

Permanent jugular catheterization in miniature pig: treatment, clinical and pathological observations

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ABSTRACT: The aim of present study was the installation of permanent jugular catheter to miniature pigs, which are frequently used as animal model for the experiments closely related to human medicine. We describe here in many details surgical interventions leading to the localization and fixation of Seldinger needle in *v. jugularis externa* and its use for extended period of time. Eight animals were included in these experiments and their health status was currently monitored and no visible problems were recorded. After two months they were euthanased and potential function of catheters was carefully inspected. Only in two instances we found pathological changes resulting in the obstruction of catheter and thrombophlebitis of *v. jugularis externa sin* and *v. cava cranialis*. In six remaining animals, it was easy to inject any time the solutions with drugs to blood system or to take safely blood samples. During whole post operation period the animals were maintained in conventional conditions, without any special care.

Keywords: miniature pig; central venous catheter; Seldinger's method

Miniature pig is currently often used as a unique model in biomedicine. The significance of the minipig, as an experimental animal, is constantly increasing, even though breeding minipigs is time and space consuming and costly process (Swindle, 1983, 1992, 1998; Stanton and Mersmann, 1986; Tumbleson, 1986; Swindle and Adams, 1988; Tumbleson and Schook, 1996). For use in biomedicine, the minipigs have considerable advantages over other experimental animals. Anatomical similarity of some organs with humans and also sufficient anatomical size of organs and tissues enable performance of different surgical interventions and executions in an extent comparable with humans (Swindle, 1983, 1998; Swindle et al., 1986; Bolton

et al., 1988; Gardner and Johnson, 1988; Smith et al., 1990; Mullen et al., 1992; Brown and Terris, 1996). Thus, the minipig model may help to create optimal pre-clinical protocol that will be safe, surgically manageable and that will enable to predict all complications associated with surgical operations and convalescence in humans.

Access to blood-vessel system represents an essential factor limiting success of a surgical experiment. Provision of a safe and reliable entry to venous system enables application of injection solutions to experimental animals, collection of blood samples for diagnostics purposes, measurement of central venous blood pressure or performance of mini-invasive surgical interventions through

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large-bore vessels. In many cases, peripheral veins can be used for these purposes, mostly auricular veins in pigs. The advantages of such peripheral system are represented by an easy introduction into well-identified vein, low risk of serious complications and last, but not least an easy treatment and maintenance. However, the peripheral system is not suitable for long-term experiments or intensive post-operation treatments. Such system often fails for obstruction of the intravenous cannula, local infection in the insertion site or insufficient fixation to skin basis. For these reasons, the use of a central intravenous catheter is unambiguously superior to the peripheral system for animals determined for long-term experiments. An implantation of the central catheter is more time-consuming and systematic treatment is more demanding, but this system eliminates complications associated with the peripheral cannulations and ensures convenient access to the central venous system.

Seldinger's method is method for introducing a catheter into a vessel via a needle puncture. The vessel is located with a special needle that contains a wire; the needle is removed. The catheter is threaded into the vein while being guided by the wire over which it is moving. The wire is then removed from the needle. This method is used in angiography, cardiac catheterization, and cannulation of the central venous system. The Seldinger method spread fast, and in the late 1950's was a routine procedure (Seldinger, 1953, 1984).

Miniature pigs have a very limited number of accessible surface veins. Ear veins (*v. auricularis caudalis* or *v. auricularis intermedia*) are suitable for intravenous administration of drugs or collection of small-volume blood samples. Course, branching and size of ear veins differ from animal to animal. Moreover, these veins are liable to formation of haematoms that complicate their repeated puncture by a needle. In small animals, up to 15 kg of weight, intravenous administration can be accomplished after proper animal fixation into *v. saphena*, localized on medial surface of thigh. The last alternative for collection of small amount of blood is *v. epigastrica superficialis cranialis*. Collection of large-volume blood samples is possible from *v. cava cranialis*. However, collection of blood from this deeply located central vein is associated with several risks, since it occurs without exact visual control. An irritation of left-side localized *nervus vagus* may lead to excessive vagotonic stimulation of heart and consequently to a cardiac arrest. Another possible

complications of this procedure are puncture of trachea or *a. carotis*.

An alternative to the peripheral access to venous system is a permanent catheterization of *v. jugularis externa*. This is a medium-bore vein; its diameter reaches in minipigs weighing 25 kg about 3–5 mm. *Vena jugularis externa* branches out of *vena cava cranialis* in front of *apertura thoracis cranialis* and courses in *sulcus jugularis* under *lamina superficialis fasciae cervicis*. In the region of the third neck vertebra is *v. jugularis externa* limited by *m. cleidomastoideus* from dorsal side and by *m. sternomastoideus* from ventral side. *Musculus cutaneus colli* then limits the vein from lateral side.

MATERIAL AND METHODS

Animals

For the study, eight miniature male pigs, six months of age and 25 kg of weight were used. All animals were clinically healthy. The pigs were kept individually in stalls and fed twice a day by a complete feeding mixture A2 (ZZN Melnik, Czech Republic). Access to water was *ad libitum*.

Catheter

A set for catheterization of central veins by Seldinger's method – Certofix Mono V 330 (B. Braun, Melsungen, Germany) was used in our experiments. The set includes Seldinger needle (18 G, 70 mm of length), guide wire with lengths marking and a flexible J-tip, in dispenser (wire of 0.89 mm in diameter, 30 cm of length) and opaque polyurethane catheter with a soft tip (16 G, 30 cm of length).

Anesthesia

The animals had not been fed 12 h before surgery. They were pre-medicated with 0.02 mg/kg of atropine (Hoechst-Biotika, Martin, Slovakia) followed 10 min later by 2.0 mg/kg of azaperone (Stresnil, Janssen Pharmaceutica, Beerse, Belgium). As soon as sedation had occurred, anesthesia was induced by intravenous administration of 10 mg/kg of ketamine (Narkamon, Spofa, Prague, Czech

Republic) into an ear vein. The animals were fixed in back position and intubated by an endotracheal cannula of 7.0 mm in diameter. Deep level of anesthesia was maintained by inhalation of halothane (Narcotan 0.5%, Zentiva, Prague, Czech Republic) with medicinal oxygen (25 ml/kg/min). SPO₂, ECO₂ and three-lead ECG were recorded during operation of the experimental animals by the use of a patient monitor MMED 6000 DP (Beijing Choice Electronic Technology Co., Ltd.).

Catheter implantation

An incision was made in caudal part of *sulcus jugularis sin.* and subcutaneous tissues were blunt-prepared up to *musculus cutaneus colli*. The muscle was cut through and two lower located muscles (*m. brachiocephalicus* and *m. sternocephalicus*) were separated. A retractor was inserted into the operation wound and *v.jugularis externa* was prepared. Two fixation ligatures (4M silk) were set around the vein, proximally and distally from the point of cannulation, about 5 cm apart, and the vein was drawn into the operation wound. To prevent vasoconstriction, the vein was moistened by 2% lidocain. The vein was punctured with Seldinger needle connected with a syringe. Penetration of the needle into lumen of the vein was checked by aspiration of blood. Afterwards, the guide wire with dispenser was hooked to the needle and inserted into the vein. When the wire was in the desired position, the dispenser and the needle were removed, retaining the position of the guide wire. The distal part of the catheter was advanced over the extracorporeal part of the guide wire up to the puncture site and, rotating it slightly, moved through the site to the desired position. The tip of the central venous catheter was introduced directly into *v. cava cranialis*. Intravasal position of the introduced catheter was checked by length markings (Figure 1A). The depth of implantation of the catheter is determined by the size of the minipig and was controlled by ECG curve on the patient monitor. Implantation of the catheter into right atrium was excluded by auscultation of heart. Following the implantation of the catheter, the site of insertion was covered by fascia of *m. brachiocephalicus* and *m. sternocephalicus* and both muscles were sutured together. The catheter was then turned into a loop, to prevent its breaks in the wound, and fixed to surrounding muscles by two positioning sutures (Vicryl). The proximal

part of the catheter was directed caudo-dorsally and through a tunnel in subcutaneous tissue was led out on surface in *regio parotidea sin.*, about 3 cm behind ear base (Figure 1B). The catheter was checked for breaks and its function was tested by aspiration and application of venous blood. Consequently, the operation wound was sutured by layers, in the following order: *musculus cutaneus colli*, subcutaneum (Vicryl) and skin (3M silk). The skin around the exterior part of the catheter was firmly sutured by 3M silk and the outlet itself was fixed to skin by a simple suture. Surgical wounds were treated by fluid bandage (Novikov sol.). The animals were given antibiotics amoxicilin (Zentiva, Prague, Czech Republic; 10 mg/kg) for seven day. To increase welfare of animals, analgesia was induced for the first four days after operation by *i.m.* administration of metamizol (20 mg/kg/day, Vetalgin, Intervet).

Catheter maintenance

The operation wounds were regularly checked and treated by sol. Novikov. Surroundings of the catheter outlet was daily washed by Betadine sol. (iodopovidonum) and then overlaid by antibiotic and disinfection ointment (Framykoin ung., Bactroban ung., Dermazin ung., Betadine ung.). The minipigs well tolerated this treatment without necessity of a fixation. An administration of drugs through catheter was preceded by aspiration of blood, to make sure that the catheter is vent and to remove a possible coagulum from lumen of the catheter. Following administration, the catheter was washed through by application of 20 ml of a sterile saline solution. Finally, 1 ml of the saline with 200 IU/kg of heparin was injected in the catheter and its orifice was closed by a screw cap. The cap was changed every seven days of the experiment.

Monitoring of animals, autopsy

Health and behavior of animals, status of operation wounds and patency of the catheter were monitored during the experiment. Next, we assessed occurrence of respiratory diseases, complications after cannulation, and ascendent inflammatory processes originating from the catheter. The animals were euthanased in two months after operation by *i.v.* application of barbiturates (Thiopental,

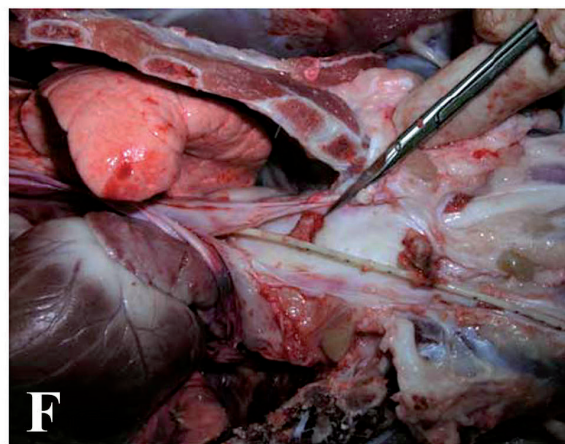
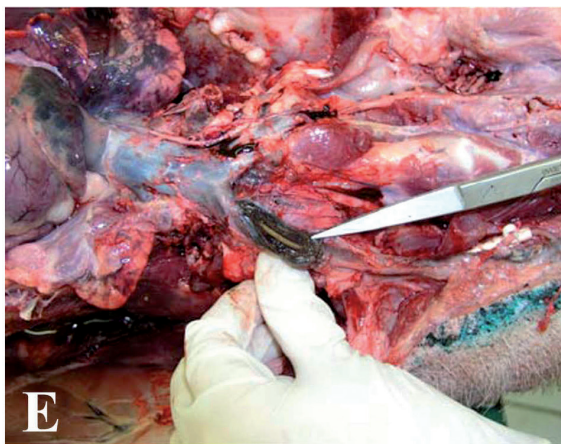
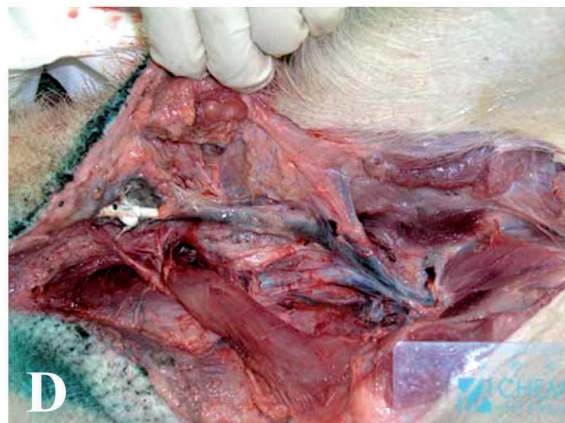
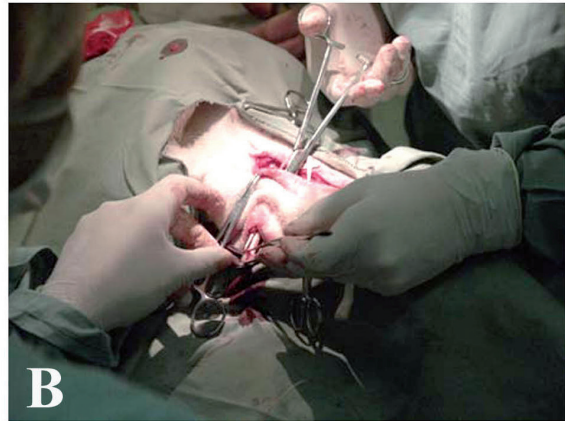
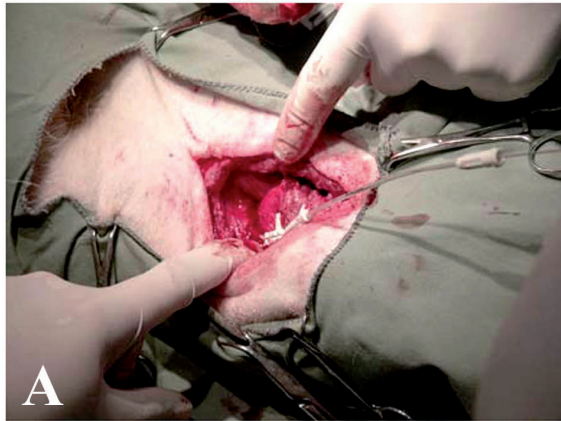


Figure 1. Implantation of the catheter and pathological findings in the experimental animals.

A – introduction of the catheter into *v. jugularis externa sin.*; B – preparation of subcutaneous tunnel for catheter outlet; C – outlet of the catheter in *regio parotidea*; D – trombophlebitis of *v. jugularis externa sin.* found in the minipig E105; E – trombophlebitis in *v. jugularis externa sin.*; F – wall-thrombi in *v. cava cranialis* found in the minipig E 103; G – *v. jugularis externa sin.* without pathological findings; H – *v. jugularis externa sin.* – catheter inside, without pathological findings

VUAB Pharma, Prague, Czech Republic; 170 mg/kg) followed by a myorelaxans (Pipcuronii bromidum – 0.08 mg/kg; Arduan, Chemical Works, Budapest, Hungary) and subjected to an anatomical autopsy. The autopsy was primarily concentrated on assessment of pathological changes in the site of cannulation and in the course of the catheter in vein, pathological changes in heart and other organs.

RESULTS AND DISCUSSION

Implantation of the catheter and complications of catheterization

The long-term cannulation of *v. jugularis externa* has been performed in pigs by surgical method (Schmitz et al., 1988; Swindle and Adams, 1988; Swindle et al., 1998; Stukelj et al., 2005) or transcutaneously (Carroll et al., 1999; Matte, 1999; Fudge et al., 2002) and proved convenient for application of medicaments or collection of blood. We have approached to the surgical method of catheteriza-

tion, using puncture of *v. jugularis externa* by the Seldinger needle. The vein is well accessible for a safe surgical preparation and is the first choice for clinics without monitoring technique since position of the implanted catheter can be reliably determined. Pigs are very liable to vasoconstriction during surgical manipulation with vessels (Swindle and Adams, 1988; Swindle et al., 1998). The vasoconstriction complicates manipulation with the vessel and the diminished lumen prevents insertion of the catheter into the vein. Such a vein is also more liable to damage or occlusion during manipulation, for example during drawing the vein into operation wound by the use of ligatures. This should be considered during preparation of the cannulated vein that must always be done carefully by the blunt method. A topical application of lidocain or papaverin proved to prevent spasm of vessels (Swindle and Adams, 1988). We have topically applied 5 ml of 2% lidocain directly into operation wound and let it work for 5–7 min. After this period of time, the vein did not exhibit tendency to vasoconstriction and manipulation with it was safe and comfortable.

Table 1. Pathological findings

Pathological finding	E66	E67	E103	E104	E105	E107	E108	E110
Granulom in operation wound	+	–	–	–	–	–	–	–
Inflammation in subcutum	–	–	–	–	–	–	–	–
Serom	–	–	–	–	–	–	–	–
Haematoma	–	–	–	–	–	–	–	–
Trombophlebitis of <i>v. jugularis externa sin.</i>	–	–	–	–	+	–	–	–
Trombophlebitis of <i>v. cava cranialis</i>	–	–	+	–	+	–	–	–
<i>Endocarditis parietalis cordis</i>	–	–	–	–	–	–	–	–
<i>Endocarditis valvularis cordis</i>	–	–	–	–	+	–	–	–
<i>Pneumonia catarrhalis acuta</i>	–	–	–	–	+	–	–	–
<i>Pneumonia interstitialis chronica</i>	–	–	–	–	–	–	–	–
Other	–	–	thrombus at catheter	–	–	–	–	–

+ = presence of pathological finding

– = absence of pathological finding

A small incision in the vein wall has been used in previous studies to facilitate insertion of catheter into vessels (Yoshikuni et al., 1984; Moritz et al., 1989; Bain et al., 1991). We used a direct penetration of the vein wall by the sharp Seldinger needle. This approach restricts manipulation with the vein, excludes bleeding into the operation wound and prevents the vein wall from an extensive damage. Following implantation of the catheter and removal of the dispenser, the vein wall firmly embraced the catheter, which safely prevented leakage of blood, but did not limit mobility of the catheter in the vein. To prevent bleeding, soaking and descendent infections at the site of cannulation, we carried out a tight suture of a part of fascia from *m. brachiocephalicus* and *m. sternocephalicus* and also sutured both muscles together. The loop in the course of the catheter well prevented the breaks but also an accidental removal of the catheter from lumen of the vein. Next, the catheter was anchored to surrounding muscle tissue by suture through the moveable fixation wings. Thus, the catheter was reliably fixed on the site of implantation.

Implantation of the catheter was carried out without complications in all experimental animals. Preparation of *v. jugularis sin.* and its catheterization by the Seldinger method proved to be a safe and well manageable procedure. No heart arrest or arrhythmias were recorded immediately after catheterization.

Maintenance of the catheter, its function and complications

Several complications have been reported to occur during or soon after the central catheterization: Catheter displacement, a wrong implantation into artery, haemotorax, haematoma, catheter embolism, arrhythmias, air embolism, damage or perforation of vein wall. Some complications occur later after catheterization, like thrombosis, thrombophlebitis, thromboembolism, local or system infection and sepsis (Sznajder et al., 1986; Swindle and Adams, 1988; Gonzales et al., 1991; Doierau et al., 1993; Yilmazlar et al., 1997; Swindle et al., 1998; Mickley, 2002; Badge et al., 2003; Yoshida, 2003). We have noticed only a local tempered swelling of subcutum, localized in the site of catheterization at the third day after operation in the minipig E66, and an obstruction of the catheter in proximal direction in the minipig E103 at the Day 20 of experiment.

The local swelling was cured by local application of Heparoid ung. and disappeared completely in a week. Obstruction of the catheter persisted until the end of the experiment, application in the distal direction worked without problems. Due to individual stalling of the pigs, the catheters were well maintained and no withdrawal or dislocation of them was observed.

Pathological assessment of animals

All experimental animals were euthanased in two months after catheter implantation. The course of the catheter was anatomically prepared and pathological changes were recorded. No macroscopically visible signs of inflammation were noticed around the outlet of the catheters (Figure 1C). All catheters were correctly introduced into *v. jugularis externa sin.* No inflammation, haematoma or blood soaking from the point of implantation was observed in operation wound. No breaks or obstructing clots were found in the catheters. All catheters were implanted into the upper third of *v. cava cranialis*. We did not observed haemothorax or pneumothorax in any of the experimental animals. Afterward, a general autopsy of all animals was carried out. The results of the autopsies are summarized in Table 1.

A trombophlebitis of *v. jugularis ext. sin.* was found in the minipig E105. The trombophlebitis afflicted the hole course of *v. jugularis* up to joining with *v. cava cranialis* that was also severely afflicted by the inflammation (Figure 1D). Clinical symptoms of a respiratory disease had been recorded in this animal and these were confirmed during the autopsy by finding *pneumonia catarrhalis acuta* in both diaphragm lung lobes. However, this finding is frequent in conventional breeding. No other clinical symptoms of a disease were observed in this animal, *v. jugularis externa dx.* was not affected by the inflammation. Catheter was functional for application as well as aspiration. The trombophlebitis displayed during autopsy by darkening and thickness of the vein wall (Figure 1E) and crepitation in the lumen. A thrombus was found in the right heart ventricle on *cuspidis septalis valvae tricuspidalis*, which argues for a descendent progress of inflammation from the proximal parts of venous system. Infection probably originated at the site of catheter outlet at the skin surface, even though no symptoms of inflammation were observed there. The autopsy

of the minipig E103 demonstrated presence of two wall-thrombi in *v. cava cranialis* (Figure 1F). No other thrombi were found in tissues and this animal did not show any clinical symptoms of a disease. An interesting finding in this animal was a thrombus attached to the distal tip of the catheter that was probably the cause of the failure of aspiration described above. An amplification of subcutaneous tissue was observed in the animal E66 and was diagnosed as a granuloma of the size 3.8×5 cm. The granuloma was freely located in subcutum, transition of inflammation to the catheterized vein was not observed. In the other catheterized animals, no symptoms of thrombophlebitis or endocarditis was noticed (Figure 1G, H). No macroscopic pathological changes were observed in other organs.

In conclusion, we have demonstrated in the present study that central venous catheterization by Seldinger method is suitable for experimental animals that must be repeatedly given large volumes of solutions into venous system or in which blood samples are often collected. The Seldinger method of catheter implantation eliminates the complications associated with the central venous catheterization. Even though the experimental animals were kept in conventional breeding conditions in this study, the autopsy showed complications in only two of eight animals.

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