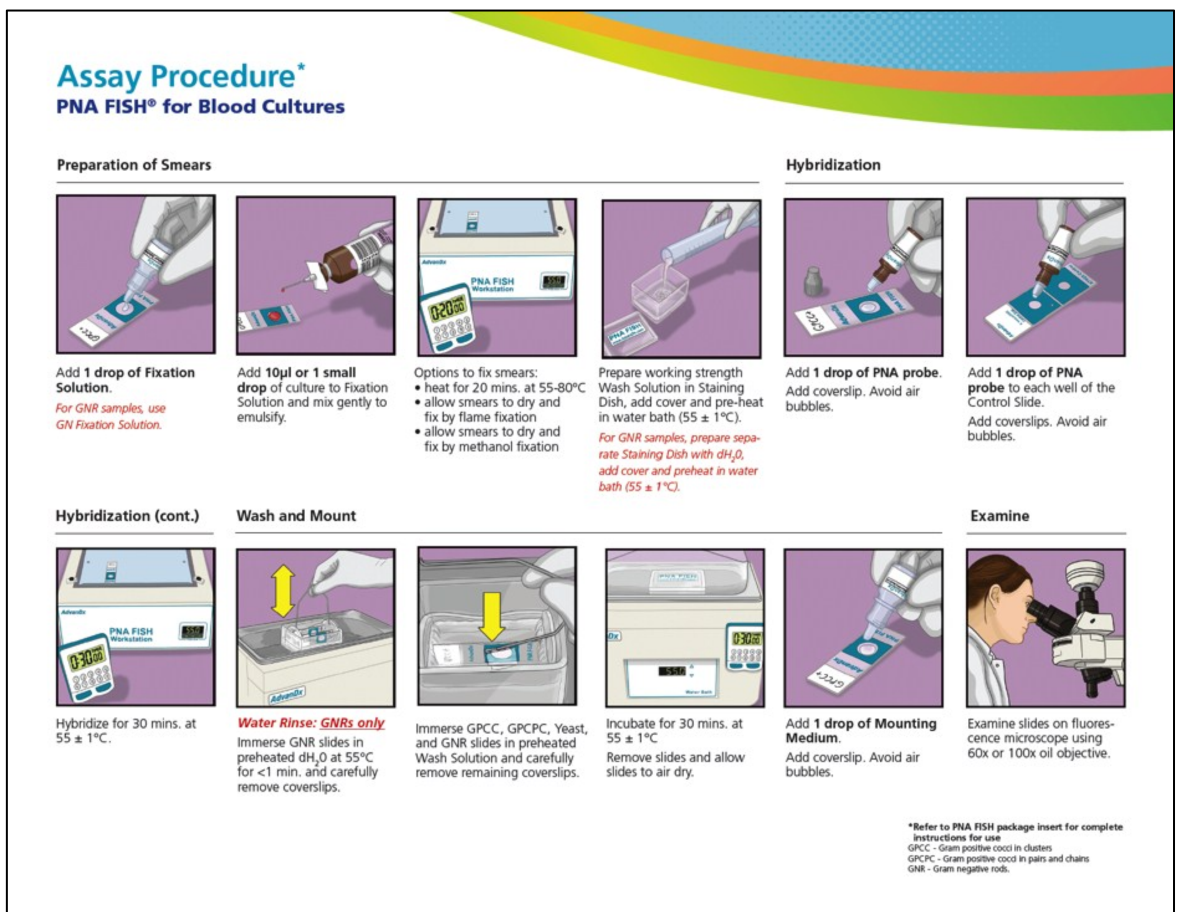
The PNA FISH test is a reliable tool for quick identification of pathogens causing bloodstream infection (28,71,72). In the last years, species-specific PNA FISH test kits have been available on the market and were used *e.g.* when blood cultures turned positive and showed Gram positive, Gram negative, or yeasts in the Gram stain. Within 90 minutes, the S. aureus/CNS PNA FISH (AdvanDx, Woburn, MA) is able to differentiate between *Staphylococcus aureus* and CNS (73). Other species-specific PNA FISH tests are also available for other Gram positive cocci (enterococci), Gram negative bacteria (identification of *Pseudomonas* spp., *Klebsiella* spp. or *E. coli*) or *Candida spp.* (identification of *C. albicans* and/or *C. parapsilosis, C. tropicalis, C. glabrata* and/or *C. krusei*).

Specific PNA FISH tests have also been used for the detection of CRBSI in EDTA blood samples without preceding culture (2,29,30,72). Unfortunately, the species-specific investigation procedure in PNA FISH tests requires an approach that combines different PNA FISH assays to identify relevant specific microorganisms, all of which increase costs and delay results. To overcome this limitation a new PNA FISH test containing universal hybridization probes for detection of a broad range of bacteria and fungi has recently become available. The universal PNA FISH tests contains PNA probes specific for ribosomal RNA (rRNA) sequences found in bacteria and fungi (BacUni and universal fungal probe) and were used in this study.

The test was performed according to the manufacturer’s instructions as described in Figure 19 in more detail. One drop of fixation solution is used and gently mixed to 10 µl of an EDTA blood sample on the FISH slide included in the test kit. Subsequently the slide is dried on the PNA FISH working station. When the smear is fully dried, one drop of universal PNA probe is added and covered with a coverslip for 30 min on the working station for hybridization. In the meantime the wash solution is prepared and preheated to 55°C. After 30 min the coverslip is carefully removed by immersing the object slide in the wash solution and

afterwards the object slide is left in the wash solution for 30 min. In the next step, the object slide is removed, and dried. Finally one drop of mounting medium and a coverslip is added for examination under the fluorescence microscope.

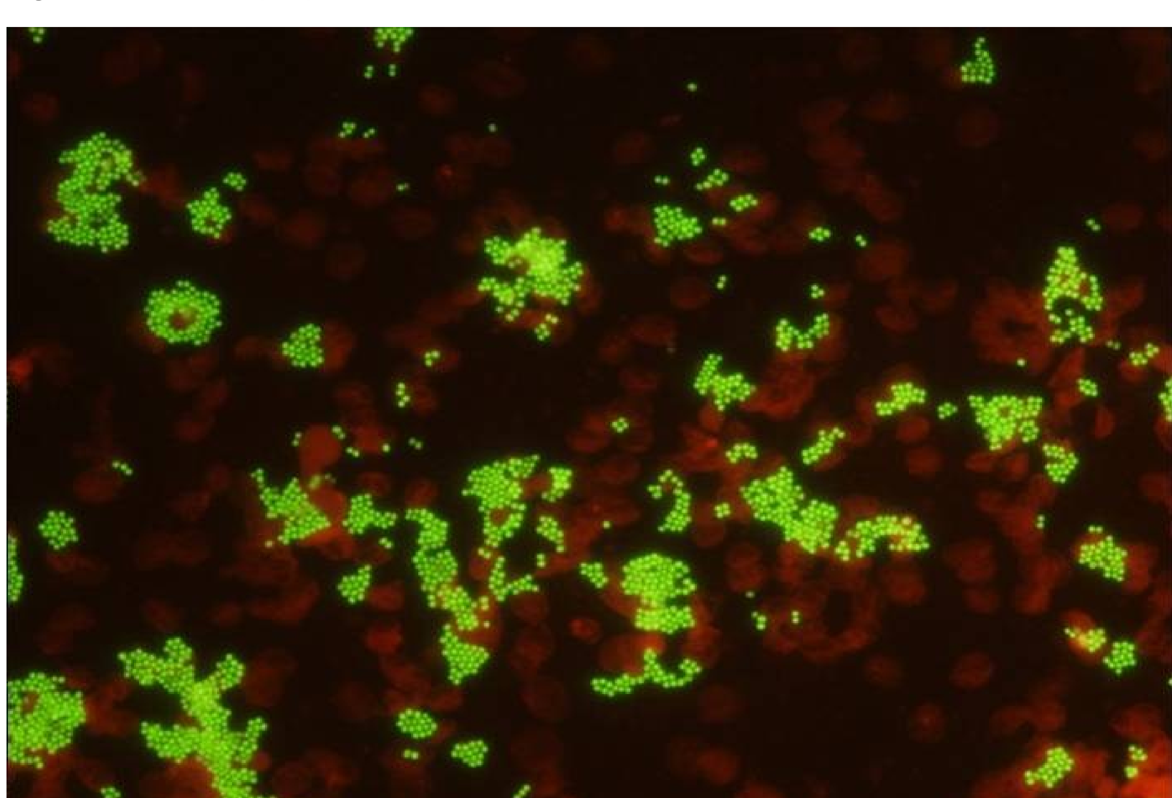
Figure 19: Manufacturer's instruction for the PNA FISH test (AdvanDx, Woburn, MA).



Assay procedure for detailed description for performing of the PNA FISH test from blood culture medium.

If the universal PNA FISH test of the screening procedure was positive (Figure 19), species-specific PNA FISH tests were subsequently performed according to our local epidemiological data regarding CRBSIs (*e.g. S. aureus*/CNS PNA FISH, in case of negative tests followed by *E. feacalis*/Other enterococci PNA FISH etc.).

Figure 20: Microscopic picture of the PNA FISH test.



Positive result of the PNA FISH test showing green fluorescence marked *Staphylococcus aureus* under the fluorescence microscope (provided by AdvanDx).

7.3.2. Gram/ AOLC stain

Additionally, EDTA blood samples were also used for the routinely performed AOLC stain and interpreted as described above on page 40 of this manuscript (3,61,62).

Gram-stained slides were no longer prepared for screening because in our recent study, Gram-stained slides did not add anything to the screening results obtained with AOLC stains (33-36,63).

7.3.3. Control cultures

To double check the universal PNA FISH test and the AOLC stain, 100 mL of the EDTA blood sample was plated evenly on a chocolate agar plate and left for 48 h at 37 °C in ambient air. Colonies were then counted, recalculated to CFU/mL and identified by routine microbiological procedures. The susceptibility pattern of every pathogen was noted and included in the data collection.

Detected pathogens were subsequently preserved and stored using Microbanks™ (Pro-Lab Diagnostics, Ontario, Canada) comprised of a cryovial system incorporating treated beads and a special cryopreservative solution until further analysis.

7.3.4. Determination of genetic relatedness

Genetic relatedness of bacterial and fungal CRBSI and screening isolates was determined by automated repetitive-sequence-based polymerase chain reaction (rep-PCR) using the DiversiLab® system (bioMérieux, Marcy l'Etoile, France). DiversiLab analysis was performed on the Institute of Hygiene, Microbiology and Environmental Health, Medical University of Graz.

The DiversiLab system is an automated technology for standardized, reproducible DNA fingerprinting analysis of bacterial and fungal samples to characterize isolates. This technology is especially used to identify the spread and source of microbial infections and for rapid determination of microbial isolates to the subspecies and strain levels. Since the test bases on the rep-PCR technology, an amplification of non-coding repetitive sequences interspersed throughout the bacterial genome using PCR is performed (74-76).

Figure 21: Schematic diagram of DiversiLab system workflow. (75)

(A) rep-PCR primer binds to genomic DNA at multiple sites and allows PCR generation of amplicons of various sizes. (B) The amplicons are separated by microfluidics with the DNA LabChip device, and data are automatically collected and analyzed with the DiversiLab software. (C) A final report is then generated. (75)

In our study cryopreserved CRBSI pathogens were cultured from Microbank beads on chocolate agar and incubated at 37°C for 24 hours. Pure culture isolates were used for further analysis with the DiversiLab system. Reagents including genus-

specific fingerprinting kits for analysis are commercially available from bioMérieux. Bacterial DNA was isolated as described by the manufacturer. For amplification the appropriate species-specific amplification kit was used followed by the electrophoresis step according to the manufacturer's instruction. Determined peak patterns were analyzed with the analysis database and compared with stored patterns.

7.4. Data Collection

Demographic, clinical, and laboratory data were extracted from computerized databases and patient’s charts.

The catheter period was defined as the time from implantation of the CVC or access to implanted ports to development of CRBSI and/or CVC removal/removal of access needles. CRBSI was defined as the presence of signs or symptoms indicating infection including core temperature of >38 °C or <36 °C, tachycardia >90/min, tachypnea >20/min, and/or transient hypotension plus a positive routine DTP result and/or routine Gram/AOLC stain and/or routine Brun-Buisson test (2,3,60,61).

8. Results

A total of 237 patients (55 hemodialysis and 182 hematooncological patients) with 402 (60 hemodialysis and 342 hematooncological) catheter periods were investigated. The mean investigation time per catheter period was 25 ± 17 days (range, 1-149 days) resulting in 10157 CVC days (3691 in hemodialysis and 6466 in hematological patients) with a total of 8098 tested blood samples (3046 hemodialysis and 5052 hematooncological patients).

A total of 19 CRBSI cases (2 hemodialysis and 17 hematooncological) were detected by routine measures resulting in a CRBSI rate of 1.9/1000 catheter days for the whole study cohort (Table 1). The CRBSI rate for the hemodialysis patients was 0.5/1000 catheter days and 2.6/1000 catheter days in hematooncological patients.

There were local signs of infection at the catheter insertion site in 4/60 hemodialysis catheter periods, with CRBSI in one case, and in 57/342 catheter periods in hematooncological patients, with CRBSI in 6 cases.

8.1. Results obtained from hemodialysis patients

Fifty-five patients were investigated resulting in 60 catheter periods. Characteristics of patients are summarized in Table 1. Five of 55 patients died during the investigation period, 4 of the lethal outcomes were unrelated to CRBSI while one was possibly related to CRBSI. The mean investigation time per catheter period was 61 ± 50 days (range 1-131 days) resulting in 3691 CVC days with a total of 2972 blood samples. Two CRBSI cases were detected by routine procedures resulting in a CRBSI rate of 0.5/1000 catheter days (Table 2).

Table 1: Baseline characteristics of hemodialysis study patients (*n*=55).

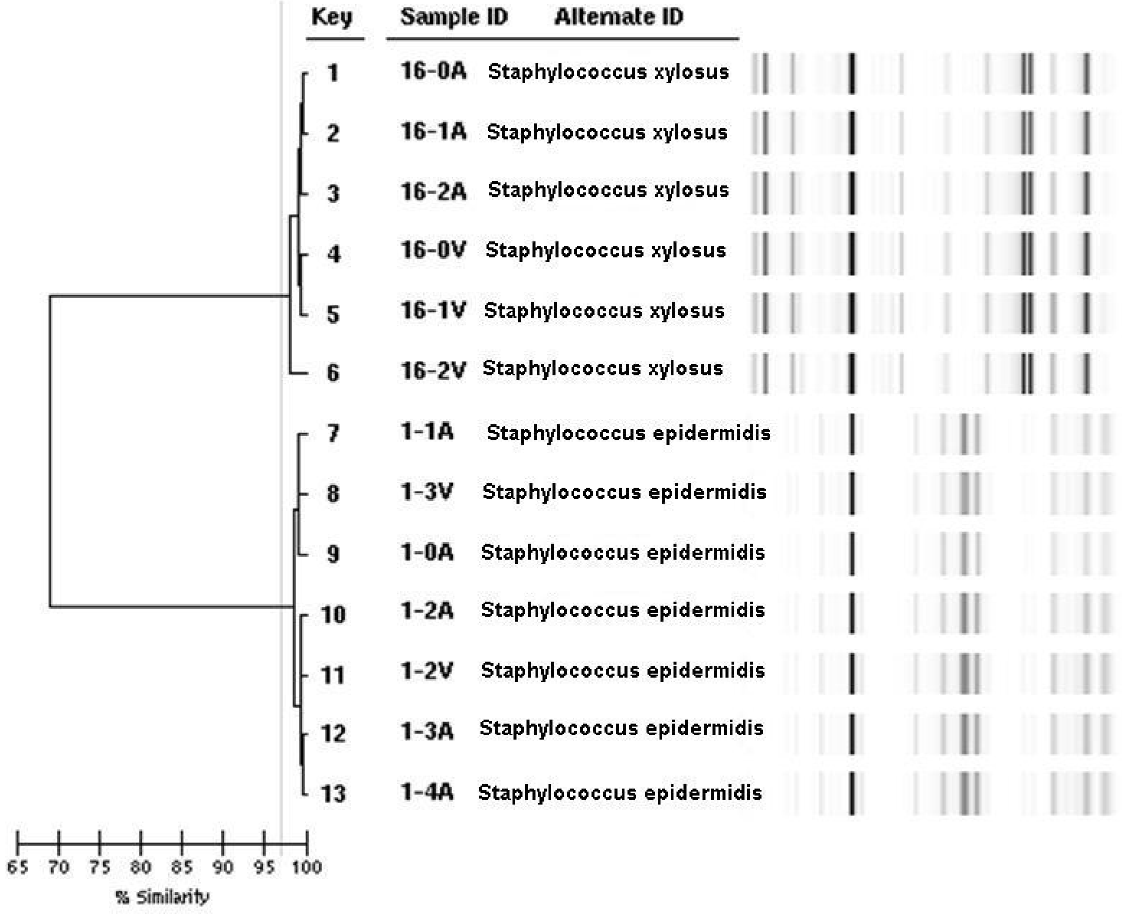
Characteristics	
Age – years (mean ± SD)	63.5 ± 15
Female sex – no. (%)	23 (41.8)
Cause of end-stage renal disease – no. (%)	
Diabetic nephropathy	11 (20)
Glomerulonephritis	2 (3.6)
Polycystic kidney disease	2 (3.6)
Hypertensive or vascular nephropathy	13 (23.6)
Other*	27 (49.1)
Coexisting or prior illnesses – no. (%)	
Diabetes mellitus	23 (41.8)
Heart disease	34 (61.8)
Cerebral disease	17 (30.9)
Hypertension	49 (89.1)
Malignancy	14 (25.5)
Medications – no. (%)	
Cortisone	10 (18.2)
Immunosuppressive agents**	12 (21.8)

* Other causes for end-stage renal disease (*e.g.*: endstage renal disease of unknown cause, amyloidosis, reflux nephropathy, analgesic nephropathy, cast nephropathy in multiple myeloma)
** Immunosuppressive agents (*e.g.*: mycophenolate mofetil, tacrolimus, cyclosporine, cyclophosphamide)

Both CRBSI cases could have been anticipated by continuously positive universal PNA FISH tests 7 days (case 1) and 8 days (case 2) before the diagnosis was

established with routine measures. Similar results were found for the AOLC stain. In both CRBSI cases, control cultures yielded the same bacterial species compared to the routine procedure with identical antibiotic susceptibility patterns showing genetic homology with a similarity index >98.5% on the DiversiLab system (Figure 22).

Figure 22: DiversiLab analysis of screening samples from hemodialysis patients with proven CRBSI.



(1-6) Positive screening samples obtained from 3 consecutive screenings *Staphylococcus xylosus* from the arterial and venous CVC lumen (case 1). (7-13) Positive screening samples obtained from 4 consecutive screenings of *Staphylococcus epidermidis* from the arterial and venous CVC lumen (case 2).

The first CRBSI case was caused by *Staphylococcus xylosus*. In addition, *Staphylococcus aureus* grew in one of the 6 central and in one of the 2 peripheral blood culture bottles. Routine Gram/AOLC stains did not show *Staphylococcus*

aureus and DTP of those blood cultures was <2 hours indicating that this strain did not cause CRBSI (2,3,19,22,37-40,60).

The second CRBSI case was caused by *Staphylococcus epidermidis* and occurred in a patient with multiple myeloma receiving palliative care. The CVC was retained after administration of vancomycin and clinical improvement. Subsequent screening tests were negative. One week later the patient was readmitted due to malaise and again had positive results with both the PNA FISH test and the AOLC stain. The patient developed fever and died of circulatory and respiratory failure. As routine CRBSI diagnostic procedures were not ordered due to the palliative setting, death related to CRBSI could not be ruled out (Case 3 in Table 2).

Three other patients with positive universal PNA FISH tests (two also had positive AOLC stains) had no signs and symptoms of bacteremia subsequently. Therefore, routine DTP and routine Gram/AOLC stain were not ordered and the patients were not considered to have CRBSI (Table 2). All 3 patients had only one positive screening test and further screenings were negative. In one of these patients (Case 4) with positive screening from the venous catheter access, line occlusion was detected before the next hemodialysis. This access was filled with rtPA providing sufficient catheter function and enabling further hemodialysis treatments via this access. Another patient (Case 5) with positive screening from the arterial catheter access received trisodium citrate 4% lock solution. The remaining patient (Case 6) with positive screening from the arterial catheter access had a red and tender catheter exit site and received local therapy with Octenisept® (octenidindihydrochlorid/phenoxyethanol).

Local signs of infection at the catheter insertion site occurred in 4/60 catheter periods; in one, CRBSI was detected (Case 1).

The sensitivity and specificity of the universal PNA FISH test in hemodialysis patients were 100% and 95%, the PPV and NPV 40% and 100%, respectively. The sensitivity and specificity of the AOLC stain were 100% and 96%, the PPV and NPV 50% and 100%, respectively.

Table 2: Screening results in hemodialysis patients.

Case	PNA FISH	AOLC	Control Culture	Screening Strain	CRBSI*	CRBSI Strain	Outcome / Follow-up
1	pos	pos	10^6 CFU/mL	*Staphylococcus xylosus*§	pos	*Staphylococcus xylosus*	Antibiotic therapy and CVC removed
2	pos	pos	10^6 CFU/mL	*Staphylococcus epidermidis*§§	pos	*Staphylococcus epidermidis*	Antibiotic therapy and CVC retained
3	pos	pos	10^5 CFU/mL	*Staphylococcus epidermidis*	id	-	Palliative care; death due to circulatory and respiratory failure,
4	pos	pos	10^6 CFU/mL	*Staphylococcus epidermidis*	n.d.	-	Occlusion of venous catheter access, instillation of rtPA, no clinical evidence of CRBSI
5	pos	neg	10^5 CFU/mL	*Staphylococcus epidermidis*	n.d.	-	Citrate 4% lock in arterial catheter access, no clinical evidence of CRBSI
6	pos	pos	10^5 CFU/mL	*Staphylococcus epidermidis*	n.d.	-	Local treatment of catheter exit site with Octentisept®, no clinical evidence of CRBSI

* = diagnosed by routine measures as DTP and/or Gram/AOLC tests.
id = indeterminable since patient had signs and symptoms of CRBSI but no routine CRBSI tests were made to rapid deterioration and palliative care.
n.d. = no routine tests since patient had no clinical signs and symptoms of CRBSI and attending physicians so did not perform routine diagnostic CRBSI test.
§ and §§ = same strain compared to the particular CRBSI strain in given patients.

8.2. Results obtained from hematooncological patients

One-hundred-eighty-two patients with 342 catheter periods were investigated. Characteristics of patients are summarized in Table 3. The mean observation time per catheter period was 19 ± 16 days (range, 1-149 days) resulting in 6466 CVC days with a total of 5052 blood samples.

Table 3: Baseline characteristics of hematooncological study patients (n=182).

Characteristics	
Age – years (mean ± SD)	56.2 ± 13.3
Female sex – no. (%)	75 (41.2)
Hematooncological malignancy – no. (%)	
Lymphoma	70 (38.5)
Acute leucemia	64 (35.2)
Myeloma	31 (17)
Chronic myeloproliferative disease	5 (2.7)
Myelodysplastic Syndrome	5 (2.7)
Aplastic anemia	3 (1.6)
Thrombotic thrombocytopenic purpura	2 (1.1)
Agranulocytosis	1 (0.6)
Chorioncarcinoma of the testis	1 (0.6)
HSCT – no. (%)	115 (63.2)
Autologous	50 (27.5)
Allogeneic	65 (35.7)
GvHD – no. (%)	34 (18.7)
CVC – no. (%)	229 (67)
2 lumina	36 (15.7)
3 lumina	189 (82.5)
5 lumina	4 (1.8)
Port – no. (%)	113 (33)
1 chamber	35 (31)
2 chamber	78 (69)

Seventeen CRBSI cases were detected by routine procedures resulting in a CRBSI rate of 2.6/1000 catheter days. Pathogens detected are displayed in Table 4. Twelve of 17 patients (71%) showed neutropenia at the time of CRBSI detection and 7/17 (41%) showed local signs of infection at catheter insertion site (*e.g.* erythema).

Table 4: Causative pathogens of CRBSI in hematooncological patients (n=17).

Causative Pathogens	
Gram positive Bacteria – no.	11
Staphylococcus epidermidis	8
Streptococcus salivarius salivarius	1
*Enterococcus faecalis**	1
Micrococcus spp.	1
Gram negative Bacteria – no.	6
Stenotrophomonas maltophilia	2
Enterobacter aerogenes	1
Escherichia coli	1
Klebsiella oxytoca	1
Pseudomonas sp.	1
Fungi – no.	1
*Candida albicans**	1

* In one patient a co-infection of *Enterococcus faecalis* and *Candida albicans* was detected.

The sensitivity and specificity of the universal PNA FISH test in hematooncological patients were 12% and 98%, the PPV and NPV 22% and 95%, respectively. The sensitivity and specificity of the AOLC stain were 29% and 97%, the PPV and NPV 33% and 97%, respectively.

A total of 15 patients showed positive screening results, but only 5/15 were diagnosed to suffer from CRBSI by routine tests. Patients with positive screening results and routinely diagnosed CRBSI are shown in Table 6. In the remaining 10 catheter periods with positive screening but no routine CRBSI diagnosis, 7 catheter samples showed positive universal PNA FISH test results, 10 positive AOLC stain results, and 10 positive control cultures. These 10 patients presented without

symptoms indicative for CRBSI; therefore, a routine procedure including blood cultures and routine Gram/AOLC stain was not ordered by the attending physician and patients were considered not to have CRBSI.

In one patient diagnosed with acute lymphatic leukemia and admitted to the hematology ward for chemotherapy, daily screening samples were drawn from the double-chamber port and revealed positive screening test (universal PNA FISH tests, AOLC stains, and control cultures with colony counts ranging from 10^3-10^6 CFU/mL). Microbiological identification revealed *Staphylococcus epidermidis* with constant susceptibility pattern and DiversiLab analyses showed >97,5% similarity, Figure 23 (f). However, the patient had no signs, symptoms, or laboratory parameters indicating CRBSI (normal body temperature, blood pressure, heart rate, CRP). The patient was discharged after completion of chemotherapy cycle and removal of port access needle. He was electively readmitted one week later, where all of the screening tests remained positive during the following days. The colony counts obtained during this second admission were lower, ranging from 10^2 to 10^6 CFU/mL. The patient still had no evidence of CRBSI and was discharged after completion of chemotherapy cycle and removal of the port access needle. One month later, the patient presented in the emergency department with 38.5°C body temperature, hypotension, diarrhea and dyspnea. Paired blood cultures were drawn and revealed *Staphylococcus epidermidis* with TTP of 10.4 h (central) and 24.4h (peripheral) indicative for CRBSI (Table 6, case 17). Antibiotic therapy was established with cefepim and extended with linezolid after receiving susceptibility testing and microbiological identification. Routine laboratory testing showed normal CRP and procalcitonin at admission to the emergency department and normal CRP on the following days. The patient recovered, relapse of the hematooncological disease was excluded with bone marrow biopsy and normal histology.

Twelve CRBSI patients were not detected by screening (Table 7). In 4 patients CRBSI originated from lumina not sampled for screening, in 3 patients CRBSI could not be allocated to certain CVC lumina due to inadequate labeling of routine blood culture bottles. Two CRBSI patients had microbial burden of CVC blood below the detection limit of PNA FISH and AOLC screening. Another two patients with CRBSI were missed by screening although microbial burden were at or above the detection

threshold of PNA FISH and AOLC tests. The remaining CRBSI patient had negative screening but the screening samples one day prior to detection of CRBSI were missing.

Thirty-three (18%) of the 182 hematooncological patients died during the observation period, in one case the death was possibly related to CRBSI. The patient was admitted to the hospital for elective chemotherapy but unfortunately developed fulminant sepsis in the following days. Routine blood cultures, routine AOLC stain as well as screening tests detected CRBSI due to *Enterococcus faecalis* and *Candida albicans*. Antibiotic therapy was immediately adapted and antifungal therapy established but the patient died 7 days later.

Table 5: Detailed information from hematooncological patients (*n*=17) with routinely determined CRBSI.

Case	Hematooncological malignancy	Neutropenia <500/µL	CVC/ Port	CRBSI Strain*	Catheter Insertion Site	Screening	Outcome / Follow-up
1	CML	yes	3 lumina CVC	*Staphylococcus epidermidis*	erythema	pos	CVC removed, cefepim therapy, patient survived
2	MDS	no	2 lumina Perm Cath	*Staphylococcus epidermidis*	inconspicuous	neg	CVC removed, linezolid therapy, patient died due to GvHD
3	Hodgkin Lymphoma	yes	3 lumina CVC	*Staphylococcus epidermidis*	inconspicuous	neg	CVC removed, piperacilin/tazobactam and linezolid therapy, patient survived
4	AML	yes	3 lumina CVC	*Stenotrophomonas maltophilia*	inconspicuous	pos	CVC removed, trimethoprim/sulfametrole therapy, patient survived
5	B-NHL	yes	3 lumina CVC	*Streptococcus salivarius salivarius*	inconspicuous	neg	CVC removed, colistin** and cefepim therapy, patient died due to acute renal failure
6	AML	yes	3 lumina CVC	*Pseudomonas sp.*	erythema	neg	CVC removed, meropenem and linezolid therapy, patient survived
7	AML	yes	3 lumina CVC	*Staphylococcus epidermidis*	erythema	neg	CVC removed, cefepim and linezolid therapy, patient survived
8	AML	yes	2 lumina CVC	*Staphylococcus epidermidis*	erythema, induration	pos	CVC removed, cefepim and linezolid therapy, patient survided.

9	ALL	yes	2 chamber-Port	*Candida albicans*	inconspicuous	pos	Port removed, meropenem, linezolid and caspofungin, patient died due to progression of underlying generalized ALL and septic multiorgan failure
10	ALL	yes	3 lumina CVC	*Micrococcus* spp.	inconspicuous	neg	CVC retained, cefepim and linezolid therapy, patient survived
11	Multiple Myeloma	no	1 chamber-Port	*E. coli*	inconspicuous	neg	Port explantation, cefepim therapy, patient survived
12	AML	yes	3 lumina CVC	*Stenotrophomonas maltophilia*	erythema	neg	CVC removed, sulfametrol/ trimethoprim and cefepim therapy, patient survived
13	Multiple Myeloma	no	1 chamber-Port	*Staphylococcus epidermidis*	inconspicuous	neg	Port explantation, cefepim and linezolid therapy, patient survived
14	AML	yes	3 lumina CVC	*Staphylococcus epidermidis*	erythema	neg	CVC removed, cefepim and linezolid therapy, patient survived
15	AML	no	3 lumina CVC	*Klebsiella oxytoca*	erythema	neg	CVC removed, tigecyclin, piperacilin/tazobactam and fosfomycin therapy, patient survived
16	CLL	yes	3 lumina CVC	*Enterobacter aerogenes*	inconspicuous	neg	CVC removed, meropenem, ciprofloxacin and vancomycin therapy, patient died due to CLL with Richter-transformation and multiorgan failure
17	ALL	no	2 chamber-Port	*Staphylococcus epidermidis*	inconspicuous	pos	Port retained, cefepim and linezolid therapy, patient survived

* = diagnosed by routine measures as DTP and/or Gram/AOLC and/or Brun-Buisson tests.
**= concomitant colistin therapy due to pneumonia and culture of multi-drug resistant *Klebsiella oxytoca* from bronchoalveolar lavage samples.

Table 6: Detailed results from hematooncological patients (*n*=5) with CRBSI and positive screening result.

Case*	PNA FISH	AOLC	Control Culture	Screening Strain	CRBSI Strain**
1	pos	pos	10^6 CFU/mL	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*
4	pos	pos	10^6 CFU/mL	*Stenotrophomonas maltophilia*	*Stenotrophomonas maltophilia*
8	neg	pos	10^5 CFU/mL	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*
9	neg	pos	10^5 CFU/mL	*Candida albicans*	*Enterococcus faecalis* *Candida albicans*
17	neg	pos	10^3 CFU/mL	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*

* = case numbers in correspond to table 5
**= diagnosed by routine measures as DTP and/or routine Gram/AOLC and/or routine Brun-Buisson tests
In cases 1, 4 and 8 screening became positive one day before the patient developed symptoms and routine measures were performed, in case 9 and 17 the patient presented with fever on the same day.

Table 7: Detailed results from hematooncological patients (*n*=12) with CRBSI and negative screening results.

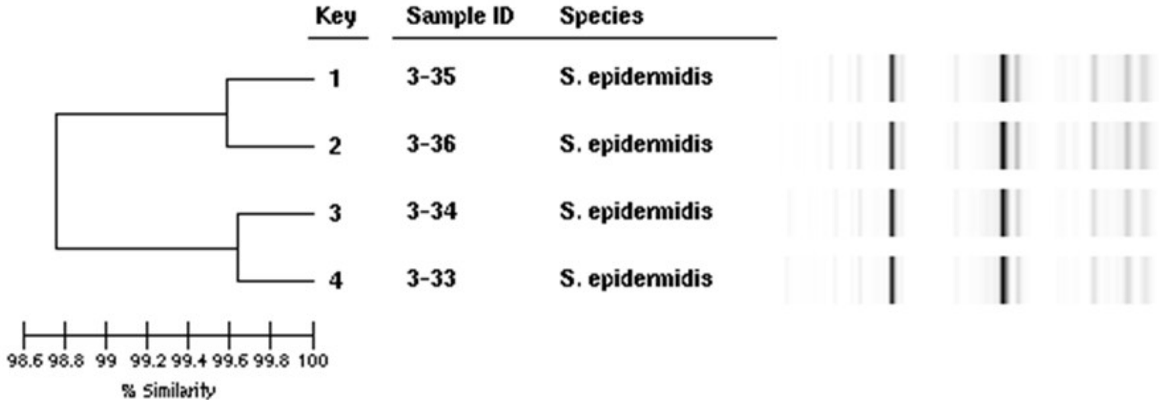
Case*	CRBSI Strain**	Screening PNA FISH and AOLC	Screenin g Control Culture	Routine DTP	Routine Gram/AOLC	Routine Brun-Buisson Test	Comments
2	*Staphylococcus epidermidis*	neg	10^1-10^5 CFU/mL	DTP 8h (cTTP 8h; pTTP 16h)	10^5 CFU/mL	10^4 CFU/mL	Positive screening control cultures on 6 consecutive days with colony counts ranging from 10^1-10^5 CFU/mL and growing *S. epidermidis*
3	*Staphylococcus epidermidis*	neg	neg	DTP 4h (cTTP 20h; pTTP 24h)	n.d.	neg	Screening samples drawn from distal CVC lumen, CRBSI originated from medial CVC lumen
5	*Streptococcus salivarius salivarius*	neg	neg	DTP 7h (cTTP 13h; pTTP 20h)	neg	neg	Screening samples drawn from distal CVC lumen, CRBSI originated from proximal CVC lumen
6	*Pseudomonas* spp.	neg	10^4 CFU/mL	DTP 9h (cTTP 14h; pTTP 23h)	10^3 CFU/mL	n.d.	***
7	*Staphylococcus epidermidis*	neg	neg	n.d.	n.d.	10^5 CFU/mL	CVC removed due to local infection of the insertion site
10	*Micrococcus* spp.	neg	neg	pos central and peripheral blood culture	10^4 CFU/mL	n.d.	Screening samples drawn from proximal CVC lumen, inadequate labeling of blood culture bottles, therefore DTP calculation impossible
11	*E. coli*	neg	neg	DTP 2h (cTTP 13h; pTTP 15h)	neg	n.d.	CVC lumen used for drawing blood cultures uncertain
12	*Stenotrophomonas maltophilia*	neg	neg	DTP 2h (cTTP 23h; pTTP 25h)	neg	n.d.	CVC lumen was used for drawing blood cultures uncertain

13	*Staphylococcus epidermidis*	neg	10^2 CFU/mL	DTP 2h (cTTP 17h; pTTP 19h)	10^4 CFU/mL	neg	Screening control culture with 10^2 CFU/mL, which is below the detection limit of PNA FISH test and AOLC test
14	*Staphylococcus epidermidis*	neg	neg	DTP 4h (cTTP 20h; pTTP 24h)	10^1 CFU/mL	n.d.	Screening samples was drawn from the distal CVC lumen, CRBSI originated from the medial CVC lumen
15	*Klebsiella oxytoca*	neg	130 CFU/mL	DTP 2h (cTTP 11h; pTTP 13h)	neg	n.d.	CVC lumen was used for drawing blood cultures uncertain; screening control culture with 130 CFU/mL is below the detection limit of PNA FISH test and AOLC test.
16	*Enterobacter aerogenes*	neg	neg	DTP 2h (cTTP 10h; pTTP 12h)	neg	Neg	Screening samples was drawn from the proximal CVC lumen, CRBSI originated from the medial CVC lumen

n.d.= not done.
cTTP= time-to-positivity of central blood culture.
pTTP= time-to-positivity of peripheral blood culture.
* = case numbers in correspond to table 5
** = diagnosed by routine measures as DTP and/or routine Gram/AOLC and/or routine Brun-Buisson tests.
***= no screening samples were collected 2 days prior to the first presentation of fever in the evening. Immediately blood cultures were drawn and antibiotic therapy was started. On the next morning the following screening sample was negative.

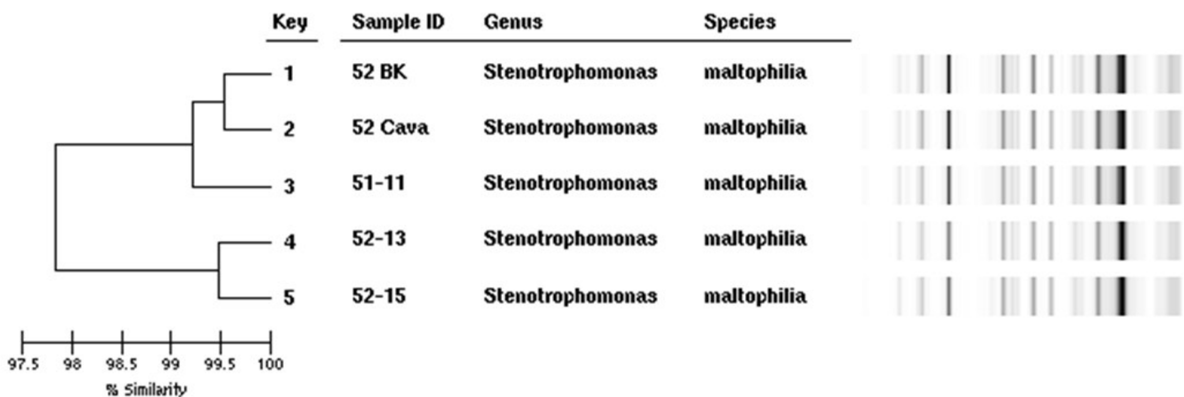
Figure 23: DiversiLab analysis of screening samples from hematooncological patients with proven CRBSI.

(a) Case 1



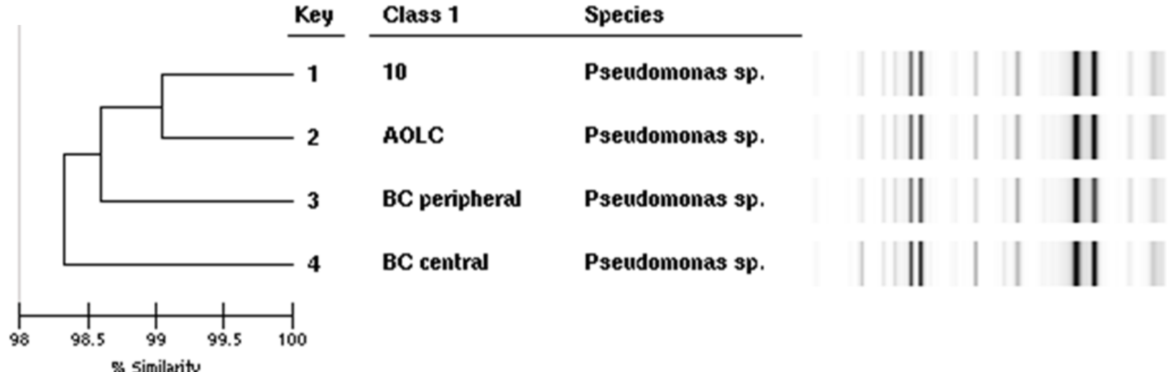
Four screening strains from 4 consecutive positive *Staphylococcus epidermidis* screenings showing >98,6% similarity in the DiversiLab analysis.

(b) Case 4

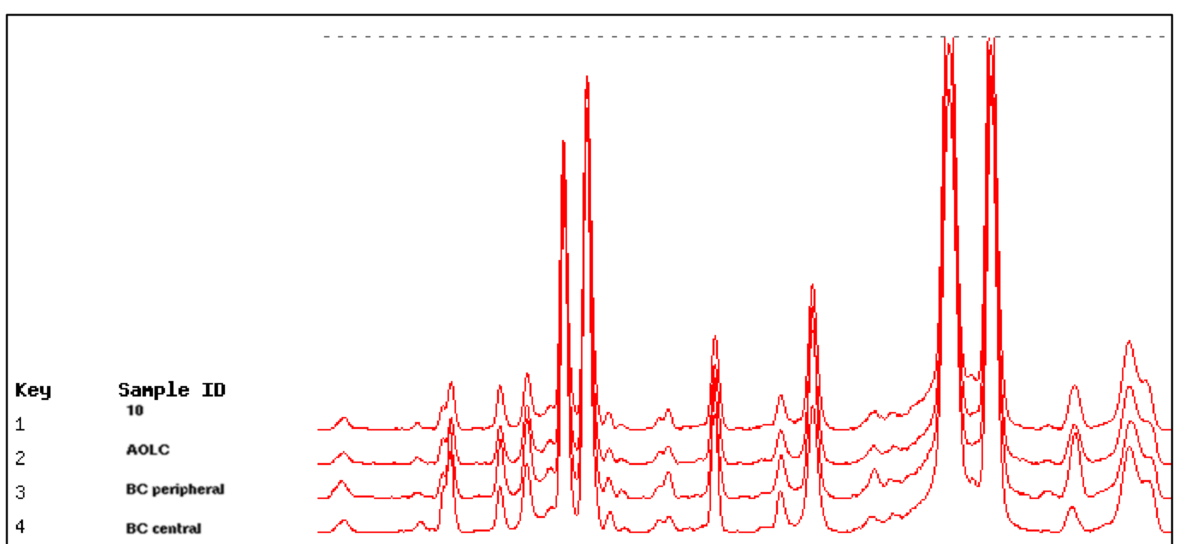


Different samples of *Stenotrophomonas maltophilia*: (Key 1) positive blood culture from the routine; (Key 2) positive Brun Buisson test after removal of the CVC; (Key 3-5) positive results from the screening. All 5 samples showed a similarity >97,5% in the DiversiLab analysis.

(c) Case 6

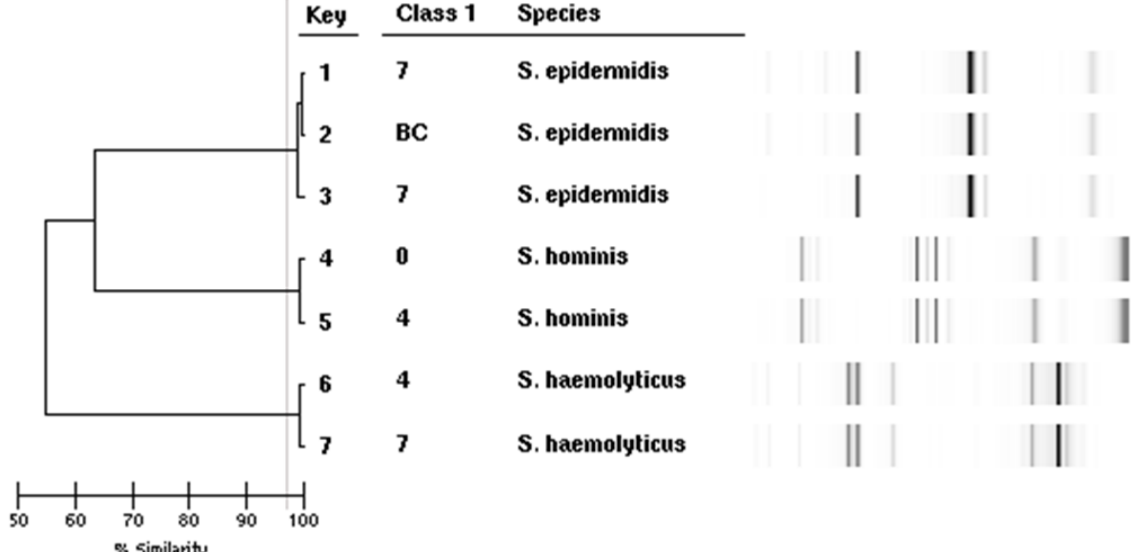


Four screening strains of Pseudomonas: (Key 1) positive screening result with the consecutive samples from AOLC and blood culture results from the routine (Key 2-4). All 4 samples showed a similarity >98% in the DiversiLab analysis.

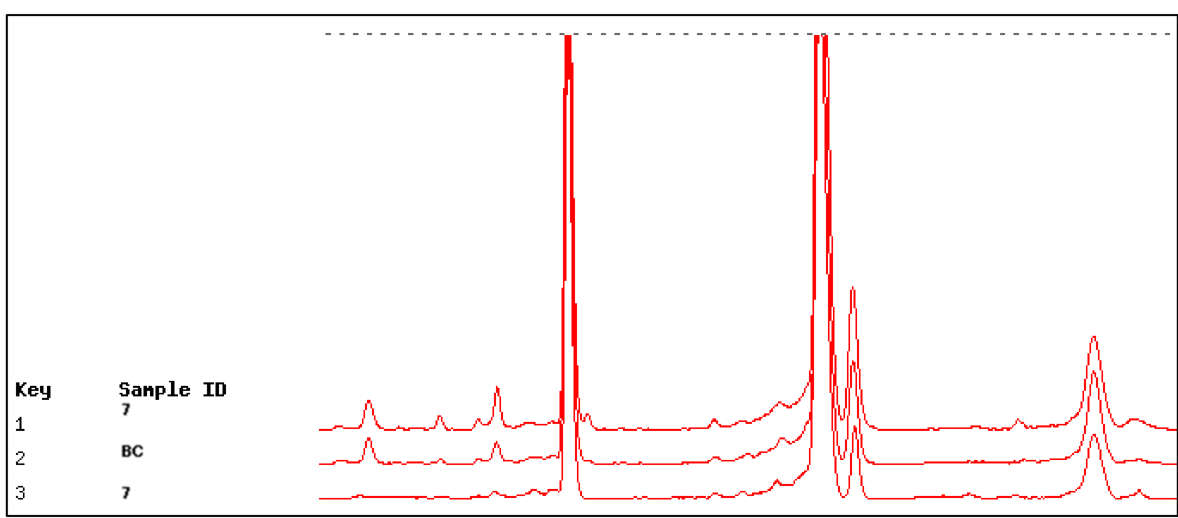


Graphic presentation of the 4 *Pseudomonas* strains determined with the DiversiLab analysis.

(d) Case 8

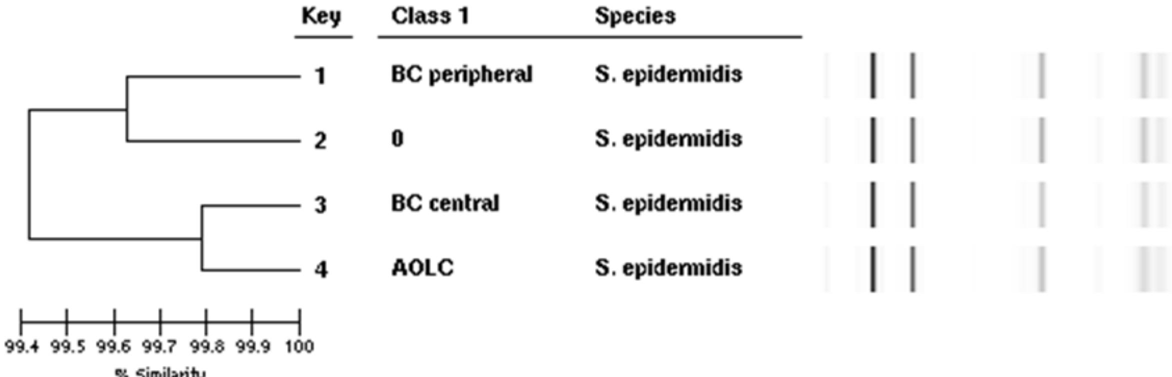


In the screening three different strains were found: *Staphylococcus epidermidis*, *Staphylococcus hominis* and *Staphylococcus haemolyticus* in three consecutive screenings of which the *Staphylococcus epidermidis* was also the responsible pathogen for the CRBSI (Key 1-3). All 3 samples from *Staphylococcus epidermidis* showed a similarity >98,5% in the DiversiLab analysis.



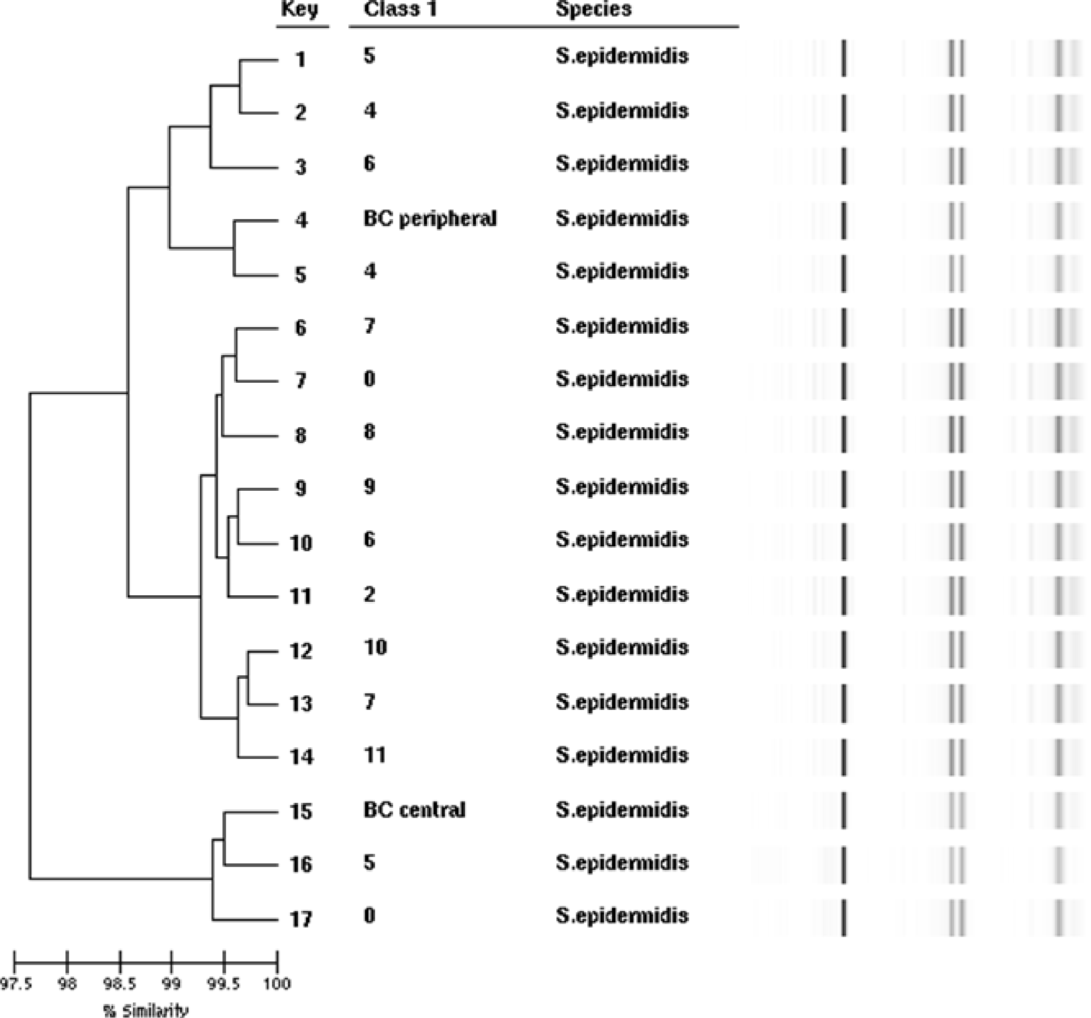
Graphic presentation of the 3 *Staphylococcus epidermidis* strains determined with the DiversiLab analysis.

(e) Case 13



Four screening strains of Staphylococcus epidermidis: positive result obtained from the screening with the consecutive samples from peripheral and central blood culture, and AOLC stain from the routine. All 4 samples showed a similarity >99,4% in the DiversiLab analysis.

(f) Case 17



Different samples of *Staphylococcus epidermidis*: (Key 4) positive peripheral blood culture from the routine; (Key 15) positive central blood culture from the routine; and the remaining strains were collected from positive screenings. All 17 samples showed a similarity >98,5% in the DiversiLab analysis.

9. Discussion

In this study, the microbiological screening using the universal PNA FISH test and the AOLC stain was investigated prospectively for anticipation of CRBSI in hemodialysis and hematooncological patients with CVCs *in situ*.

Results obtained from the screening were evaluated regarding different aspects. The following questions were investigated:

1) Is routine screening useful for prediction of CRBSI?
2) Is the bacterial count indicative for differentiation between contamination, colonization, and/or infection?
3) Should screening be performed by using universal PNA FISH test, AOLC stain, or both assays?
4) How often should the screening be performed?
5) Should each CVC lumen and port chamber be screened?

9.1. Hemodialysis patients

The 55 hemodialysis patients included in this study build a representative study cohort regarding baseline characteristics (Table 1, Page 63). Duration of investigation time was 61 ± 50 days resulting in a regular-performed screening for CRBSI within a mean of 2 months. Five patients died during the study period due to other reasons than CRBSI.

Over 3691 CVC days, 2 CRBSI cases were diagnosed resulting in a CRBSI rate of 0.5/1000 catheter days. This rate is markedly lower than CRBSI rates previously published, despite the lack of antimicrobial locking solutions in our patient cohort (3,14,36,41,42,77,78).

The low CRBSI rate in our patient cohort might be in part due to the specially trained nursing staff of the hemodialysis ward. According to the literature, trained nurses are of special importance to avoid CRBSI. In addition, at our hemodialysis ward, the no-touch CVC connection technique is used. During connection of both CVC lumina with the hemodialysis machine, the patient wears a mask and is advised to turn the head in opposite direction of the CVC and nursing personal. The nursing personal also wears a mask, gown, and sterile gloves. Connection is performed with help of gauze, soaked with the skin disinfectant Octenisept®, without touching the CVC hub itself. Disconnection is performed the same way. This connection technic may support the low rate of CRBSI in our study collective.

In this study, sensitivity and specificity of the universal PNA FISH test were 100% and 95%, and PPV and NPV 40% and 100%. Data for the AOLC stain were 100%, 96%, 50% and 100%, respectively. Data suggest that patients with negative screening results are very unlikely to develop CRBSI enabling clinicians to focus diagnostic measures in febrile hemodialysis patients more efficiently on other origins of infections. Screening tests performed of both CVC lumina did not miss a single CBRSI in our study cohort. Therefore, the major advantage in our hemodialysis patients was that blood had to be removed routinely from both CVC lumina prior to connection to hemodialysis machines and therefore both lumina

could be investigated without additional blood loss necessary for screening of both lumina.

Another advantage of screening with the universal PNA FISH test was that screening results turned positive 7 and 8 days before the diagnosis of CRBSI was started by routine measures. Therapeutic approaches targeting CRBSI may therefore be introduced at an earlier stage to avoid CRBSI and its associated complications. This is of paramount importance in hemodialysis patients since the majority has no possibility for an arteriovenous fistula and therefore depends on a hemodialysis CVC with adequate function.

In 4/60 catheter periods, the catheter insertion site showed signs of infection but only one patient had CRBSI diagnosed with the routine procedure. As recently described in the literature, local signs of infection have low sensitivity and are unspecific (2,29,30,43).

Only 2 cases of CRBSI were detected in our hemodialysis study cohort for evaluation of the screening tests. Because the CRBSI rate in these patients was very low, we decided to terminate the study prematurely on our hemodialysis cohort.

9.2. Hematooncological patients

In this study, a total of 182 hematooncological patients with 342 catheter periods including patients with percutaneous CVCs (67%) or with totally implanted ports (33%) were analyzed.

The CRBSI rate was 2.6/1000 catheter days in hematooncological patients, which is lower than that recently published (44,63). In 2009, our study group evaluated the prospective potential of AOLC screenings in HSCT patients. In this pilot study, 8 CRBSI cases were routinely diagnosed in 1859 catheter days resulting in a CRBSI rate of 4.3/1000 catheter days and was therefore higher compared to the present study (3,45,63). The reasons for the decrease in CRBSIs remain speculative. In the present study, the lower CRSBI rate might be due to a mixed study cohort including HSCT and non-HSCT patients (hematooncological patients with suspected hematological disease, staging or disease-specific treatment; chemotherapy; complications of chemotherapy; stem cell apheresis; HSCT; and treatment of GvHD) compared to the recent study, which included HSCT patients only. In recent years, catheter hygiene measures have also been improved. The type of catheter was similar but catheter flushing technique changed as ready-to-use saline solution flushing was used in the present study period.

The sensitivity and specificity of the universal PNA FISH test were 12% and 98%, the PPV and NPV 22% and 95%, respectively. The sensitivity and specificity of the AOLC stain were 29% and 97%, the PPV and NPV 33% and 97%, respectively. These data suggest, similar to our data obtained from the hemodialysis study cohort, that patients with negative screening results are very unlikely to develop CRBSI. When compared to the hemodialysis cohort, the lower values for the hematooncological patients for sensitivity and the PPV might be due to screening of only one lumen in the hematooncological study population.

In contrast to hemodialysis patients, the universal PNA FISH test and the AOLC stain turned positive 1 day before CRBSI was detected with routine procedures, compared to 7 and 8 days in the hemodialysis cohort. One might hypothesize that neutropenic patients may be more susceptible to bacterial spread from a

contaminated catheter, resulting in earlier clinical symptoms compared to hemodialysis patients. This hypothesis is supported by the fact that 71% of our hematooncological study patients with diagnosed CRBSI had neutropenia in the study cohort.

9.3. PNA FISH and AOLC screening

In the literature, the Gram/AOLC stain has been shown to be a reliable diagnostic tool for the detection of CRBSI in the routine (3,20,45,46,61). Additionally, in our recent pilot study investigating Gram/AOLC stain for CRBSI screening in patients receiving HSCT, sensitivity and specificity were 62.5% and 98% and the PPV and NPV 83% and 94.4% respectively. Two possible reasons for the moderate sensitivity in the recent study were discussed: the absolute threshold of 10^3–10^4 CFU/mL of the Gram/AOLC stain and investigation of only one routinely used lumen in multilumen CVCs (3,47,48,63). In the present study, the latter drawback was excluded in the hemodialysis patients because both lumen of the double lumen CVCs were investigated leading to a sensitivity of 100% for the universal PNA FISH and the AOLC stain. This hypothesis is encouraged in the present study because sensitivity decreases to 12% for the universal PNA FISH and 29% for the AOLC stain in hematooncological patients in which only one lumen was screened. This result is underlined by hematooncological patients (Table 7) with negative screening from one catheter lumen and subsequent CRBSI originating from the other catheter lumen (3,21,45,64). The results demonstrate that each lumen in a multilumen CVC and each chamber in a double-chamber port is a potential source of CRBSI and should ideally be screened to achieve reliable laboratory results. To the best of our knowledge, no study regarding screening for CRBSI using AOLC has been published.

In vitro investigations and quantitative control cultures from EDTA blood samples in the present study showed that the universal PNA FISH test had a detection limit of 10^5 CFU/mL (data not shown) and is therefore above the absolute threshold of the Gram stain/AOLC stain (10^3–10^4 CFU/mL). Regarding sensitivity, specificity, PPV and NPV, the lower absolute threshold of the AOLC stain clearly is a benefit for screening procedures for CRBSI. Especially in hematooncological patients lower colony counts seem to be sufficient for development of a CRBSI: 5 cases of CRBSI were diagnosed anticipatively with AOLC stain, but only 2 with the universal PNA FISH test (Table 6, page 73).

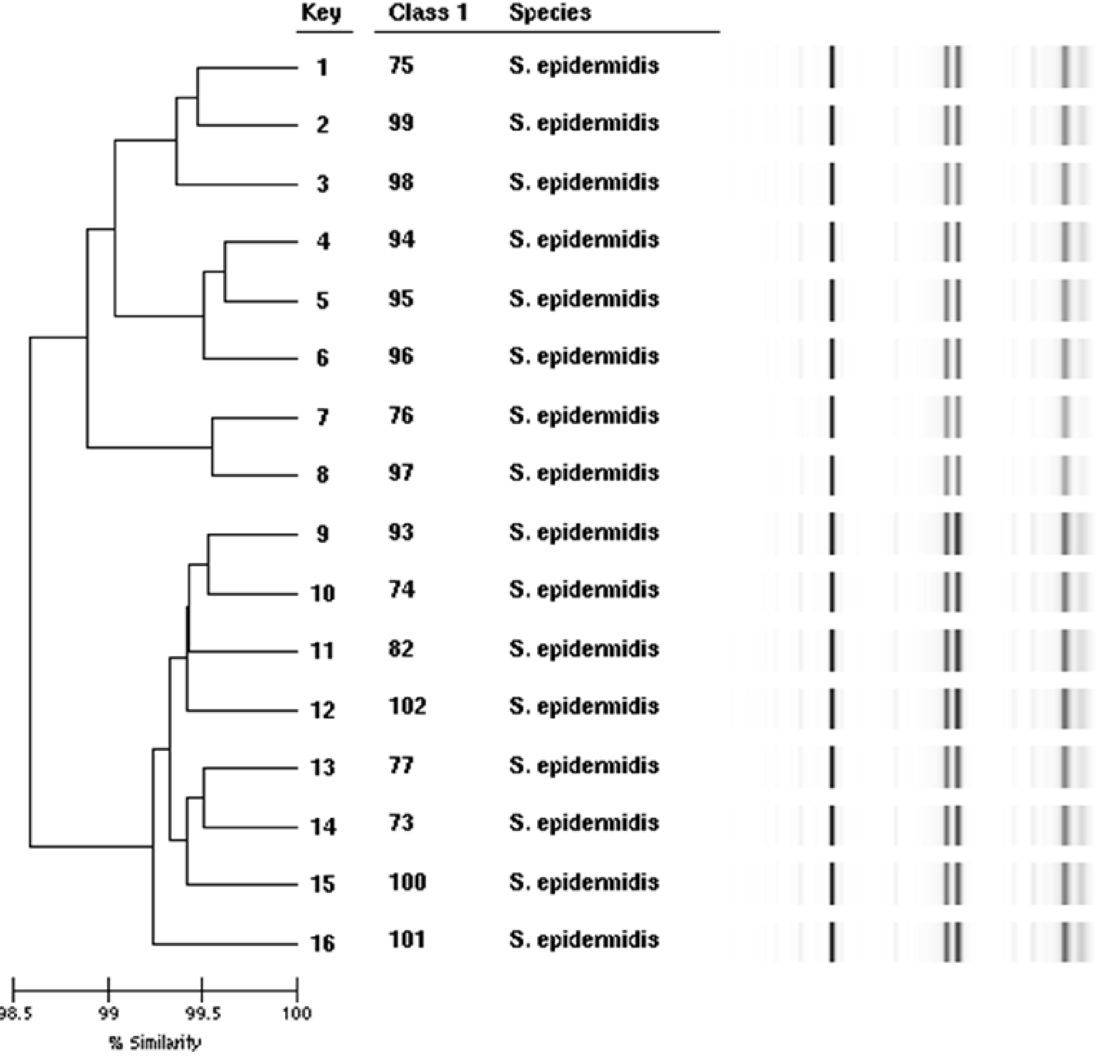
In our study, we had 4 hemodialysis and 10 hematooncological patients with

positive PNA FISH test and AOLC stain without subsequent signs and symptoms of bacteremia; routine DTP test therefore was not ordered, and the patients were considered not to have CRBSI. Possible explanations are either slight bacterial shedding of the biofilm into the EDTA blood sample during blood sampling but no or not enough bacterial shedding into the bloodstream to cause signs and symptoms compatible with CRBSI or contamination of the hub during EDTA blood sampling.

DiversiLab analysis performed from positive screening samples in patients without signs and symptoms indicating CRBSI also showed high similarity (Figure 24).

Figure 24: DiversiLab analysis of screening samples from hematooncological patients with no proven CRBSI.

(a)



16 consecutive positive screening strains with a similarity of >98,5% in the DiversiLab analysis. The patient presented without signs and symptoms indicative for CRBSI so no routine measures were taken. Colonization might be the explanation for these results.

2/4 hemodialysis patients received either local catheter treatment (rtPA) or catheter insertion site treatment (Octenisept®). The natural course of the microbial growth and possible subsequent spread of microorganisms into the peripheral bloodstream might have been influenced by these treatments. In 10 hematooncological patients 8 patients showed multiple positive screenings with CNS without symptoms indicating infection. One patient had one positive screening with CNS and one with *Klebsiella pneumoniae* (positive universal PNA

FISH, AOLC and 10^5 CFU/mL in the control culture). On the following day the patient was discharged after removal of CVC, which was no longer necessary, in good clinical condition without signs and/or symptoms compatible with CRBSI. The remaining patient showed positive screening results from the universal PNA FISH test, the AOLC stain and the control culture with *E. faecalis*. The patient developed fever whereas immediately diagnostic procedures were initiated. The routine Gram/AOLC stain was performed and blood culture bottles for determination of DTP were drawn. Routine Gram/AOLC stain was negative but control culture of the AOLC stain revealed 10^3 CFU/mL *E. faecalis* (detection limit of 10^3-10^4 CFU/mL for AOLC stain). Central blood cultures showed *E. faecalis* but in the blood cultures drawn peripherally only CNS were detected; these results were not indicative for CRBSI. However, since the patient's condition did not improve, the CVC was removed and the qualitative catheter culture showed *E. faecalis*. Unfortunately, a quantitative catheter culture (*i.e.* Brun-Buisson method) could not be performed due to inadequate catheter transport conditions (CVC was sent within an uncertain volume of transport fluid).

We additionally had positive control cultures but negative universal PNA FISH tests and AOLC stains from EDTA samples drawn from 10 hemodialysis patients and additionally 23 hematooncological patients without signs or symptoms indicative of CRBSI. The colony counts of these control cultures were below the threshold of both the universal PNA FISH test ($\leq 10^5$ CFU/mL) and the AOLC stain ($\leq 10^3$ CFU/mL). All hemodialysis and 13 hematooncological patients only had one positive control culture, and further screenings were negative. In the remaining 10 hematooncological patients more than one control culture was positive, but both screenings remained negative. A possible explanation might be contamination during blood sampling or beginning colonization.

"False" negative screening results also occurred in the hematooncological study group. The main reason was that only one lumen of a multilumen CVC was screened and when CRBSI originated from the other lumen of which no screening sample was drawn; it was not possible to anticipate CRBSI. In another 3 patients CRBSI could not be allocated to certain CVC lumina due to inadequate labeling of routine blood culture bottles. Two CRBSI patients had microbial burden of CVC blood below the detection limit of PNA FISH and AOLC screening. Another two

patients with CRBSI were missed by screening although microbial burden were at or above the detection threshold of PNA FISH and AOLC tests. The remaining CRBSI patient had negative screening but the screening samples one day prior to detection of CRBSI were missing.

Despite advances in the diagnosis of CRBSI due to the introduction of catheter-sparing *in situ* techniques, all diagnostic measures currently focus on patients actually suffering from CRBSI and thus represent a reactive diagnostic policy. CRBSI is considered to be due to increasing extra- and/or intraluminal microbial load and subsequent spread of microorganisms into the peripheral bloodstream followed by clinical signs and symptoms of bacteremia/fungemia (45,79). Detection of this relevant increase in the microbial burden could help to prevent the development of CRBSI and its complications. In neutropenic patients receiving hematopoietic stem cell transplantation it has been shown that, using this anticipative strategy, CRBSI could be anticipated with the Gram stain/AOLC screening stain an average of 2 days before the diagnosis was established by routine procedures (3,63). In this recent study, this hypothesis could not be proven.

Continuous screening on a regular basis as performed in this study is a prerequisite for anticipating CRBSIs because irregular screening failed to predict CRBSI in patients receiving total parenteral nutrition (3,51,52,80). In the study published by Catton et al., screening was performed once in 3 months using endoluminal brushing or quantitative blood cultures and was not considered to be a useful screening procedure (80).

In another study of patients with hemodialysis catheters, 21 of 31 asymptomatic patients had positive blood cultures drawn from CVCs for screening purposes (53,81). Of these 21 patients, 12 had concordant microbial growth from peripheral blood cultures at a mean time of 32 days after the first positive catheter-drawn blood cultures were obtained. Unfortunately, no clinical data regarding signs or symptoms of CRBSI of these patients were provided. The clinical relevance of positive blood cultures remains obscure regarding that study.

Another screening study investigating 21 patients showed that 7 of 20 patients with positive CVC screening performed by quantitative blood cultures

subsequently had septicemia (1,54,82). Only one of 21 patients had negative blood cultures drawn from CVCs. This high rate of catheter colonization could not be confirmed in subsequent investigations (2,81).

Ramanathan and colleagues described that if CVCs were removed in hemodialysis patients with bacteremia, 62% showed a positive catheter culture. If CVCs were removed for non-infection-related reasons, in 30% catheter cultures were also positive. They also investigated the biofilm thickness of the CVC in different segments of the catheter, intra- and extravascular segment and inner and outer surface each. The outer surface of the extravascular segment in bacteremic and non-bacteremic patients showed the thickest biofilm and the highest microbiological yield, and the biofilm was thicker in bacteremic patients (54,79).

Guembe and colleagues analyzed the value of superficial cultures of CVC for prediction of CRBSI. Cultures were collected from skin insertion site and all CVC hubs and could help to rule out the catheter as the source of bacteremia (NPV of >90%) (83).

Regarding test costs, the AOLC stain is much cheaper than the universal PNA FISH test (0,2 Euro vs. 20 Euro) and time-to-result of the AOLC stain is also shorter (30 min) compared to the universal PNA FISH test (completed in about 2 h). However, the AOLC stain provides no species identification (52).

The main disadvantage of both screening tests is the missing susceptibility testing. In the last years an increase of multidrug resistant pathogens has been observed including MRSA, vancomycin-resistant *Enterococcus faecium* and fluconazole-resistant *Candida* spp. (53). Therefore reliable and quick information about the resistance pattern would be of benefit for an adequate antibiotic and/or –fungal therapy. However, the aforementioned strains are very rare at our institution.

Our study also has some limitations since it would have been of interest to also screen all CVC lumen in hematooncological patients, which was not possible due to ethical reasons. Unfortunately even if patients developed fever, collection of samples was not always adequate (*e.g.* inadequate labeling of blood culture bottles, drawing of only central blood culture bottles, transport of removed CVCs in sterile tubes containing betaisodona® solution or discarding of removed CVCs).

9.4. Answers to study hypotheses

Regarding our study hypotheses which have been described above (page 48), answers are as follows:

1) Is routine screening useful for prediction of CRBSI?

Screening on a regular basis seems to be useful in hemodialysis patients. CRBSI was anticipated 7 and 8 days prior to the routine.

In hematooncological patients, CRBSIs were missed due to investigation of only one lumen. Because screening for study purpose cannot be performed from every CVC lumen due to ethical reasons screening is not useful in this group of patients.

2) Is the bacterial count indicative for differentiation between contamination/colonization/infection?

The bacterial count is **not** indicative for differentiation between contamination/colonization/infection. Whereas in hemodialysis patients CRBSI was present with bacterial loads of 10^5 to 10^6 CFU/mL, in hematooncological patients CRBSI occurred with bacterial loads between 10^1 and 10^6 CFU/mL. Therefore, CRBSI detection methods based on certain cut-off levels (*e.g.* Gram/AOLC method with cut-off between 10^3 and 10^4 CFU/mL) may miss CRBSIs in cases with low bacterial burden.

3) Should screening be performed by using the universal PNA FISH test, AOLC stain, or possibly both?

The AOLC stain seems to be superior to the universal PNA FISH because the AOLC has a lower limit of detection. Additionally, the AOLC is cheaper and showed better performance regarding sensitivity, specificity, PPV, and NPV. In future studies, screening might be performed using AOLC. If the AOCL staining

revealed a positive result, species-specific PNA FISH tests might be done subsequently for microbiological identification of the causative agent.

4) How often should the screening be performed?

Screening in hemodialysis patients prior to every hemodialysis seems to be a good procedure for screening on a regular basis with no additional blood loss.

In hematooncological patients, screening on a daily basis of only one lumen seems not to be useful. If screening is performed, all lumina of a multilumen CVC should be screened but the additional blood loss in patients with pancytopenia has to be considered.

5) Should each CVC lumen and port chamber be screened?

If screening is performed, adequate results can only be obtained if each lumen of a CVC and each chamber of a port were screened.

9.5. Conclusion

The proactive anticipative strategy to detect CRBSI in a subclinical stage using direct microscopic examination of catheter blood samples may be a cost saving diagnostic approach in patients with a CVC and a high risk for developing CRBSI. Hemodialysis patients with negative screening results are very unlikely to develop CRBSI, but due to the low CRBSI-rate of 0.5/1000 catheter days in this study cohort, further studies are necessary.

Screening for CRBSI using the universal PNA FISH and the AOLC stain in hematooncological patients does not appear to be a useful and cost-efficient tool for anticipative diagnosis of CRBSI. Reasons for false negative results might include concomitant antibiotic therapy during sampling, dilution of microbial burden due to concomitantly administered infusions during sampling, and origin of CRBSIs from another lumen not sampled for screening. False positive results might origin from catheter colonization without subsequent spread of microorganisms into the peripheral bloodstream followed by clinical signs and symptoms of bacteremia and/or fungemia.

10. References

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