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Research article

Bacterial colonization of non-permanent central venous catheters in hemodialysis dogs

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ABSTRACT

Non-permanent central venous catheters (CVCs), are the most commonly used vascular access in veterinary patients undergoing hemodialysis. In human dialysis patients, CVC infection represents a common cause of morbidity and mortality. The aim of this retrospective observational study was to evaluate the prevalence of bacterial colonization of CVCs in dogs submitted to hemodialysis treatment at time of CVC removal.

The CVCs of all dogs submitted to hemodialysis (n = 23) at the Veterinary Teaching Hospital "Mario Modenato" of the University of Pisa between January 2015 and December 2016 were considered. For all dogs, data regarding signalment, reason for hemodialysis treatment, duration of catheterization (≤15 or >15 days), CVC complications, and 30-day survival were considered. Statistical analysis was performed using Graph Pad Prism™. Five over 23 dogs (22%) showed positive bacterial culture of CVC (+), and 18/23 dogs (78%) negative culture of CVC (-). The most prevalent microorganism was Staphylococcus Spp (3/5; 60%). No significant difference was found in the prevalence of CVC infection according to age, gender, reason for hemodialysis, CVC complications, duration of catheterization, and outcome. No statistically significant difference (p = 0.64) in survival curves was reported at log rank analysis between dogs with CVC - and CVC +.

The prevalence of bacterial CVC contamination in our dialysis dogs showed relatively low. Exclusive use of CVC for hemodialysis, good hygiene practice during CVC management, and use of chlorhexidine as an antiseptic should be strongly encouraged.

1. Introduction

Non-permanent central venous catheter (CVC), represents the most commonly used vascular access in veterinary patients undergoing hemodialysis. CVC can be associated with different complications, which may occur at time of catheter insertion, during catheter's dwell period, or at time of removal (Napalkov et al., 2013). Risk of infection of the skin exit site, and subsequent bacterial migration along the extraluminal catheter surface to the bloodstream can be associated frequently with the use of percutaneous catheters (Shah et al., 2013). Material of the catheter seems to be an important determinant in prevention, fewer infectious complications have been associated with Teflon® or polyurethane catheters than polyvinyl chloride or polyethylene catheters (Frasca et al., 2010). Using antiseptic or antimicrobial coating of catheters can reduce the risk of catheter-related blodstream infection (CRBSI). Intraluminal infection of the catheter and extension of that infection to the bloodstream can be given also by bacterial or fungal contamination of CVC hub. Breaks in aseptic technique during placement and maintenance of the catheter, or with increases in the frequency of catheter access increases the risk of CRBSI (Shah et al., 2013). CRBSI should be suspected in a patient who develops clinical or laboratory signs of systemic inflammatory response. Identification and prevention of catheter-related complications is a critical point to improve hemodialysis patient care (Shah et al., 2013). Infection, is the second most common cause of mortality in human dialysis patients, accounting for 14% of deaths, with the majority of infections being catheter related (28–33% in one study) (Evers, 1995; Tokars et al., 2005; Katneni and Hedayati, 2007). In one study, bacterial infection is responsible for more than 30% of all cause morbidity and mortality in human patients, with vascular access infection being the culprit in 73% of all bacteremias (Langston, 2011). In

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human medicine bacteremia occurs in 0.6–1.7% of human dialysis patients per month, with vascular access infections occurring in 1.3–7.2% of patients (Tokars et al., 2005).

In veterinary medicine there are no studies about the prevalence of CVC infections in hemodialysis patients.

The aim of the study is to retrospectively evaluate the prevalence of bacterial colonization of central venous catheters in dogs submitted to hemodialysis treatment at time of CVC removal.

2. Materials and methods

The retrospective observational study was conducted on all dogs submitted to intermittent hemodialysis at the Veterinary Teaching Hospital "Mario Modenato" of the University of Pisa between January 2015 and December 2016. For all dogs, data regarding signalment (age, gender), reason for hemodialysis treatment (acute kidney injury-AKI; acute on chronic kidney disease-CKD; and end-stage renal disease-ESRD), duration of catheterization (days), and CVC complications (malfunctioning, clots) were collected from medical records. According to age, dogs were divided in three groups: young (0–1 years old), adult (2–7 years old), and aged (≥8 years old). According to duration of catheterization, dogs were divided in two groups: ≤15 days and >15 days. Dogs were then classified in survivors (S) and non-survivors (NS). The group of survivors included dogs, which were still alive 30 days after discontinuation of hemodialysis, or 30 days after replacement of CVC. The group of non-survivors included dogs, which died or were euthanized in the intra- or inter-dialysis time. CVC was considered "contaminated" if any bacterial growth was present. Dogs with CVC positive for bacterial growth were classified as (CVC +), while dogs with CVC negative for bacterial growth were classified as (CVC -).

The diagnosis of central venous catheter-related infection (CVC-RI) based on the following criteria: isolation of the same organism from a quantitative culture of the distal segment of the catheter and from the blood of a patient with clinical symptoms of sepsis in the absence of any other noticeable source of infection (Gupta, 2016).

2.1. CVC placement

Prior to CVC placement, all dogs underwent general anesthesia. Both sides of the neck were clipped. In the surgery theatre, each dog was initially placed in left lateral recumbency for CVC placement. A large area of the right side of the neck was surgically prepared with alcoholic chlorhexidine scrub. The right jugular vein was isolated by the use of sterile barriers. The CVC was aseptically placed by percutaneous Seldinger technique, and secured to the skin with non-absorbable, 2-0, nylon suture (Ethicon™, Somerville, NJ, USA). Double-lumen, 11.5 Fr x 24 cm length, silicone CVC (medCOMP®, Harleysville, Pennsylvania) was placed in dogs between 10 and 25 kg of body weight; double lumen, 13.5 Fr x 28 cm length, silicone CVC (medCOMP®, Harleysville, Pennsylvania) was placed in dogs >25 kg of body weight; double lumen, 8 Fr x 20 cm length, polyurethane CVC (Arrow-Teleflex Medical Europe Ltd, Athlone, Ireland) was placed in dogs <10 kg of body weight. After the placement, the CVC ports and the skin insertion site were isolated with vetrap, and dogs were placed in right lateral recumbency for aseptic placement of esophagostomy feeding tube (FT). At the end of the surgery, an x-ray in left lateral projection was performed to check for the CVC and the esophagostomy feeding tube position.

The CVC was dedicated to hemodialysis only, and the access was restricted to dialysis staff. Prior to each access, a standardized catheter care protocol, and the operator had to wear gloves, mask, and surgical cap. (see supplementary file).

All CVCs were removed at the Veterinary Teaching Hospital "Mario Modenato" by the dialysis staff. Reasons for CVC removal were: 1) discontinuation of hemodialysis, 2) CVC replacement due to malfunction or infection, 3) death or euthanasia.

In order to avoid CVC contamination, FT care was always performed after CVC care, by changing gloves and using a clorexidine solution.

The dialysis records of the dogs of the study group were retrospectively evaluated to check CVC complications and current antibiotic therapy. Major complications of CVC were defined as conditions responsible for premature discontinuation of the dialysis session (CVC break, severe coagulation of ports). Minor complications of CVC included small intra- and extra-lumen clots responsible for multiple stops and starts of the blood flow during the dialysis session.

2.2. Culture method and antibiogram

About 2 ml of TSB culture broth (Tryptic Soy Broth) was added to the sterile container containing the tip of the extracted catheter. The container was then incubated for 24 h at 37 °C. Subsequently, an aliquot of the broth (corresponding to a few microlitres) was sown on three different culture media: Agar Blood, Agar Salt Mannite, Mac Conkey Agar. Once sowing was obtained, these three soils were incubated for 24 h at 37 °C. At this point, some colonies were removed and transferred into a slide for Gram staining. This coloration allowed to differentiate Gram positive from Gram negative bacteria, highlighting the cell wall properties of microorganisms. Bacterial identification was achieved through the semi-automated miniAPI (bioMérieux) system and the API 20 Strep tunnels, for the identification of Streptococcaceae and related germs, ID 32 GN for the identification of Gram negative bacilli, and ID 32 STAPH for the identification of staphylococci.

Multidrug resistance was defined as non-susceptibility to at least one agent in three or more antimicrobial categories.

2.3. Statistical analyses

Statistical analysis was performed using Graph Pad Prism™ (GraphPad Software, San Diego, CA). Categorical variables were reported as number (percentages) and continuous variables were reported as mean (SD). Fisher test was used for univariate analysis for factors in relation to CVC infection. Log rank analysis was used to compare survival curves of CVC – and CVC + dogs. Results were considered statistically significant for $p < 0.05$.

3. Results

Our study population included a total of 23 dogs submitted to hemodialysis treatment. Five dogs were females, and 18 dogs were males, with a mean age of 5.7 ± 4.2 years old, and a mean body weight of 23.5 ± 11.6 kg. The study population was composed by 8/23 mix-breed dogs, 3/23 Labrador Retrievers, and one each of the following breed: Australian Shepard, Springer Spaniel, American Staffordshire, English Setter, Border Collie, Boxer, Chinese Sharpei, Dogue de Bordeaux, Golden Retriever, Duchshund, Giant Poodle, Italian Spinone. According to reason for hemodialysis, 14/23 (61%) dogs were affected by AKI, 8/23 (35%) by AKI on CKD, and 1/23 (4%) by ESRD. Median duration of catheterization was 8 days (1–135 days). Major CVC complications (break and severe CVC obstruction) occurred in 2/23 (8%) dogs at 121 and 135 days from placement respectively. In both cases CVC break needed immediate discontinuation of hemodialysis and replacement over the wire. Minor CVC complications (clotting, bleeding, malfunctioning) occurred in 6/23 (26%), 4/6 (67%) occurring in <50% of the hemodialysis sessions, and 2/6 (33%) occurring in >50% of the hemodialysis sessions.

Five over 23 dogs (22%) showed positive bacterial culture of CVC, and 18/23 dogs (78%) negative culture of CVC. Gram + bacteria showed a prevalence of 67% (4/6), and Gram – bacteria showed a prevalence of 33% (2/6). The most prevalent microorganism was *Staphylococcus Spp*, which was present in 3/5 (60%) CVC cultures; followed by *Kleibsiella Pneumoniae* (1/5; 20%), *Pseudomonas Viridis* (1/5; 20%), and *Streptococcus Spp* (1/5; 20%). One CVC was contaminated with both

Staphylococcus Spp and *Streptococcus Spp*. Sensitivity profiles of the CVC + are reported in Table 1. All the bacteria responsible for CVC infection showed multidrug resistance (Table 1). No significant difference was found in the prevalence of CVC + according to age, gender, reason for hemodialysis, CVC complications, duration of catheterization, and outcome (Table 2). No statistically significant difference (p = 0.64) in survival curves was reported at log rank analysis between dogs with CVC – and CVC +.

All the 5 dogs with CVC+ were already under antibiotic therapy at time CVC was removed (Table 3).

4. Discussion

In the present study, the prevalence of CVC contamination was relatively low (22%), compared to a 78% of no CVC contamination. As the elevated rates of blood-stream infections in dialysis patients using CVCs, anti-infective and antimicrobial surveillance protocols have been revised and potentiated (Miller et al., 2016). Prevention of catheter-related infection (CVC-RI) involves several measures, which should be used in combination to reduce CVC-RI rate. Improved CVC care, good hand hygiene and continue education of the dialysis staff have been considered as critical points. In our cohort of dogs, a rigid asepsis has been observed during CVC placement, and the access to the dialysis catheter has been restricted to dialysis staff. Moreover, no fluid therapy, parenteral nutrition, or blood sampling from the dialysis CVC have been authorized. The rationale behind this was to limit the number of accesses to the dialysis CVC, in order to reduce the probability of CVC contamination. These preventative measures might contribute to limit the rate of CVC contamination in our dogs. The exclusive use of chlorhexidine (both as alcoholic and soapy solution) might contribute to reduce CVC positivity. Alcoholic chlorhexidine solution was used instead of povidone-iodine for preparation of the surgery field in all dogs, while prior- and after each hemodialysis session both alcoholic and soapy chlorhexidine solutions were used to scrub the CVC. Chlorhexidine has been reported to significantly reduce the incidence of catheter related bacteremia and hospitalization time in human hemodialysis patients (Onder et al., 2009). Moreover, the use of an antibiotic ointment at the site of skin insertion might limit CVC contamination. Topical antibiotics showed to lower bacteremia rates, and exit site infections in hemodialysis patients. In particular, the use of mupirocin, or povidone-iodine ointments have been associated with a reduced risk of catheter-related blood stream infections, while no data are available for chloramphenicol (James et al., 2008). All dogs received a locking solution of a mixed solution of sodium chloride and heparin. The relatively low prevalence of CVC contamination of the present study seemed also to support the use of a locking solution with no antibiotic. This finding was in agreement with current

Table 2. Univariate analysis of factors related to CVC infection.

Variable	Total number of dogs	CVC +	CVC -	p-value
Age (years)				
0–1	6/23	2/6	4/6	0.64
2–7	9/23	2/9	7/9	
>8	8/23	1/8	7/8	
Gender				
Female	5/23	1/5	4/5	0.99
Male	18/23	4/18	14/18	
Reason for HD				
AKI	14/23	3/14	11/14	0.84
AKI/CKD	8/23	2/8	6/8	
ESRD	1/23	0/1	1/1	
Complications				
C	12/23	3/12	9/12	0.99
NC	11/23	2/11	9/11	
Duration of catheterization (days)				
≤15	11/23	2/11	9/11	0.99
>15	12/23	3/12	9/12	
Outcome				
S	12/23	3/12	9/12	0.99
NS	11/23	2/11	9/11	

C: complications of CVC; NC: no complications of CVC; S: survivors; NS: non-survivors; HD: hemodialysis.

Table 1. Sensitivity profiles of the CVC+.

	Staphylococcus Spp	Staphylococcus Spp	Kleibsiella Pneu	Pseudomonas Viridis	Staph + Streptococcus Spp
Amikacin	S	S	S	S	R
Amoxicilline	R	R	R	R	R
Ampicilline	R	R	R	R	R
Amoxi + Clav	R	R	R	R	S
Cefalexine	R	R	R	R	S
Cefalotine	R	R	R	R	S
Cefotaxime	R	R	R	R	S
Doxycycline	MS	MS	R	R	S
Enrofloxacine	R	R	R	R	R
Erythromycin	R	R	R	R	R
Gentamicin	R	R	S	MS	MS
Streptomycin	R	R	R	R	R
Tetracycline	R	R	R	R	S
Trimethoprim/Sulfa	R	R	R	R	R
Ceftazidime	R	R	R	S	S
Ciprofloxacin	R	R	R	R	R
Clindamycin	R	NE	R	NE	NE
Colistin	R	R	R	S	R
Neomycin	R	R	R	R	R
Piperacillin	R	R	R	S	R
Rifampicin	NE	S	R	R	MS
Tobramycin	R	MS	R	S	MS

S: sensitive; R: resistant; MS: median susceptibility; NE: not tested.

Table 3. Antibiotic therapy of dogs with CVC contamination at time of CVC removal.

Case	Infection agent	Antibiotic therapy
#1	*Staphylococcus Spp*	Ampicillin + Metronidazole
#2	*Staphylococcus Spp*	Ampicillin + Enrofloxacin
#3	*Kleibsiella Pneumoniae*	Doxycycline
#4	*Pseudomonas Viridis*	Ampicillin + Enrofloxacin
#5	*Staph Spp + Strept Spp*	Cefazolin + Marbofloxacin

human guidelines, which do not recommend the routine use of antibiotic in locking solution, due to the potential for promoting fungal infections, antibiotic resistance, and systemic toxicity (KDIGO Guidelines, 2012).

Anyway, our study was only able to evaluate CVC contamination rate, with no information about possible localized insertion-site infections, catheter-associated bloodstream infections, or catheter-associated clinical sepsis.

In our study, Gram + bacteria showed a prevalence of 67% (4/6), and Gram – bacteria showed a prevalence of 33% (2/6). Polymicrobial infection was present in only one CVC. These findings seem to be in agreement with human medicine. In human dialysis patients, the majority of CVC-RI has been associated with Gram + bacteria. Gram – bacteria have been isolated in the remaining 20%–40% of the infections. Polymicrobial infections accounted for 10%–20%, while fungal infections were less than 5% (Miller et al., 2016). In our cohort of dogs, *Staphylococcus Spp* was the most prevalent bacterium, as it was present in 3/5 infected CVCs. As *Staphylococcus Spp* is commonly found on canine skin (Hoffmann et al., 2014), it is plausible that CVC infection was the result of an alteration of the epidermal barrier, *Staphylococcus Spp* overgrowth, or both. Similarly, in a human study on CVC-RI of non-permanent CVCs, *Staphylococcus Aureus* was responsible for the majority of infections (Gupta, 2016). Potential risk factors for CVC contamination are represented by colonization of the CVC with skin flora, environmental bacteria, hematogenous seeding of the CVC from another infection site, or contaminated dialysate (Gahlot et al., 2014). Although the exact mechanism of CVC contamination was unknown, we hypothesized that CVCs were accidentally contaminated by skin, or environmental bacteria in 4/5 dogs. Hematogenous seeding from other infection sites was considered less probable. Only one of the five dogs had a diagnosis of CVC-RI, with positive blood culture, fever (39.8 °C), chills, and marked weakness. In this dog leukocytosis, elevated serum C-reactive protein and fibrinogen were also found. Although 4/5 dogs with contaminated CVC did not show clinical signs of CVC-RI, sensitivity panel showed multidrug resistance in all dogs. Moreover, the majority of contaminated CVC showed sensitivity only to potentially nephrotoxic agents, such as aminoglycosides. This finding may represent a severe medical problem, as in hemodialysis humans with CVC-RI, mortality was significantly associated with inadequate antibiotic therapy (Sahli et al., 2017). It should also be noticed that 4/5 dogs with CVC contamination were already on two antibiotics at time of CVC culture. It is possible that the higher susceptibility to infections of dogs with renal failure, and the consequent use of antibiotics, might contribute to cause selective pressure on bacteria, as reported in human medicine (Wong et al., 2007).

In our study prevalence of CVC infection did not differ significantly among dogs of different age. Influence of age on infection rate of CVC is controversial in human medicine. In one study (Marschall et al., 2008), patients less than 1 year old, and above 60 years old showed a significantly higher risk of CVC infection, while in another study (Gupta, 2016) conducted on non-permanent CVCs, age was not a significant predictor of CVC-RI. In a recent veterinary study (Reminga et al., 2018) age was not reported as risk factor to develop CVC complications.

In our population of dialysis dogs no significant difference in the prevalence of CVC bacterial contamination was found according to CVC complications. In human dialysis patients, increased risk of CVC infection has been showed to be independently associated with CVC thrombosis (Wang et al., 2015). In our population, small blood clots of the CVC were considered minor complications. Although a 33% of dogs showed minor CVC complications (blood clots included) for >50% of hemodialysis sessions, this finding was not associated with an increased risk of CVC bacterial contamination. It should also be considered that the only 2 dogs with major CVC complication (CVC break), underwent CVC change over the wire. The immediate change of damaged CVCs might contribute to reduce the risk of bacterial contamination in the two dogs.

In our study population, no significant difference in the median CVC permanence time was found between CVC + and CVC – dogs (11 days vs 8 days). Moreover, no significant difference in the prevalence of CVC contamination was found between dogs with CVC permanence time ≤15 days and >15 days. In the other hand, Reminga et al. reported as the longer dwell time was a risk factor for the development of complications (Reminga et al., 2018). Non-cuffed CVCs are the first choice type of vascular access suggested to initiate dialysis in human AKI patients, but their use over 1–3 weeks is not recommended, due to higher risks of infections (KDIGO Guidelines, 2012; Gupta, 2016; Sahli et al., 2017). The lack of significant increase in CVC infection rate in the group of dogs with CVC >15 days was of particular importance, and seemed to reflect an accurate care of the CVC by the dialysis staff. Moreover, no significant difference in 30 day survival was seen between dogs with CVC – and CVC +. We hypothesized that prompt removal, or change over the wire of the contaminated CVC in the 5 dogs with CVC + reduced the risk of CVC-RI and associated mortality.

The present study has several limitations. Firstly, blood culture was performed in only 1/5 dogs. We opted not to run blood cultures in the remaining 4 dogs, as no clinical or laboratory signs of blood stream infection were present. Routine use of blood cultures in veterinary patients on hemodialysis is currently difficult due to large volumes of blood required (8–10 ml), and associated costs. Although no clinical and laboratory signs were present, we could not exclude that the remaining 4 dogs had some degree of CVC-RI. Another limitations of the study were the relatively small sample size (n = 23) and the absence of an exit-site culture. As the retrospective nature of the study, no information regarding possible exit-site infections in both dogs with CVC – and CVC + were available and it is possible the lack of patient information about suggestive of CVC-RI or localized infections.

Although fungal infections of dialysis CVCs have been reported to be rare in human dialysis patients (Miller et al., 2016), we could not exclude fungal contamination, as fungal cultures were not run.

5. Conclusion

In conclusion, the prevalence of bacterial CVC contamination in our canine dialysis population showed relatively low. Despite the current human guidelines do not recommend the use of non-permanent dialysis CVCs longer than 2 weeks, due to the risks of skin contamination, our findings did not show any association between CVC contamination and time of CVC permanence, or mortality. Exclusive use of CVC for hemodialysis, good hygiene practice during CVC management, and use of chlorhexidine as an antiseptic should be strongly encouraged.

Declarations

Author contribution statement

F. Perondi and I. Lippi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

C. Brovida: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

V: Petrescu: Analyzed and interpreted the data; Wrote the paper.

F. Porciello and G. Ceccherini: Analyzed and interpreted the data.

F. Fratini: Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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