UPPSALA
UNIVERSITET

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 1337

Experimental Studies on Diagnostic and Therapeutic Aspects of Intraosseous Access

GUNNAR STRANDBERG

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2017

ISSN 1651-6206
ISBN 978-91-554-9937-2
urn:nbn:se:uu:diva-321403

Dissertation presented at Uppsala University to be publicly examined in Hedstrandsalen, Akademiska Sjukhuset, Uppsala, Friday, 1 September 2017 at 13:00 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in Swedish. Faculty examiner: Docent Stefan Lundin (Göteborgs Universitet).

Abstract
Strandberg, G. 2017. Experimental Studies on Diagnostic and Therapeutic Aspects of Intraosseous Access. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 1337. 55 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-554-9937-2.

Reliable access to the circulation is paramount in most medical and surgical emergencies. When venous access cannot be expediently established, intraosseous (IO) access is indicated. This method has a high success rate even in relatively inexperienced hands and there is considerable clinical experience of IO administration of drugs and fluids. There is however limited evidence on the use of IO samples for laboratory analysis. Also, uptake of drugs during shock has not been extensively studied. Further, there have been concerns that analysis of IO samples may damage laboratory equipment. We have studied, in a porcine model, the use of IO samples for point of care analysis of blood gases, acid base parameters and blood chemistries in stable circulation, in experimental septic shock, and in hypovolemia after major hemorrhage, comparing IO samples with arterial and venous samples, and comparing IO samples from different sites. We have also studied coagulation assays on IO samples in stable circulation and after major hemorrhage. Furthermore, we have compared IO and intravenous administration of antibiotics in experimental sepsis.

Average differences between IO and arterial/venous samples varied between the studied analytes. During stable circulation, average IO levels of blood gases, acid-base parameters, hemoglobin/hematocrit and several blood chemistries approximated venous levels relatively well. Differences in acid-base and blood gas parameters, and lactate, were more pronounced in hypovolemia, as well as in sepsis. The dispersion of the differences was often relatively large, indicating limited precision. Average differences between two intraosseous sites were small.

Intraosseous samples were clinically hypercoagulable with a strong tendency to clot in vitro, and thromboelastography demonstrated shortened reaction times compared with venous samples. Major bleeding and hemodilution moderately affected the studied coagulation parameters.

In endotoxemic animals with circulatory instability, concentrations of cefotaxime and gentamicin in samples from the pulmonary artery were comparable at 5 minutes after intraosseous and intravenous administration, and during a 3 hour observation period.

In summary, agreement between analytes in intraosseous and conventional blood samples was variable and often unpredictable, especially during circulatory compromise. Intraosseous samples clinically appeared hypercoagulable, and thromboelastography confirmed this. High and comparable concentrations of cefotaxime and gentamicin were found after intraosseous and intravenous administration of equivalent doses, suggesting that uptake is acceptable during septic instability.

Keywords: Infusions, Intraosseous, Sepsis, Point-of-care Systems, Blood Coagulation, Antibiotics

Gunnar Strandberg, Department of Surgical Sciences, Anaesthesiology and Intensive Care, Akademiska sjukhuset, Uppsala University, SE-75185 Uppsala, Sweden.

ISSN 1651-6206
ISBN 978-91-554-9937-2
urn:nbn:se:uu:diva-321403 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-321403)

Tillägnas min familj

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I Strandberg G, Eriksson M, Gustafsson M, Lipcsey M, Larsson A. Analysis of intraosseous samples using point of care technology – an experimental study in the anaesthetised pig. Resuscitation 2012; 83: 1381-1385

II Strandberg G, Larsson A, Lipcsey M, Berglund L, Eriksson M. Analysis of intraosseous samples in endotoxemic shock – an experimental study in the anaesthetised pig. Acta Anaesthesiologica Scandinavica 2014; 58: 337-344

III Strandberg G, Larsson A, Lipcsey M, Michalek J, Eriksson M. Intraosseous and intravenous administration of antibiotics yields comparable plasma concentrations during experimental septic shock. Acta Anaesthesiologica Scandinavica 2015; 59: 346-353

IV Strandberg G, Lipcsey M, Eriksson M, Lubenow N, Larsson A. Analysis of thromboelastography, PT, APTT and fibrinogen in intraosseous and venous samples – an experimental study. Scandinavian Journal of Trauma Resuscitation and Emergency Medicine 2016; Nov 3; 24(1):131

V Strandberg G, Larsson A, Lipcsey M, Eriksson M. Comparison of intraosseous, arterial and venous blood sampling for laboratory analysis in haemorrhagic shock. In manuscript.

Contents

Abbreviations

ADP	Adenosine Diphosphate
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
APTT	Activated Partial Thromboplastin Time
AST	Aspartate Aminotransferase
ATLS	Advanced Trauma Life Support
AUC	Area Under the Curve
BE	Base Excess
CE	Conformité Européenne
CI	Confidence Interval
CK	Creatine Kinase
CO	Cardiac Output
CPR	Cardiopulmonary Resuscitation
CV	Coefficient of Variation
DO_2	Delivery of O_2
FVIIa	Factor VIIa
GP1b	Glycoprotein 1b
γ-GT	γ-glutamyltransferase
Hb	Hemoglobin
ICU	Intensive Care Unit
INR	Internationalized Normalized Ratio
IO	Intraosseous
IQR	Interquartile Range
IV	Intravenous
K	Kinetics
LoA	Limits of Agreement
Ly30	Lysis at 30 minutes
MA	Maximal Amplitude
MAP	Mean Arterial Pressure
MIC	Minimal Inhibitory Concentration
NO	Nitric Oxide
PCO_2	Partial Pressure of CO_2
POCT	Point of Care Testing
PT	Prothrombin Time
R	Reaction Time
SD	Standard Deviation

SIRS	Systemic Inflammatory Response Syndrome
SOFA	Sequential Organ Failure Assessment
SO_2	Hemoglobin oxygen saturation
TEG	Thromboelastography
TEM	Thromboelastometry
TF	Tissue Factor
TFPI	Tissue Factor Pathway Inhibitor
TLR4	Toll Like Receptor 4

Introduction

The rapid establishment of vascular access is paramount in most medical and surgical emergencies in order to administer drug or fluid therapy. Securing blood for various laboratory analyses is also an essential part of emergency care. Peripheral venous catheterization is normally the preferred first line vascular access. However, this can sometimes be a demanding task in the critically ill patient. A special consideration is the pediatric patient where venous access is frequently difficult even under stable conditions, and obesity is another factor negatively affecting the success rate.(1, 2) In emergencies, intraosseous (IO) access is often recommended as an alternative when peripheral venous access fails.(3, 4) This method is reported to be fast and have a high success rate even in relatively inexperienced hands.(5, 6)

Documentation of the successful use of IO access in resuscitation is plentiful.(7, 8) IO infusion flow rates of up to 200 ml/min using a pressure bag have been reported in the clinical setting.(9, 10) Laboratory investigations have demonstrated delivery of injected substances to the central circulation to be as fast as with intravenous (IV) administration although the delivered dose was sometimes smaller for the IO route.(11-13) Antibiotics are examples of drugs that are highly dependent on adequate concentrations for clinical efficacy.(14, 15) IO administration of antibiotics has been previously studied in animal models under hemodynamically stable conditions.(16, 17)

The possibility of using IO aspirate for laboratory analysis has also been studied. Animal laboratory investigations have found acceptable agreement between pH and PCO2 in IO and central venous samples during cardiopulmonary resuscitation.(18) Also, comparable values for common blood chemistries including hemoglobin, creatinine and electrolytes have been demonstrated in human and animal subjects. (19-21) However, studies are not always in agreement over the reliability of analyses and the studies on human subjects were conducted during hemodynamically stable conditions, which is not generally the case in clinical situations when IO access is used. Furthermore, questions have been raised concerning the possible damage to laboratory equipment from analyzing IO samples.(22, 23)

The analysis of coagulation studies, important in many medical and surgical emergencies, in IO samples has, to the best of our knowledge, not been previously studied or published.

The present work partially concerns the feasibility of using point of care equipment for the analysis of IO samples. This could enable clinicians to acquire laboratory data in remote or prehospital emergencies and also circumvents the risk of damage to laboratory instruments, if cartridge based analyzers are used. Furthermore, we have studied the analysis of IO samples in experimental septic and hemorrhagic shock models, and coagulation analyses on intraosseous samples, none of which have been thoroughly investigated previously. We have also studied the delivery of antibiotics to central circulation after IO administration in an experimental sepsis model.

Background

Bone marrow anatomy and intraosseous access

The bone marrow accounts for approximately 5 % of the body weight of an adult human. It is the primary hematopoietic organ and a major lymphatic tissue.(24) In the newborn, all bones contain red bone marrow with active hematopoiesis. With ageing, an increasing portion is transformed into yellow marrow with an abundance of adipocytes and no active hematopoiesis, although red marrow is found in the flat bones, such as the sternum and the ilium, and in the epiphyseal parts of long bones. The yellow marrow has the potential to reassume hematopoiesis if necessary.(25)

The red marrow consists of compartments of blood cells and precursors, surrounding the marrow blood vessels, together with adipocytes, connective tissue cells and matrix found within a mesh of trabecular bone.(24)

The bone marrow, both red and yellow, is a highly vascularized tissue. Arteries enter the long bones through nutrient foramina which are most abundant in the diaphyseal and epiphyseal areas. The bone marrow and vessels are also supplied by myelinated and unmyelinated nerves entering through the foramina.(24) Within the bones, arteries branch into arterioles that traverse the cortical bone in perforating (Volkmann´s) and longitudinal (Haversian) canals. Within the bone marrow, arterioles supply a system of sinusoids, small and highly permeable vessels with a single layer of endothelium, which eventually drain through venules into a central venous sinus. From here, veins reenter the systemic circulation through the nutrient foramina.(24, 25) Thus, by cannulating the marrow cavity, one may gain access to the central circulation, by the same route as is used by the mature blood cells from the marrow. In contrast to peripheral veins, which may collapse during shock and exsanguination, the IO route is accessible also under such conditions, and the uptake of substances administered IO has been demonstrated to be comparable with that for IV administration.(11-13)

In experimental swine models, the pressure in the marrow of long bones has been reported to be in the order of 20-30% of the arterial pressure and related to changes in the same.(26, 27) Also in a swine model, a blood flow in the tibia of approximately 15 ml / 100 g / minute was demonstrated during stable conditions, and was substantially reduced during hypovolemia and after catecholamine administration.(28) Reduced tibial blood flow has also been demonstrated in rats upon adrenergic stimulation with norepinephrine.(29)

Administration of phentolamine in the iliac artery, in a human study, increased local blood flow, suggesting alpha-adrenergic regulation.(30)

The method of IO access was first described by Drinker in 1922 and further investigated by Tocantins and O´Neill.(31-33) At this time, venous catheterization was performed with reusable steel needles and was far less straightforward than with today's plastic catheters. IO access was used during World War II but with the advent of the plastic IV catheter in the 1950s the method was essentially abandoned.(32) In the 1980s, however, the method regained interest as a rescue alternative for difficult vascular access, especially in children where IV access is often challenging.(34) Several medical devices exist for the insertion of IO catheters, including hand-powered, spring-loaded and battery-powered instruments.(35) Commonly used sites for insertion are the proximal tibia, the humeral head or the sternum but several other sites including the distal tibia or the distal femur have been described.(35) A site of insertion is identified and a correct needle length is chosen. The latter will depend on site of insertion and patient habitus, with humeral access often requiring a longer needle than insertion in the lower extremity.(36) Specialized devices for sternal access are commercially available and are primarily used by military medical personnel.(37) Equipment for IO access is widely available in Swedish emergency departments and prehospital units. Complications from IO cannulation are rare, but include fractures, infections and extravasation of fluids from infusion in previously punctured or fractured bone, or with improperly positioned catheters. Such extravasation has been reported on several occasions to cause compartment syndrome and ischemia.(38) For sternal puncture, serious intrathoracic injury is also a potential hazard.(35) Fat embolism has been discussed as a potential problem, and animal studies have demonstrated that this phenomenon frequently occurs after IO infusion, although it is not commonly reported as a clinical complication. (39, 40)

In pediatric use, growth plate injury has also been discussed. A radiographic study, however, found no evidence of this.(41)

Absolute contraindications are fracture or previous recent IO cannulation of the bone in question, due to the abovementioned risk of extravasation. Further contraindications are infection at the intended site of puncture and the rare bone diseases osteogenesis imperfecta and osteopetrosis, due to an increased risk of fracture in these conditions. Also, the inability to confidently identify a site of insertion is a contraindication to the procedure. IO cannulation in itself is reported to be only moderately painful, but IO injection and infusion causes increased IO pressure and is very painful. It is therefore often recommended that a small dose of local anesthetic is first administered in awake subjects.(42)

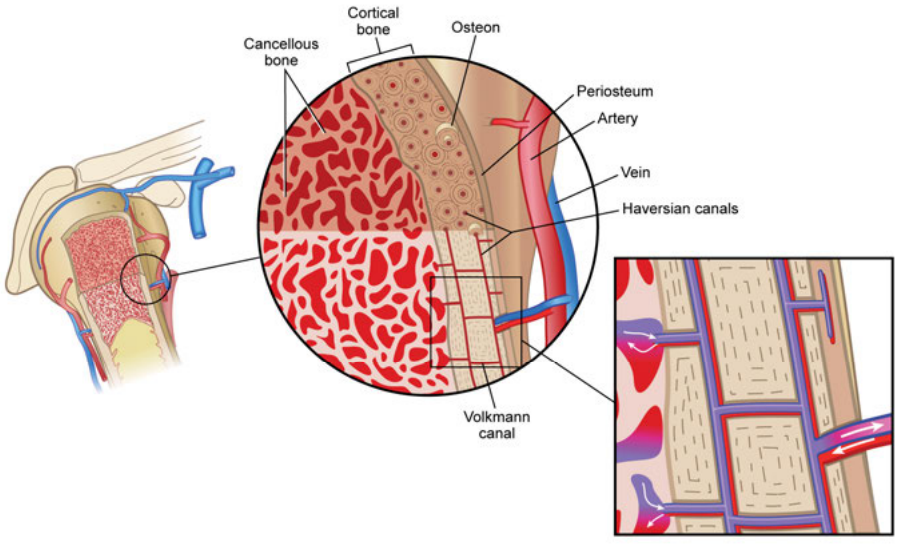


Figure 1: Intraosseous Anatomy. Image Courtesy of Teleflex Incorporated. © 2017 Teleflex Incorporated. All rights reserved.

Intraosseous fluid and drug administration

Drinker and others described the use of the bone marrow for infusion of blood products and other substances already during the first half of the 20th century.(31, 33) Since then, considerable clinical experience with the method has accumulated. Experimental studies have been undertaken to determine maximal infusion flow rates. A recent study on human cadavers found the highest mean flow rates (93. 7 ml/min), with sternal IO access followed by humeral access (57. 1 ml/min) and tibial access (30. 7 ml/min), all using 300 mm Hg infusing pressure.(43) However, small clinical studies have reported flow rates as high as 204 ml/min (tibia) and 153 ml/min (humerus), but with large variations.(9, 10)

Experimental data also exist on emergency drug effects and concentrations after IO administration. In a 1990 study, Orlowski et al. studied, after femoral IO administration in stable anesthetized dogs, the clinical effect of adrenaline and bicarbonate, and plasma concentrations of lidocaine, glucose and calcium, and found the IO route to be comparable with central venous administration.(44) More recently, Burgert et al studied adrenaline uptake in a porcine cardiopulmonary resuscitation (CPR) model and found both tibial and sternal IO administration to be inferior to IV administration.(45) Hoskins et al. studied central venous, sternal IO and tibial IO administration of tracer dye in a porcine CPR model and found that dose delivery was lower and time to peak

concentration longer after tibial IO administration compared with the other sites.(13)

Recently, Yost et al. found similar pharmacokinetics after IO and IV administration of atropine during hypovolemia in a porcine model.(46) In what is, to the best of our knowledge, the only human study to date, von Hoff et al. studied pharmacokinetics of morphine injection IO (iliac crest) or IV in 14 volunteers and found no significant differences between the sites regarding maximal plasma concentrations and dose delivered.(11)

Jaimovich et al. studied tibial IO administration of cefotaxime, vancomycin, chloramphenicol and tobramycin in a porcine model during stable circulation.(17) Compared with IV administration, peak concentrations and the area under the time- concentration curve (AUC) of all studied drugs were lower for the IO route. Although differences were not always statistically significant, only cefotaxime reached therapeutic concentrations. Postmortem examinations revealed high drug concentrations at the site of injection, suggesting sequestration.

Pollack et al. studied IO administration of ceftriaxone, cefotaxime, ampicillin and gentamicin, also in a porcine model, and found lower levels of ceftriaxone after IO administration, while the remaining drugs demonstrated comparable levels.(16)

Intraosseous sampling for laboratory analysis

Although the primary objective when establishing IO access will most likely be administration of drugs and fluids, sampling for laboratory analysis is also desired in medical emergencies. Examples of laboratory analyses that may be of immediate diagnostic and prognostic importance in an emergency are hemoglobin, glucose, acid base parameters, electrolytes, blood gases, c-reactive protein, lactate, cardiac enzymes, cultures, toxins, drug levels and coagulation parameters. If an IO catheter is the only available vascular access, it is important to know if samples from this can be used for analysis.

Orlowski et al. demonstrated no significant differences between electrolytes, blood chemistries and hemoglobin among IO, arterial and venous samples in healthy anesthetized dogs.(21)

Hurren compared venous samples with simultaneous IO samples obtained during scheduled bone marrow biopsy in children and found acceptable agreement for hemoglobin, sodium, calcium and creatinine but not for potassium and glucose.(47)

In another study performed in conjunction with scheduled bone marrow biopsy, Ummenhofer et al. found hemoglobin, sodium, chloride, creatinine, urea, bilirubin, glucose and pH but not PCO_2 and PO_2 in IO samples to be good predictors of venous values.(48)

Abdelmoneim et al. compared levels of pH and PCO_2 in IO and mixed venous samples in anesthetized pigs during CPR and found clinically significant differences in pH after 15 minutes of CPR and when bicarbonate was administered. Differences in PCO_2 were highly variable.(49)

Miller et al, in a study on ten healthy volunteers, found significant correlation between IO and venous levels of glucose, creatinine and albumin but not for sodium, potassium, calcium and PCO_2.(20)

Point of care laboratory analysis

As described above, among other tests, hemoglobin, glucose, electrolytes, lactate, acid-base status and blood gases can help to identify the cause and severity of illness and are often requested in emergencies. The abovementioned analyses could all immediately influence medical decision making in critical situations and are often available on point of care testing (POCT) instruments in various clinical settings. The goal of POCT is to provide short turnaround times for the analyses, allowing faster medical decisions.(50) This is always important in an emergency, but point of care testing may also be valuable in remote or prehospital settings where a central laboratory is not available.(51)

Point of care analyses may be performed on whole blood samples for example in an emergency department or even during transport using smaller portable instruments. Depending on the analysis in question, arterial, venous or capillary blood may be used. Medical devices for POCT are subject to a European directive on in vitro diagnostics and requires CE marking before being allowed on the European market.(52)

Benchtop blood gas analyzers are widely available as point of care instruments in Swedish intensive care units, operating theatres and emergency departments. A disadvantage with such instruments, when analyzing IO samples, is that they are normally not using disposable cartridges. Blood clots, lipids and bone fragments in the samples could possibly block the narrow tubings in the instrument and cause damage or prevent further testing until service has been performed. It may therefore theoretically be advantageous to use single-test cartridges when analyzing IO samples.

Sepsis and septic shock

Sepsis has until recently been defined as a systemic inflammatory response syndrome (SIRS) caused by infection, where SIRS is defined as the presence of two or more of the following findings: hyper - or hypothermia, tachypnea or hyperventilation, tachycardia, and leukocytosis or leucopenia. The defini-

tion of severe sepsis is sepsis with signs of organ dysfunction or hypoperfusion, and when hypotension is fluid refractory the condition is termed septic shock.(53, 54)

Recently, a new definition of sepsis was proposed in a consensus paper by the Sepsis definitions task force.(55)

In this paper, sepsis is defined as organ dysfunction, manifested as an increase in the Sequential Organ Failure Assessment (SOFA) score of at least 2 points, in the setting of suspected infection.(56) Baseline SOFA score is assumed to be 0 unless previous organ dysfunction is known to be present. Thus, this definition shifts the focus from inflammation to organ failure.

Although modern health care has improved the prognosis, mortality in septic shock is still high and it is the most common cause of death among critically ill patients in non-coronary intensive care units (ICU) in the United States.(57, 58)

Recent studies suggest an increased incidence of sepsis in developed countries, possibly attributable to an ageing population and increased recognition, while the mortality of the condition seems to have decreased over the last decades.(55, 59, 60)

Sepsis is often accompanied by hemodynamic instability to which vasodilation, microvascular leakage and myocardial dysfunction may all be contributing.(54, 61) Underlying mechanisms are still not completely understood but interactions between the infecting agents and the host immune system and tissues involving complex neurohumoral mechanisms are implicated.(61, 62) Present research highlights the possible importance of the endothelial glycocalyx for vascular integrity and barrier function, and suggests that this structure is disturbed by reactive oxygen species and cytokines in sepsis.(63) Nitric oxide (NO) has long been implicated as an important mediator of septic vasoplegia although interventions with NO inhibitors has not been convincingly successful.(64, 65) Myocardial dysfunction is also recognized as a common finding in sepsis and can lead to a hypodynamic state with decreased contractility and stroke volume where the cardiac output (CO) may or may not be conserved by tachycardia.(61, 66, 67) In contrast, a hyperdynamic vasoplegic state is often attributed to the early phase of sepsis, at least when fluid resuscitation has been commenced.(68) Hypodynamic shock in sepsis is reported to be a more common finding in the pediatric population.(69)

Although the pathophysiology of sepsis remains to be fully elucidated, great effort is made to improve the level of care for this life-threatening condition. It has been demonstrated that protocol-based, goal-directed treatment improves outcome in sepsis, although this is challenged in recent trials, and evidence based guidelines are updated and published regularly.(53, 70, 71)

The timely administration of adequate antibiotic therapy is of undisputed importance in sepsis in order to increase the likelihood of survival, and supportive therapy with fluid infusions and vasoactive drugs is frequently

needed.(53, 72) Therefore, gaining reliable access to the circulation is an important early goal when caring for a septic patient. Laboratory analyses are also important in confirmed or suspected septic patients for diagnostic and prognostic purposes. Notably, both initial lactate and its clearance have been reported to have prognostic value in sepsis.(73, 74)

In the critically ill patient with a suspected serious infection, antibiotic treatment should be initiated with broad-spectrum agents likely to cover all potential causative microorganisms. When a probable culprit organism is identified, therapy should be narrowed in order to prevent the selection of resistant pathogens and also to prevent adverse drug effects and opportunistic infections.(53) The choice of agents should take into account patient-specific factors such as the suspected focus of infection, immunosuppression, known microbial colonization and recent antibiotic therapy as well as the local microbiologic panorama including drug resistance patterns. In Sweden, recommended first line therapy for community acquired serious infections commonly includes a broad-spectrum beta-lactam, such as cefotaxime, and in severe sepsis or septic shock an aminoglycoside is often added.(75) Plasma concentrations of antibiotics are important both for clinical effect and toxicity. For beta-lactam antibiotics, the time above minimal inhibitory concentration (MIC) is crucial for antimicrobial activity, while for the aminoglycosides the peak concentration is regarded as more important.(14, 15)

Depending on bioavailability, antibiotics may be administered orally or parenterally. In the circulatory unstable septic patient, however, the uptake of orally administered drugs may be questioned, and when bowel function is compromised parenteral administration is the only option. Also, the time taken to reach peak concentrations is longer with oral administration. When a very rapid effect is desired, intravenous injection may be the preferred alternative.(76)

Hemorrhage, hemostasis and coagulation assays

Hemorrhage with significant loss of intravascular volume is a medical emergency that may lead to impaired tissue perfusion and ultimately ischemic organ damage and death.(77) Both a low circulating volume and the isovolemic anemia following resuscitation of hemorrhage with crystalloid and colloid fluids impair oxygen transport, as evident from the equation for oxygen delivery: $DO_2 = CO \times (Hb \times 1.39 \times SaO_2 / 100) + (0.003 \times PO_2))$

Here, DO_2 is the delivery of O_2 (mL/min), CO the cardiac output (L/min), Hb the hemoglobin level (g/L), SaO_2 the arterial oxygen saturation in % and PO_2 the arterial partial pressure of O_2 in mm Hg. The constant 1.39 denotes the maximal theoretical oxygen carrying capacity (mL) of 1 g of hemoglobin and 0.003 ml is the volume in mL corresponding to 1 mm Hg of dissolved oxygen.(78)

When the DO_2 becomes critically low, tissue oxygenation is insufficient and anaerobic metabolism will occur. If cellular energy supplies are depleted, eventually membrane integrity is compromised and the cell dies.

The volume of blood loss that may be tolerated, or compensated for, before symptoms and clinical findings are apparent, will be dependent on patient factors, e.g. cardiovascular and pulmonary status. Based on clinical findings, hypovolemia is often divided in classes or stages from I (mild) to IV (preterminal).(77) Such classifications, deriving from the Advanced Trauma Life Support (ATLS®) concept, however, are sometimes criticized for being unreliable.(79) A hypothetical explanation for this may be the abovementioned variations in ability to compensate due to age, comorbidity or medication.

The physiological response to major hemorrhage and hypovolemia includes tachycardia and tachypnea, decreased diuresis and endogenous plasma volume expansion driven by a decreased capillary pressure and the osmotic effect of an elevated blood glucose due to glycogenolysis.(77, 80) Another crucial part of the endogenous response to injury and hemorrhage is hemostasis.

Blood clotting is regulated by a complex system of cellular and humoral mechanisms. The primary hemostasis results from interaction between platelets and subendothelial collagen, exposed upon tissue damage, with von Willebrand factor, binding to the platelet glycoprotein 1b (Gp1b), acting as a bridge. This triggers degranulation with release of various substances from the platelet, including the vasoactive substances serotonin, thromboxane A2, and adenosine diphosphate (ADP), stimulating further platelet activation.(81) The activated platelets aggregate and expose the phospholipid phosphatidylserine, an important catalyst for the clotting cascade, on their outer surfaces.(82)

The secondary hemostasis in vivo starts with interaction between factor VIIa and Tissue Factor, the latter exposed in endothelial damage.

The FVIIa -TF complex then activate other factors, finally resulting in the generation of a small amount of thrombin. The thrombin thus generated augments the clotting reaction, now taking place on the surfaces of activated platelets, generating much larger amounts of thrombin. Thrombin then converts fibrinogen to fibrin, forming a blood clot, which is reinforced by cross-linking of the fibrin mesh with factor XIIIa. This process is also restricted by several inhibitors, the best known being antithrombin III, protein C and Tissue Factor Pathway Inhibitor (TFPI).(83) Both the primary and the secondary hemostasis are frequently targets of therapeutic agents; platelet inhibitors and anticoagulants.

In parallel with the clotting process starts a process of fibrinolysis, serving to control and eventually break down the clot. This is accomplished by the action of plasmin, a serin protease, on the fibrin in the clot. Plasmin is generated from its precursor plasminogen primarily by the enzyme tissue plasminogen activator (tPa), released from the endothelium.(84) This mechanism is utilized in thrombotic conditions such as myocardial infarction, ischemic

stroke and pulmonary embolism, when exogenous plasminogen activator may be administered to accomplish clot lysis.(85)

Both clotting and fibrinolysis may be affected in various pathologic conditions, with major trauma and sepsis being well known examples. In these conditions, platelets and the coagulation cascade may be activated by molecules released from microbial pathogens or damaged tissues, causing thrombotic complications.(86) Secondarily, consumption of platelets and coagulation factors, as well as inhibitors of fibrinolysis, may result in bleeding diathesis. The latter may be further augmented by hypothermia, acidosis and hemodilution.(85, 87)

Thus, there are several reasons why laboratory analysis of blood clotting may be important in medical emergencies, and the various parts of this intricate system are studied with different assays. The prothrombin time (PT) measures the time taken to form a fibrin clot in a decalcified, platelet poor plasma sample after the addition of calcium, TF and phospholipid. The time is reported in seconds or as the International Normalized Ratio (INR), gained by dividing the measured time by a normal PT. This test is sensitive to deficiencies of factors II, V, VII, and X.(83, 88) The Activated Partial Thromboplastin Time (APTT), is the time, given in seconds, to clotting of a decalcified, platelet poor plasma sample after the addition of calcium, phospholipid and a contact activator, often kaolin. This test is sensitive to deficiencies of factors other than FVII.

Fibrinogen may be measured by comparing the clotting time, after the addition of thrombin to the sample, with a standard curve of known concentrations.(83)

The abovementioned tests are often performed in central laboratories, although point-of care tests are also available, and analyze specific parts of the clotting system. In contrast, thromboelastography (TEG) and thromboelastometry (TEM) are functional assays that test blood clotting on a global level and may be performed at the point of care. Although the technology is not new, these analyses are presently receiving increased attention, and a US 2014 consensus report recommended their use during trauma resuscitation. However, a 2015 Cochrane review stated that there is insufficient data on their accuracy in this setting.(89-91)

Both TEG and TEM analyze the viscoelastic properties in decalcified whole blood samples after the addition of calcium and contact activation. The sample is placed in a cup and a pin is suspended in the sample. The pin and cup rotate relative to each other (the cup rotates in TEG and the pin in TEM) and as a clot is formed, torque is created, resulting in a curve, the morphology of which can be analyzed. Relevant parameters are the time to initiation of clot formation, the time from initiation of clotting until a defined clot strength is reached and the maximal clot strength, as well as evidence of clot lysis. In the TEG assay, these are reflected by the reaction time (R), kinetics (K) and

alpha-angle (α), maximal amplitude (MA) and lysis at 30 minutes (Ly30), respectively.

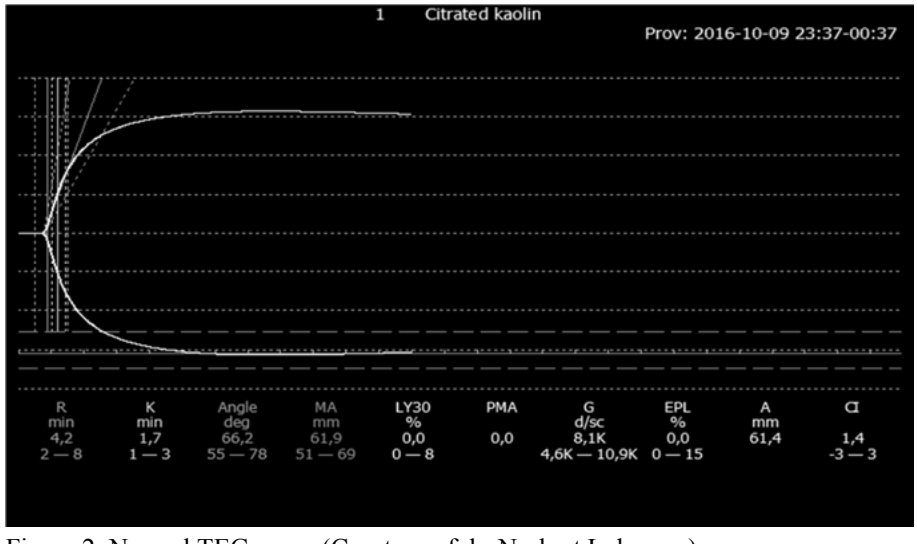


Figure 2: Normal TEG curve (Courtesy of dr. Norbert Lubenow)

The animal model

When a research question concerns human physiology, it should ideally be studied on human subjects. However, this is not always possible for various reasons.

In a clinical scenario where IO access is used, the patient will often not have another access suitable for sampling. Also, in a time-critical emergency, it may be both logistically difficult and ethically problematic to draw extra samples for research purposes. Therefore, to compare IO samples with arterial or venous samples during critical illness, an animal model is a reasonable alternative. It would also be ethically unacceptable to randomize critically ill patients to receive drug treatment IV or IO, or indeed to insert a possibly redundant IO access when venous access is already in place.

When choosing a suitable animal model species, the nature of the research questions must be considered.

The circulatory system of the pig is in many aspects, such as the relative size of heart and blood vessels, similar to that of humans, and the species is often used for experimental studies and surgical training.(92) It is sufficiently large to allow instrumentation and use of human medical equipment, and the blood volume allows repeated sampling, making it suitable for intensive care related research. Also, pigs are readily available from local producers without long and potentially stressful transports.

In these experiments, general anesthesia was induced with a combination of tilétamin-zolazepam, ketamine and morphine and maintained with pentobarbital, morphine and, with the exception of paper V, rocuronium. This general model of anesthesia is well established in our animal laboratory.(93, 94)

In paper II, an experimental sepsis model with endotoxin infusion was used.

Endotoxins, or lipopolysaccharides (LPS), are molecules found in the cell walls of gram-negative bacteria and are potent immunostimulators. The LPS consists of an inner lipid region (Lipid A), primarily responsible for its toxicity, an intermediate core oligosaccharide and an outer polysaccharide, the O-antigen.(95)

LPS binds to the Toll-like receptor 4 (TLR4) complex in immunocompetent cells of humans and various animal species, leading to the activation of inflammatory pathways.(96, 97) As endotoxin can be stored, quantified and administered in standardized doses, it offers a simple and reproducible model of sepsis.

Porcine endotoxemic shock models have been widely utilized in experimental sepsis research.(98) The porcine pulmonary vasculature is sensitive to endotoxin, causing significant pulmonary hypertension on endotoxin exposure.(99) This initially leads to hypodynamic circulation with decreased cardiac output (CO) in porcine sepsis models, in contrast to the hyperdynamic circulation often seen in the early phase of human septic shock. However, hypodynamic circulation is reported to be a common finding in children with community acquired septic shock (68, 69) (100, 101)

There is also a scarcity of information on the natural history of unresuscitated sepsis. The definition of septic shock implies hypotension refractory to fluid treatment. By the time cardiac output measurements are available, the patient is likely to have received significant volumes of fluid and may be more prone to exhibit the classically described hyperdynamic picture.

In this experiment, an infusion of 4 μg/kg/h of endotoxin (Sigma Chemical, St. Louis, MO) was administered. This is a relatively high dose, in our previous experience able to quickly induce a syndrome of multi-organ failure.(94)

In paper IV, animals were subjected to hemorrhage and resuscitation with crystalloid to induce hypovolemia and hemodilution, and in paper V, hemorrhage was also induced.

Research Aims

To evaluate the feasibility of analyzing of IO samples with a cartridge based point-of-care instrument

To study the possibility of using IO samples, under stable physiologic conditions, during septic shock and during hypovolemia, as a substitute for arterial or venous samples for emergency laboratory analyses including blood gases, acid base parameters, hemoglobin, electrolytes, glucose and liver enzymes.

To compare simultaneous samples from different IO insertion sites for laboratory analysis

To study the performance of the IO route for administration of selected antibiotics during experimental sepsis and compare this with IV administration.

To study the possibility of using IO samples for coagulation studies, during stable conditions and after hemorrhage and hemodilution.

Material and Methods

Protocols

Paper I

Five apparently healthy young domestic-breed pigs were anesthetized and received an arterial catheter and bilateral tibial IO catheters. Samples were taken hourly during 6 hours from the arterial and both IO sites and analyzed directly for hematocrit, pH, PCO_2, PO_2, sodium, potassium, calcium and lactate. Secondary calculated parameters were hemoglobin, base excess, HCO_3^- and SO_2. The analyses were performed using an Istat® (Abbott Point of Care, Princeton NJ) portable point-of-care instrument with disposable cartridges. Hemodynamic parameters were monitored continuously and registered hourly during the experiment. Mean levels of the measured analytes from all sampling sites and average differences between simultaneous samples from different sites were calculated.

Paper II

Eight anesthetized pigs were exposed to a high dosed endotoxin infusion for 6 hours to induce experimental septic shock. IO accesses were inserted at three different sites, the tibia bilaterally and the humerus. Arterial, peripheral venous and central venous catheters were also inserted. A continuous infusion of Ringer´s acetate was given at a constant rate of 40 ml/h in one of the tibial IO catheters. Noradrenaline infusion was administered to keep mean arterial pressure (MAP) $\geq$ 60 mm Hg. Hourly samples were collected from the arterial, central venous and all three IO access points during 6 hours and analyzed for hematocrit, glucose, PO_2, PCO_2, pH, and lactate using point of care equipment as described in paper I. Secondary calculated parameters were hemoglobin, base excess and HCO_3^-.Analyte levels were compared among the different sampling sites.

Paper III

Eight anesthetized pigs were exposed to endotoxin infusion during 6 hours. At the onset of severe hypotension (defined here as a reduction of MAP of $\geq$

30% of baseline level recorded after induction of anesthesia) alternatively after 3 hours of endotoxemia, if the first criterion was not satisfied during this time, they were randomly administered 75 mg/kg of cefotaxime and 7 mg/kg of gentamicin as bolus injections either in a proximal tibial IO cannula (n=4) or in a peripheral IV catheter (n=4). Samples from the pulmonary artery were then taken after 5, 15, 30, 60, 120 and 180 minutes and antibiotic concentrations were analyzed as described below. Noradrenaline was administered if necessary, aiming for a MAP ≥ 60 mm Hg. Central concentrations of the drugs were compared between IO and IV administration.

Paper IV

Ten pigs were anesthetized and received an arterial catheter, standard size and large bore central venous catheters and a tibial IO catheter. Samples were taken simultaneously from the IO and central venous sites and analyzed with TEG, PT, APTT and fibrinogen concentration. 50% of the calculated blood volume was then removed through the large bore catheter and replaced with the same volume of Ringer´s acetate. A small dose of heparin was administered in six of the animals. Sampling and analysis was then repeated. The measured parameters were compared between IO and venous samples and between baseline and second samples.

Paper V

Ten pigs were anesthetized and received arterial and central venous catheters and a large bore dialysis catheter. An IO catheter was inserted in the proximal tibia. IO, arterial and central venous samples were taken and analyzed at the point of care for blood gases, acid base parameters, hematocrit and electrolytes. IO and venous samples were also centrifuged and sent to the laboratory for analysis of glucose, ALT, AST, γ-GT, ALP, creatinine and CK. 20% of the calculated blood volume was then removed through the dialysis catheter. A new IO catheter was inserted and the sampling and analysis repeated. After this another 20% of the starting blood volume was removed, and the analyses were repeated once more after insertion of a third IO catheter. Average levels of all analytes from the different sites and average differences between sites at the three sampling time points were calculated.

Anesthesia and preparation

All animals were anesthetized with intramuscular injections of tilétamin-zolazepam and xylazin shortly after arrival in the laboratory. They then received auricular IV catheters and a continuous infusion of pentobarbital, morphine and rocuronium was administered. In paper V, rocuronium was not used. After

deepening of analgesia with bolus doses of ketamine and morphine the animals were tracheotomized and mechanical ventilation initiated. A right cervical artery was catheterized and central venous and pulmonary artery catheters were inserted through the right internal jugular vein. A vesicotomy was performed and a urinary catheter inserted. IO accesses, and additional venous accesses, were inserted as described above. In papers I – III, a 10 ml/kg infusion of succinylated gelatin, 40 mg/ml, was administered and a stabilization period of 30 minutes preceded the experiments. This model of anesthesia is well established from previous studies in our animal laboratory.

Laboratory analysis

An Istat® point of care analyzer (Abbott Point of Care, Princeton, NJ) was used for the analyses in paper I, II and V. This is a handheld instrument which uses disposable cartridges containing the components necessary for the analysis, such as sensors, calibrants and reagents, and the sample is contained within the cartridge. EG7+ and CG4+ cartridges were used.

pH and PCO_2 were measured by direct potentiometry and HCO_3^- and BE were calculated from these values. PO_2 and lactate were measured amperometrically. Sodium and potassium were measured with ion selective potentiometry. Hemoglobin in papers I and III was calculated from the hematocrit, which was measured by conductometry.

In paper II, hemoglobin was measured spectrophotometrically on an OSM 3® (Radiometer AS, Copenhagen, Denmark) instrument, and glucose on a

Precision XP® glucometer (Abbott Point of Care, Princeton, NJ). The glucometer measures glucose amperometrically and the blood sample is applied to a disposable test strip. The remaining analyses were performed on the Istat®.

In paper III, cefotaxime concentrations were quantified with a protein precipitation-based method using reversed-phase high-pressure liquid chromatography separation coupled to mass spectrometry. Gentamicin was analyzed on an Architect Ci8200 analyzer (Abbott Laboratories, Abbott Park, IL).

In paper IV, TEG was performed on a TEG 5000® (Haemoscope, Niles, IL) using recalcified kaolin activated whole blood. Activated partial thromboplastin time (APTT, reagent STA PTT automate 5), fibrinogen (clot method, reagent 00673) and prothrombin time (PT, reagent STA SPA +) were analyzed with a STA-R® instrument (Diagnostica Stago S.A.S, Asnières sur Seine, France) with reagents from the same manufacturer. The prothrombin time was reported as International Normalized Ratio (INR) values. The total coefficients of variation for the methods were 1.9% at 34.6 seconds for APTT, 3.0% at 3 g/L for fibrinogen and 1.1% at INR 1.0 for the prothrombin time.

In paper IV, hemoglobin was analyzed spectrophotometrically on an ABL 800 blood gas analyzer.

In paper V, pH, PCO_2, PO_2, Sodium, Potassium, Calcium, Hematocrit and Lactate were analysed on the Istat® as described for paper I above. ALT, AST, ALP, Creatinine, Creatine kinase, γ-GT and Glucose were analyzed on a BS380 instrument (Mindray, Shenzhen, China). The reagents were from Abbott Laboratories (Abbott Park, IL, US). The total coefficient of variation (CV) for the methods were 3.1% at 0.34 μkat/L for ALT, 3.7% at 0.80 μkat/L for AST, 3.8% at 1.58 μkat/L for alkaline phosphatase, 1.5% at 87 μmol/L for creatinine, 4.5% at 2.5 μkat/L for creatinine kinase and 3.2% at 0.56 μkat/L for gamma-GT and 1.8% at 5.8 mmol/L for glucose.

Statistical analysis

In paper I, sampling from two IO and one arterial catheter was performed hourly, at seven times, in five pigs. Mean values for the different sampling points were calculated for all studied parameters hourly and for all samples. Mean differences in simultaneous samples between the two IO sites, and between mean IO and arterial sample values, were calculated. Differences were expressed as percentages of sample value means (relative differences). Coefficients of variation (CV) were calculated for IO and arterial sample values and were based on all samples.

In paper II, sampling from three IO catheters, one central venous and one arterial catheter was performed hourly, at seven times in eight pigs. A test was performed to evaluate whether differences between sample values from the different sites interacted with time of sampling. Means of the repeated IO and arterial samples for each animal and analyzed parameter were presented in Bland-Altman diagrams with calculation of mean differences.(102) When the differences were not correlated (Spearman $r > 0.5$) with the level of the analyte, limits of agreement corresponding to ± 2 standard deviations for the differences were presented. For the remaining comparisons, corresponding data were tabulated.

Mean values of IO, arterial and venous samples from all animals were also presented hour by hour for each parameter.

In paper III, median and range of the concentrations of both antibiotics at each sampling time were calculated for each mode of administration. The area under the time - concentration curve (AUC) was calculated and the difference and ratio between mean AUC for the two modes of administration were determined.

In paper IV, one IO and one venous sample was taken at two time points in each animal. Median and interquartile range (IQR) were calculated for the TEG parameters and median and range for, PT, APTT and fibrinogen concentrations at both sampling times. Correspondingly, median (IQR or range) differences between simultaneous IO and venous sample values, and between first and second samples, were calculated. Differences between simultaneous

IO and venous samples, and first and second samples, were also assessed with Wilcoxon´s signed rank test.

In paper V, IO, arterial and venous samples were analyzed at three times in ten animals. Mean levels of all analytes were calculated separately for the different accesses at all times. Differences between simultaneous IO and venous analyte levels were presented in Bland-Altman diagrams where the inter-method difference is plotted against the mean of both methods for each measurement. IO-venous and IO-arterial differences were also tabulated.

Results

Paper I

IO sampling was possible from the same catheters during the 6 hour study period, although it was sometimes more difficult than the arterial sampling, and point-of-care analysis was technically straightforward.

When comparing all IO and arterial samples, average levels of hemoglobin, sodium and calcium were similar. PO_2 in IO samples were lower than the arterial values, on a level normally associated with venous samples. PCO_2, lactate and potassium were higher and pH lower in the IO samples. CV of all samples were generally smaller in the IO samples. Average differences between simultaneous IO and arterial samples were clinically insignificant for hemoglobin, sodium and calcium but not for the remaining analytes. PCO_2 and lactate were higher and pH lower in the IO samples at all times during the experiment.

When comparing all samples from the two IO sites, average levels, as well as average differences between simultaneous samples, were small for all analytes except potassium and the calculated base excess.

Figure 3 presents mean IO and arterial levels over the duration of the experiment.

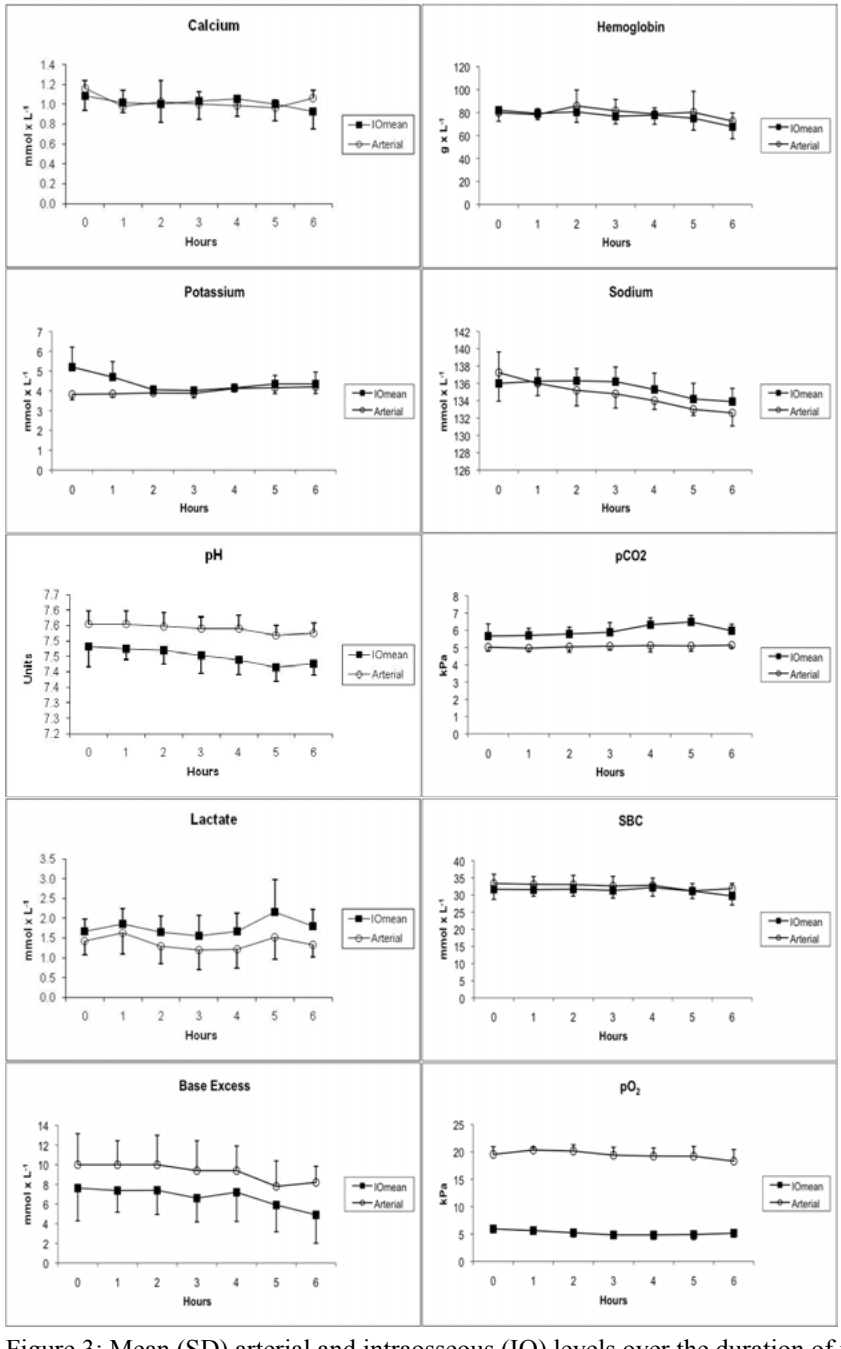
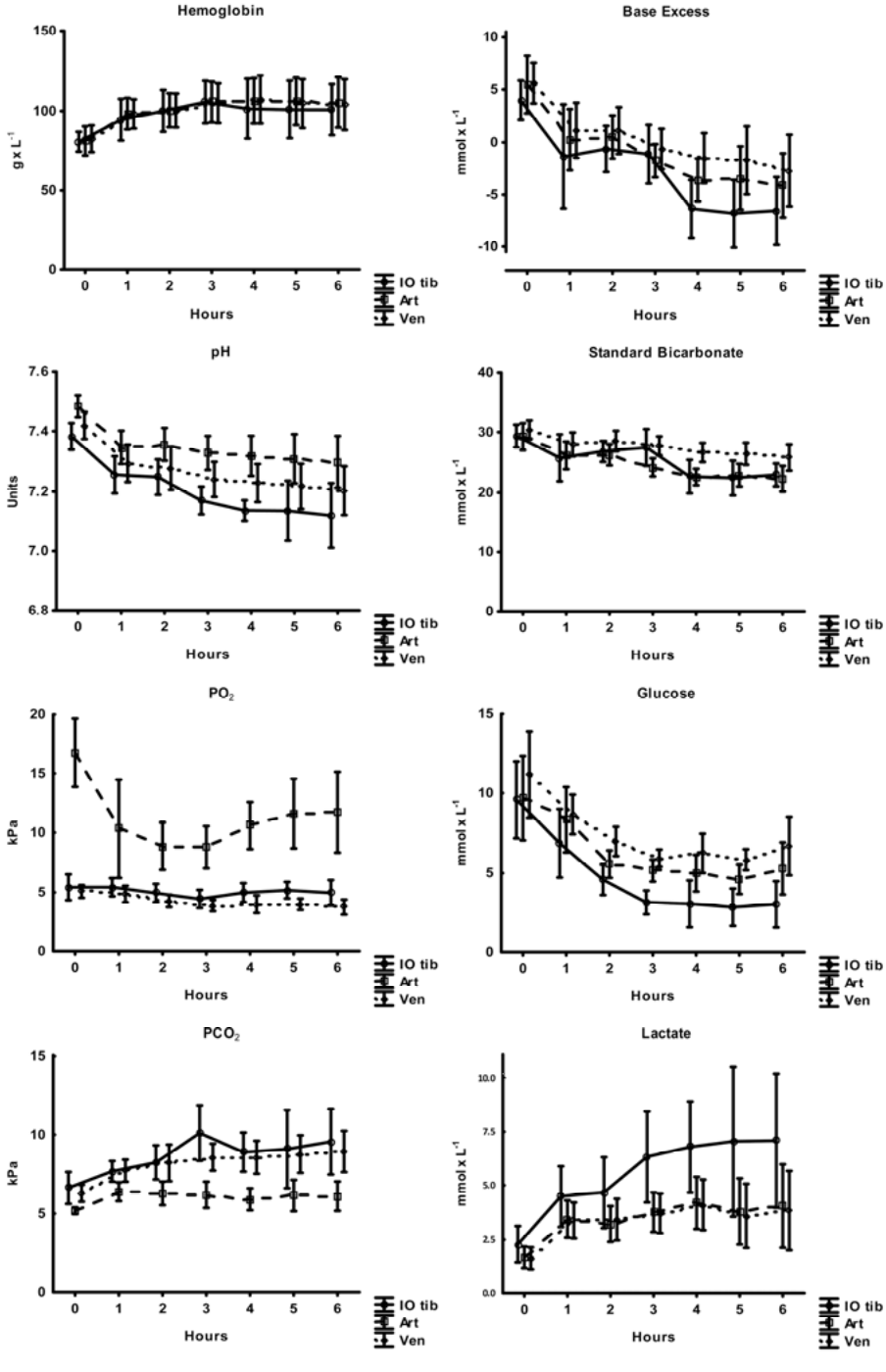


Figure 3: Mean (SD) arterial and intraosseous (IO) levels over the duration of the experiment in paper I. N = 5 animals. IO levels are means of left and right.

Paper II

Differences between sample values from IO and arterial and venous accesses were clinically relevant for most of the studied analytes. Also, the dispersion of the differences was generally large. For some analytes, notably glucose, pH, PCO_2 and lactate, the direction of bias was consistent. Graphically, the bias seemed to increase during the experiment, but an interaction test could not verify this. Average differences between sample values from the tibial (without infusion) and humeral IO catheters were generally small while agreement between values from the tibial catheters with and without ongoing infusion was limited and unpredictable. Figure 4 presents mean IO, arterial and venous levels over the duration of the experiment.

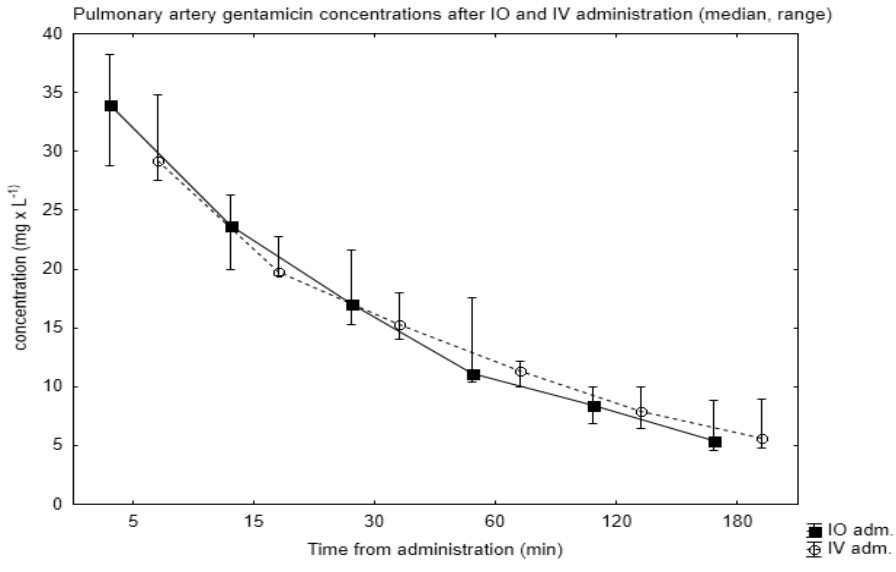
(Figure 4 (next page): Levels of the studied analytes in intraosseous (IO), arterial and venous samples in endotoxemic pigs during the experiment in paper II (n = 8 animals, mean, SD).

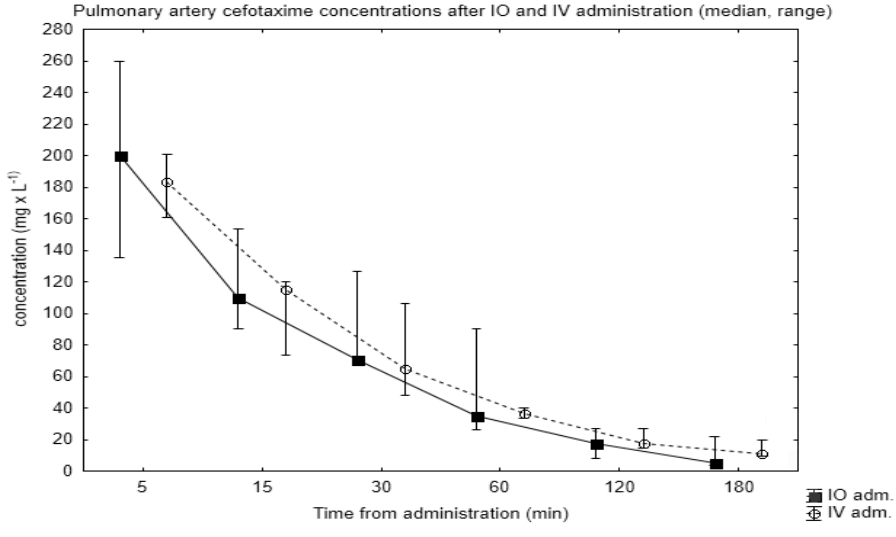

Hemoglobin
g x L-1
Hours
Base Excess
mmol x L-1
Hours
pH
Units
Hours
Standard Bicarbonate
mmol x L-1
Hours
PO2
kPa
Hours
Glucose
mmol x L-1
Hours
PCO2
kPa
Hours
Lactate
mmol x L-1
Hours
IO tib
Art
Ven

Paper III

The administration of endotoxin resulted in hemodynamic derangement and all animals except one received antibiotics based on the criterion of a reduction in MAP. Median (range) concentration of cefotaxime at 5 minutes after IO administration was 200 mg / L (135 - 260) versus 183 mg / L (161 - 201) after IV administration. For gentamicin, median concentrations at 5 min were 34 mg / L (29 - 38) after IO and 29 mg / L (28 - 35) after IV administration. Mean cefotaxime area under the concentration-time curve (AUC mg x h / L) was 108 ± 20 after IO and 117 ± 11 after IV administration; p 0.48, ratio 0. 93 (95% CI 0.7 – 1. 2). Mean AUC for gentamicin was 28.1 ± 6.8 for IO and 32.2 ± 3.5 for IV administration; p 0.32, ratio 0.87 (95% CI 0.62 - 1.19).

Figure 5 (next page): Concentrations of gentamicin and cefotaxime in pulmonary artery blood after intraosseous (IO, n = 4 animals) and intravenous (IV, n = 4 animals) administration in paper III (median, range).


Pulmonary artery gentamicin concentrations after IO and IV administration (median, range)
concentration (mg x L-1)
Time from administration (min)
IO adm.
IV adm.


Pulmonary artery cefotaxime concentrations after IO and IV administration (median, range)
concentration (mg x L-1)
Time from administration (min)
IO adm.
IV adm.

Paper IV

The IO samples clinically appeared hypercoagulable compared with the venous samples and premature clotting in vitro was a common finding during handling in the laboratory, making the analysis of PT, APTT and fibrinogen difficult.

When comparing TEG parameters analyzed using heparinase, the R time was invariably and significantly shorter in the IO samples at both first and second analysis (median (IQR) 1.6 (1.2 – 2.2) vs 4.6 (4.4 - 6.7) min and 1.6 (1.3 - 2.2) vs 4.7 (4.4 - 5.3) min). The MA was smaller in the IO samples at first, but not second, analysis. For the other parameters, no significant differences were demonstrated.

When comparing TEG parameters in baseline and second (after hemodilution) samples, the MA and α-angle decreased, while the other parameters were unchanged.

TEG parameters are presented in table 1.

In the small number of cases where IO levels of PT, APTT and fibrinogen were available for comparison, major differences were observed for APTT, but not for PT and fibrinogen. In the venous samples, fibrinogen decreased significantly between the first and second sampling while no important differences were seen for PT and APTT.

Table 1: TEG reaction time (R), kinetics (K), α-angle (α), Maximal Amplitude (MA) and Lysis at 30 minutes (LY30) in intraosseous (IO) and central venous (CV) samples at baseline (upper table) and after hemodilution (lower table) and average differences between simultaneous samples IO-CV from the same individuals in paper IV. Values are median and interquartile range (Q1-Q3)

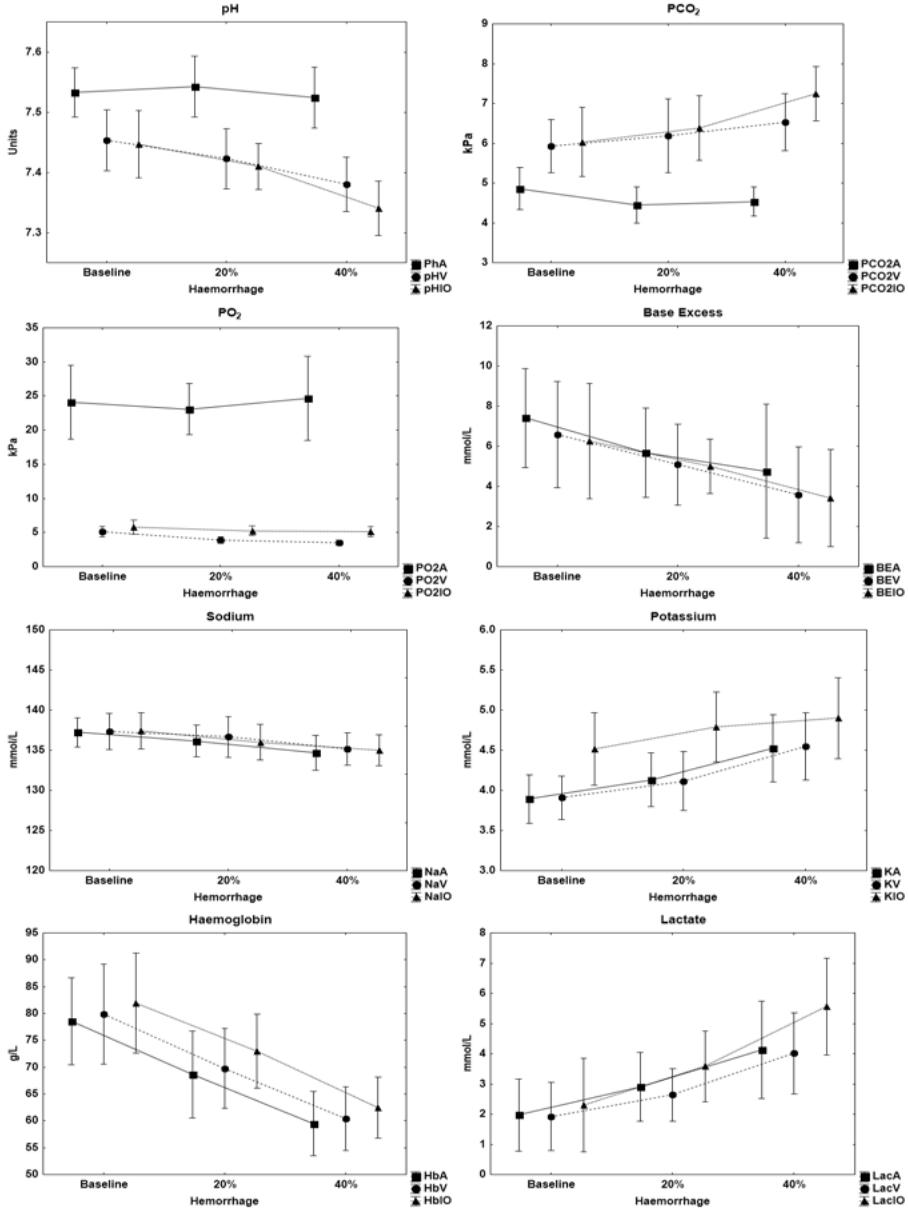
Variable	Unit	n IO	n CV	IO	CV	IO – CV
R	min	9	10	1.6 (1.2 – 2.2)	4.6 (4.4 – 6.7)	-2.8 (-5.8 – (-1.8))
K	min	9	10	0.9 (0.8 – 1.0)	1.0 (0.8 – 1.1)	0 (-0.1 – 0)
α	°	9	10	77.3 (75.6 – 78.8)	77.2 (74.3 – 78.5)	-0.4 (-1.5 – 0.1)
MA	mm	9	10	68.3 (68.2 – 71.3)	76.4 (71.9 – 77.8)	-8.9 (-9.6 – (-2.6))
LY 30	%	9	10	3.2 (2.2 – 4.9)	3.2 (2.3 – 4.9)	0.7 (-1.5 – 0.9)

Variable	Unit	n IO	n CV	IO	CV	IO – CV
R	min	8	8	1.6 (1.3 – 2.2)	4.7 (4.4 – 5.3)	-2.8 (-3.5 – (-2.3))
K	min	8	8	1.2 (0.9 – 1.7)	1.2 (1.1 – 1.4)	-0.1 (-0.3 – 0.7)
α	°	8	8	75.3 (67.5 – 77.9)	73 (71.6 – 74.9)	2.5 (-7.3 – 4.7)
MA	mm	8	8	64.6 (54.8 – 69.3)	67.3 (65.8 – 69.3)	-0.9 (-14.9 – 2.5)
LY 30	%	8	8	3 (1.2 – 5.4)	2.4 (1.2 – 6.4)	0.1 (-1.4 – 0.4)

Paper V

Sampling from all accesses was straightforward at all times. With progressive hypovolemia the animals became increasingly hypotensive and the cardiac index (CI) decreased. At baseline, average differences between IO and venous samples were clinically insignificant for most of the studied parameters but with increasing hypovolemia especially the difference in lactate and glucose increased. Differences in blood gases and acid-base parameters between IO/venous and arterial samples existed at baseline and increased after hemorrhage. Dispersion of the differences was often large, indicating limited precision. IO, venous and arterial levels at all sampling times are presented in figure 6.

Figure 6 (next page). Levels of selected analytes in intraosseous (IO), venous and arterial samples, in paper V, at baseline and after hemorrhage corresponding to 20% and 40% of the calculated blood volume.


pH
Units
7.6
7.5
7.4
7.3
Baseline
20%
40%
Haemorrhage
PhA
pHV
pHIO
PCO2
kPa
9
8
7
6
5
4
3
Baseline
20%
40%
Hemorrhage
PCO2A
PCO2V
PCO2IO
PO2
kPa
35
30
25
20
15
10
5
0
Baseline
20%
40%
Haemorrhage
PO2A
PO2V
PO2IO
Base Excess
mmol/L
12
10
8
6
4
2
0
Baseline
20%
40%
Haemorrhage
BEA
BEV
BEIO
Sodium
mmol/L
150
145
140
135
130
125
120
Baseline
20%
40%
Hemorrhage
NaA
NaV
NaIO
Potassium
mmol/L
6.0
5.5
5.0
4.5
4.0
3.5
3.0
Baseline
20%
40%
Hemorrhage
KA
KV
KIO
Haemoglobin
g/L
95
90
85
80
75
70
65
60
55
50
Baseline
20%
40%
Hemorrhage
HbA
HbV
HbIO
Lactate
mmol/L
8
7
6
5
4
3
2
1
0
Baseline
20%
40%
Haemorrhage
LacA
LacV
LacIO

Discussion

Intraosseous access is increasingly recognized as a rescue alternative in emergencies with difficult vascular access both in-hospital and out of hospital, and in civilian and military settings (37, 103, 104). The indication for insertion of IO access will most likely be an immediate need for fluid or drug treatment, and there is now substantial clinical experience of this method. Experimental data on drug delivery from IO administration sites to central circulation are also available and indicate that IO administration may equal IV (11, 13, 46). There are however also experimental data demonstrating that both hypovolemia and the administration of catecholamines significantly decreases IO blood flow, raising concern regarding the uptake of intraosseously administered drugs (28).

In antibiotic therapy, plasma drug concentrations are crucially important for antimicrobial activity (14, 15). Plasma concentrations of antibiotics after IO administration have previously been studied experimentally, but not in a sepsis model with circulatory instability. (16, 17).

Another potential use of intraosseous catheters is sampling for laboratory analyses. This is sometimes endorsed in local guidelines as well as commonly accessed online resources, although the scientific data on accuracy and precision are relatively sparse and not always in agreement. (20, 21, 47, 48, 105)

It may be questioned whether the intraosseous space can be considered a homogenous compartment for sampling purposes or whether local variations in blood flow or metabolism may significantly affect blood composition and analyses. It may also be discussed if pathophysiologic conditions such as shock of various origin, which is likely to be present when IO access becomes necessary, affects the reliability of analyses performed on IO samples. Further, it has been argued that the analysis of IO samples may confer a risk of damaging laboratory equipment, and many hospital laboratories are reluctant to analyze them (22, 23).

In paper I, we studied the analysis of blood gases, acid base status, hematocrit/hemoglobin and electrolytes in IO and arterial samples in a steady state model with anesthetized swine. Sampling and analysis was feasible using a portable point of care instrument with disposable cartridges, which implies that the risk of equipment damage can be circumvented. This was recently confirmed in a human study by Veldhoen et al. who compared, also using the Istat® analyzer, IO and venous samples taken at scheduled diagnostic bone marrow aspiration in children who were hemodynamically stable (106).

In our study, average levels of hemoglobin, sodium and calcium were similar in IO and arterial samples and average differences between simultaneous samples from the sites were clinically insignificant. This indicates that levels probably are fairly equal in the two compartments. A limitation of this study, however, is that agreement was not fully assessed, as dispersion of the differences was not calculated. The CV of all samples, presented in the paper, is also a blunt indicator of precision as it incorporates all animals and time points. Although the variation related to individual and time can be expected to be equal for both modes of analysis this is at best a rough estimate. When studied separately for each individual, CV for the IO samples were still smaller than for the arterial for all parameters. This can however not be considered a true replicate analysis since the physiologic conditions are not identical throughout the experiment, although it is a steady state model and a post hoc analysis demonstrated no significant changes in analyte levels over time.

An interesting finding is that average levels from the two IO sites were very similar and average differences between simultaneous IO samples were generally small. This indicates that the IO compartment may be reasonably homogenous in this regard, at least in the lower extremity and under stable conditions.

However, as pointed out earlier, data on the variation of differences would have added useful information and for this reason, average levels and differences from paper I were recalculated and presented in the additional table 2 below.

Table 2: Average levels and differences between simultaneous samples IO left (L) – Arterial and IO left – IO right (R) in non-endotoxemic pigs. (n= 33 samples (mean (SD)).

	IO L	**Art**	**IO L - Art**	**IO L – IO R**
Hb (g/L)	77 (9)	80 (10)	-2 (11)	0.2 (12)
pH	7.46 (0.05)	7.54 (0.04)	-0.07 (0.04)	0.03 (0.04)
PCO_2 (kPa)	5.8 (0.6)	5 (0.2)	0.8 (0.6)	-0.3 (0.5)
PO_2 (kPa)	5.2 (0.8)	19.4 (1.5)	-14.2 (1.5)	-0.1 (0.8)
Lactate (mmol/L)	1.7 (0.5)	1.4 (0.4)	0.3 (0.2)	-0.1 (0.2)
Na (mmol/L)	136 (2.5)	135 (2.2)	1 (1.7)	0.4 (2)
K (mmol/L)	4.2 (0.4)	4 (0.3)	0.18 (0.3)	-0.3 (0.7)
Ca (mmol/L)	1.02 (0.18)	1.02 (0.13)	0.001 (0.19)	0.01 (0.24)

Taken together, the data from paper I suggest that, during stable circulation, IO and arterial levels may be reasonably similar for hemoglobin, sodium, potassium and calcium while systematic differences seem to exist for pH, PCO_2 and PO_2. However, large variations in the differences between simultaneous IO and arterial samples suggest that agreement is not reliable and that the IO samples therefore are not suitable for detailed diagnostics. The more recent human study by Veldhoen et al. found similar differences between IO and venous samples and concluded that IO samples may be an acceptable substitute in an emergency but not otherwise (106).

In paper II, we compared samples from IO and arterial as well as central venous samples, tibial and humeral IO samples, and IO samples from tibial catheters with and without infusion. This was done in a model of septic shock using endotoxin infusion. All except one of the animals in this study presented obvious signs of circulatory instability. Like in the first study, data indicated systematic differences between IO and arterial samples for the measured acid base parameters pH and PCO_2, which were more pronounced than in the steady state model, while IO and venous levels were more similar. The latter is consistent with previous findings during unstable circulation. (18, 49, 107) Arteriovenous PCO_2 and pH differences have previously been reported to be useful as indicators of hypoperfusion and in guiding resuscitation (108-110).
The average difference in hemoglobin level between IO and arterial or venous samples was, in our opinion, clinically acceptable. We also measured glucose, which was consistently lower in the IO than in arterial and venous samples, and lactate, which was higher in the IO samples.
Average differences of analyte levels between tibial and humeral IO samples were generally small, in analogy with the findings from the comparison of bilateral tibial samples in paper I, while major differences were seen between samples from the tibial catheters with and without infusion.

Here we also studied the correlation between the analyte levels and differences between methods, and presented limits of agreement (LoA) (calculated as ± 2 SD for the differences) if the correlation coefficient (R) was ≤ 0. 5. By convention, if the LoA are considered clinically acceptable, the methods can be used interchangeable for diagnostic purposes.(102). In most cases the LoA, when presented, would be considered unacceptably large for diagnostic purposes. It is notable that pH was invariably lower and PCO_2 higher in the IO than in the arterial samples. Also, lactate was nearly invariably higher and glucose lower in the IO samples. The latter finding was however not reproduced in the human study mentioned earlier (106). On some occasions, it may be useful to know whether analyte levels are in the high, low or normal range, and IO samples might give some information on this.

Although the visual impression from figure 1 in paper II is that differences between sampling sites increased during the experiment for some analytes, an interaction test failed to verify this. Despite this, data from the second paper

indicate that IO samples in the setting of septic shock may be less reliable than during steady state, at least for the analytes affected by local metabolic processes. In the additional table 3 below, average differences in simultaneous IO and arterial samples, and in two simultaneous IO samples, are compared between papers I and II, for the common parameters.

Table 3. Difference between simultaneous samples from IO and arterial (IO - Art) and two IO sites (IO – IO) in endotoxemic (E, n = 53 samples) and non - endotoxemic (n = 33 samples) pigs. Values are mean (SD)

	IO – Art	**IO – IO**	**IOE - Art E**	**IOE - IOE**
Hb	-2 (11)	0.2 (12)	-2 (6)	-2 (6)
pH	-0.07 (0.04)	0.03 (0.04)	-0.14 (0.08)	-0.01 (0.06)
PCO_2	0.8 (0.6)	-0.3 (0.5)	2.6 (1.6)	-0.4 (1.5)
Lactate	0.3 (0.2)	-0.1 (0.2)	2.0 (1.7)	0 (1.0)

In paper III, concentrations of cefotaxime and gentamicin were measured in samples from the pulmonary artery during three hours after administration of weight adjusted doses randomly administered either IO or IV in pigs during endotoxemic shock.

The two modes of administration resulted in comparable plasma concentrations after 5 minutes, although the range of concentrations were larger for the IO route. While slightly higher after IV administration, concentration - time AUC was also comparable for the two modes of administration. Although the study is small and the confidence intervals for the AUC ratios are relatively wide, it nevertheless indicates that IO access may be an effective way to administer the studied antibiotics in hemodynamically unstable sepsis, and the concentrations reached by both modes of administration, with the high doses given, by far exceeds commonly observed values of minimal inhibitory concentration (MIC) for community-acquired pathogens such as Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes and Escherichia coli where MIC often is below 1 mg/L.(111). For beta-lactams, the time over MIC is considered the most important parameter for antimicrobial effect, and the time-concentration curves were similar for the two modes of administration (14). For aminoglycosides, the peak concentration is considered as the most important parameter. The latter value is probably not captured in the experiment, since the first sample is taken at 5 minutes, but the concentrations at this point are comparable. In the similar study by Jaimovich et al. cefotax-

ime time-concentration curves were also comparable for IO and IV administration of cefotaxime, while IO injection of Tobramycin resulted in a lower peak concentration than did IV administration.(17) Ideally, in a study like this, a crossover design using washout periods would be utilized. This was however not possible in our study from a practical or animal ethical perspective, and animals received similar treatment. Further, larger studies on human subjects would of course be preferable to confirm the results. However, randomizing critically ill patients to IO or IV antibiotic administration would hardly be ethically acceptable. Also, the results from this study should not be extrapolated to antibiotics with other pharmacokinetic properties than the ones studied.

In paper IV, samples were taken from anesthetized pigs during steady state for analysis of TEG, PT, APTT and fibrinogen concentration. Analyses were repeated after major hemorrhage and subsequent hemodilution.

The clinically most striking finding was a strong tendency for the IO samples to coagulate in vitro, often discovered during handling of the samples in the central laboratory, but on some occasions shortly after aspiration. The explanation for this may hypothetically be found either in the composition of the IO aspirate or the sampling technique. IO samples have previously been demonstrated to have a similar hematocrit, higher leukocyte count and lower platelet count than venous blood.(112) A higher cellularity and different phospholipid content in the bone marrow might hypothetically affect coagulation.(113, 114) Technical aspects of sampling are undoubtedly important in all laboratory analysis, and hemolysis resulting from forceful aspiration and shear stress may affect the results of coagulation analyses.(115) Contact activation in the sampling device might also be a problem and a previous study describes using anticoagulant in the syringes when drawing IO samples.(112) However, the technique was the same for the IO and venous samples, and in vitro coagulation was not observed in the latter.

The TEG analysis demonstrated a significantly shortened reaction time in the IO samples, which is consistent with the clinical observations, and a lower MA, which may hypothetically be explained by premature consumption of coagulation elements, although a different platelet content might also be a contributing factor. Also, the effect of heparin was less readily seen in the IO samples, indicating a stronger clotting tendency.

The hemodilution between baseline and second samples was quite pronounced with an average decrease in hemoglobin from 100 to 57 g/L. Despite this, besides decreased MA and α, TEG parameters were not significantly affected. This is analogous with a previous porcine study where a decrease in MA was the only observed TEG abnormality after 35% hemorrhage and crystalloid resuscitation.(116)

Coagulopathy associated with trauma is a complex disorder where tissue damage, hypoperfusion, acidosis and hypothermia as well as hemorrhage and hemodilution may be contributing factors.(117)

In paper V we returned again to the study of point of care analysis, this time measuring blood gases, acid base parameters, electrolytes, hematocrit and lactate in IO, venous and arterial samples during stable circulation and two levels of hypovolemia after hemorrhage. We also compared glucose and enzymes, analyzed at the central laboratory, in IO and venous samples. Average differences between venous and IO samples were relatively small during stable circulation, but increased in hypovolemia, in particular for glucose and lactate. Lower pH and higher PCO_2 levels were seen both in IO and venous samples, compared with arterial, in hypovolemia. This finding is in line with the observations in paper II discussed above.

Analyses known to be affected by hemolysis, i.e. potassium, AST, and CK, also demonstrated clinically relevant differences between IO and venous samples. (118) ALP was higher in IO than in venous samples, and this may hypothetically be of either skeletal or leukocyte origin.(119) In this study, in contrast to in our previous studies on IO sampling, a new IO cannula was placed before each sampling time. With this approach, aspiration of appropriately sized samples was uncomplicated at all times. Despite this, differences between IO and arterial/venous levels seemed to increase between baseline and subsequent samples. This suggests that technical issues are not solely responsible for the observed differences. Given that there seems to exist clinically relevant differences between IO and venous or arterial samples during unstable circulation, one question is whether capillary samples, often utilized in children, is a better alternative. Although many analyses may be performed on capillary blood, the available sample volumes will likely be smaller than with IO sampling. Arterialized capillary samples from the earlobe have been demonstrated to be accurate for blood gas measurement and, if available, is probably a good option.(120) In unstable patients, capillary lactate, similarly to IO lactate in our studies, seems to be higher than in arterial blood, and an animal study also demonstrated limited agreement between capillary and arterial PCO_2 in hemorrhagic shock. (121, 122)

Limitations

Although this thesis contributes data on several previously unstudied topics, the individual papers have a number of limitations which are discussed above. Certain general limitations also apply to all studies included in the thesis.

They are experimental animal studies, and the results are therefore not necessarily applicable in humans. Although there are major similarities in the circulatory system between the species, there are obvious differences in the skeletal system, which may be of importance. Further, the number of animals in each individual study is small, which increases the risk of random errors, but the design of repeated measurements, when applied, may strengthen the anal-

yses. Also, when examining the repeated studies of the same laboratory analyses in different pathophysiologic conditions, we come to similar conclusions. Larger studies, preferably on human subjects with critical illness, where data are almost completely lacking, could be expected to provide more reliable and clinically useful information, although such studies could be both ethically and practically difficult to conduct.

Conclusions

Bearing the abovementioned limitations in mind, the data in our studies suggest that laboratory analysis of the studied parameters in IO samples is possible and that point of care equipment may be used for such analysis, but that the agreement with arterial and venous samples is variable and sometimes limited. Caution is therefore recommended in the interpretation of the results, especially during unstable circulation, and IO samples should not be relied upon for detailed diagnostics. Analysis of samples from different IO accesses generally seems to yield similar results but ongoing intraosseous infusion apparently decreases reliability.

IO injection of the studied antibiotics during endotoxemic shock rapidly yielded central concentrations comparable to those seen after peripheral IV administration, and should be considered in an emergency.

Analysis of coagulation parameters in intraosseous aspirate seems particularly problematic due to hypercoagulability and premature clotting in vitro of the IO samples.

Future plans

A clinical study of IO laboratory analysis in critically ill patients, focusing on emergency analyses such as acid base parameters, electrolytes, glucose, C-reactive protein and cardiac enzymes would be of interest, as data from this population are lacking. Such a study, as discussed previously, will probably be difficult, but maybe not entirely impossible, to conduct. IO sampling in heparinized syringes and TEG analysis using heparinase to counteract a potential activation of clotting during the sampling procedure is another potential project of interest. Also, the impact of IO adrenaline administration on the pharmacokinetics of simultaneously administered drugs, especially in combination with other potentially important factors such as hypovolemia or impaired cardiac output from other causes is a relevant study topic since IO access in an emergency may have to be used for administration of all necessary drugs.

Acknowledgements

Stort tack till min huvudhandledare **Mats Eriksson** vars oupphörliga stöd, entusiasm och idérikedom gjort detta arbete möjligt.

Stort tack också till mina handledare **Miklós Lipcsey** och **Anders Larsson** för era fantastiska insatser och för att ni alltid funnits till hands när det behövts.

Tack till mina medförfattare **Mats G. Gustafsson**, **Lars Berglund**, **Joel Michalek** och **Norbert Lubenow** för era stora insatser under detta arbete.

Tack till **Sten Rubertsson** och **Anders Larsson** för ert stöd och för att jag fått tiden och möjligheten att arbeta med detta.

Tack till **Monika Hall**, **Anders Nordgren, Kerstin Ahlgren och Mariette Andersson** utan vilkas kvalificerade arbete och hjälp detta heller inte varit möjligt.

Tack till **Katja Andersson** för hjälp med det mesta

Tack till **Uppsala Universitet, Teleflex Incorporated, Laerdal Foundation for Acute Medicine och Selanders Stiftelse** för finansiellt stöd.

Tack till **alla mina kollegor** för stöd och uppmuntran.

Slutligen ett **stort tack till hela min familj** för ert stöd och tålamod som varit den grundläggande förutsättningen för att göra detta möjligt.

References

1. Rauch D, Dowd D, Eldridge D, Mace S, Schears G, Yen K. Peripheral difficult venous access in children. Clinical pediatrics. 2009;48(9):895-901.
2. Nafiu OO, Burke C, Cowan A, Tutuo N, Maclean S, Tremper KK. Comparing peripheral venous access between obese and normal weight children. Paediatric anaesthesia. 2010;20(2):172-6.
3. Deakin CD, Nolan JP, Soar J, Sunde K, Koster RW, Smith GB, et al. European Resuscitation Council Guidelines for Resuscitation 2010 Section 4. Adult advanced life support. Resuscitation. 2010;81(10):1305-52.
4. International Liaison Committee on R. The International Liaison Committee on Resuscitation (ILCOR) consensus on science with treatment recommendations for pediatric and neonatal patients: pediatric basic and advanced life support. Pediatrics. 2006;117(5):e955-77.
5. Ong ME, Ngo AS, Wijaya R. An observational, prospective study to determine the ease of vascular access in adults using a novel intraosseous access device. Annals of the Academy of Medicine, Singapore. 2009;38(2):121-4.
6. Gazin N, Auger H, Jabre P, Jaulin C, Lecarpentier E, Bertrand C, et al. Efficacy and safety of the EZ-IO intraosseous device: Out-of-hospital implementation of a management algorithm for difficult vascular access. Resuscitation. 2011;82(1):126-9.
7. Lewis P, Wright C. Saving the critically injured trauma patient: a retrospective analysis of 1000 uses of intraosseous access. Emergency medicine journal : EMJ. 2014.
8. Sunde GA, Heradstveit BE, Vikenes BH, Heltne JK. Emergency intraosseous access in a helicopter emergency medical service: a retrospective study. Scandinavian journal of trauma, resuscitation and emergency medicine. 2010;18:52.
9. Ong ME, Chan YH, Oh JJ, Ngo AS. An observational, prospective study comparing tibial and humeral intraosseous access using the EZ-IO. The American journal of emergency medicine. 2009;27(1):8-15.
10. Ngo AS, Oh JJ, Chen Y, Yong D, Ong ME. Intraosseous vascular access in adults using the EZ-IO in an emergency department. International journal of emergency medicine. 2009;2(3):155-60.
11. Von Hoff D, Kuhn J, Burris Hr, Miller L. Does intraosseous equal intravenous? A pharmacokinetic study. American Journal of Emergency Medicine 2008;26:31-8.
12. Buck ML, Wiggins BS, Sesler JM. Intraosseous drug administration in children and adults during cardiopulmonary resuscitation. The Annals of pharmacotherapy. 2007;41(10):1679-86.
13. Hoskins SL, do Nascimento P, Jr., Lima RM, Espana-Tenorio JM, Kramer GC. Pharmacokinetics of intraosseous and central venous drug delivery during cardiopulmonary resuscitation. Resuscitation. 2012;83(1):107-12.

14. Turnidge JD. The pharmacodynamics of beta-lactams. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 1998;27(1):10-22.
15. McLean AJ, IoannidesDemos LL, Li SC, Bastone EB, Spicer WJ. Bactericidal effect of gentamicin peak concentration provides a rationale for administration of bolus doses. J Antimicrob Chemother. 1993;32(2):301-5.
16. Pollack CV, Jr., Pender ES, Woodall BN, Parks BR. Intraosseous administration of antibiotics: same-dose comparison with intravenous administration in the weanling pig. Annals of emergency medicine. 1991;20(7):772-6.
17. Jaimovich DG, Kumar A, Francom S. Evaluation of intraosseous vs intravenous antibiotic levels in a porcine model. American journal of diseases of children. 1991;145(8):946-9.
18. Kissoon N, Idris A, Wenzel V, Murphy S, Rush W. Intraosseous and central venous blood acid-base relationship during cardiopulmonary resuscitation. Pediatric emergency care. 1997;13(4):250-3.
19. Grisham J, Hastings C. Bone marrow aspirate as an accessible and reliable source for critical laboratory studies. Annals of emergency medicine. 1991;20(10):1121-4.
20. Miller LJ, Philbeck TE, Montez D, Spadaccini CJ. A new study of intraosseous blood for laboratory analysis. Archives of pathology & laboratory medicine. 2010;134(9):1253-60.
21. Orlowski JP, Porembka DT, Gallagher JM, Van Lente F. The bone marrow as a source of laboratory studies. Annals of emergency medicine. 1989;18(12):1348-51.
22. Nicoll SJ, Rochester SJ. Blood sampling through intraosseous needles: time to stop? Resuscitation. 2008;79(1):168; discussion -9.
23. Cervinski MA. Laboratory analysis of intraosseous blood: bad to the bone? Clin Chem Lab Med. 2014;52(8):e187-9.
24. Travlos GS. Normal structure, function, and histology of the bone marrow. Toxicologic pathology. 2006;34(5):548-65.
25. Ross MH, Romrell L. J, Kaye, G. K., . Histology, a text and atlas - 3:rd edition: Williams & Wilkins; 1995.
26. De Lorenzo RA, Ward JA, Jordan BS, Hanson CE. Relationships of intraosseous and systemic pressure waveforms in a Swine model. Academic emergency medicine : official journal of the Society for Academic Emergency Medicine. 2014;21(8):899-904.
27. Frascone RJ, Salzman JG, Adams AB, Bliss P, Wewerka SS, Dries DJ. Evaluation of intraosseous pressure in a hypovolemic animal model. The Journal of surgical research. 2015;193(1):383-90.
28. Voelckel WG, Lurie KG, McKnite S, Zielinski T, Lindstrom P, Peterson C, et al. Comparison of epinephrine with vasopressin on bone marrow blood flow in an animal model of hypovolemic shock and subsequent cardiac arrest. Critical care medicine. 2001;29(8):1587-92.
29. Feitelson JB, Kulenovic E, Beck DJ, Harris PD, Passmore JC, Malkani AL, et al. Endogenous norepinephrine regulates blood flow to the intact rat tibia. Journal of orthopaedic research : official publication of the Orthopaedic Research Society. 2002;20(2):391-6.
30. Dinenno FA, Tanaka H, Stauffer BL, Seals DR. Reductions in basal limb blood flow and vascular conductance with human ageing: role for augmented alpha-adrenergic vasoconstriction. The Journal of physiology. 2001;536(Pt 3):977-83.

31. Tocantins LM, O'Neill J F, Price AH. Infusions of Blood and Other Fluids Via the Bone Marrow in Traumatic Shock and Other Forms of Peripheral Circulatory Failure. Annals of surgery. 1941;114(6):1085-92.
32. Foex BA. Discovery of the intraosseous route for fluid administration. Journal of accident & emergency medicine. 2000;17(2):136-7.
33. Drinker CK DK, Lund CC. The circulation in the mammalian bone marrow. American journal of physiology. 1922;62:1-92.
34. Orlowski JP. My kingdom for an intravenous line. American journal of diseases of children. 1984;138(9):803.
35. Luck RP, Haines C, Mull CC. Intraosseous access. The Journal of emergency medicine. 2010;39(4):468-75.
36. Kehrl T, Becker BA, Simmons DE, Broderick EK, Jones RA. Intraosseous access in the obese patient: assessing the need for extended needle length. The American journal of emergency medicine. 2016;34(9):1831-4.
37. Vassallo J, Horne S, Smith JE. Intraosseous access in the military operational setting. Journal of the Royal Naval Medical Service. 2014;100(1):34-7.
38. Hallas P, Brabrand M, Folkestad L. Complication with intraosseous access: scandinavian users' experience. The western journal of emergency medicine. 2013;14(5):440-3.
39. Hasan MY, Kissoon N, Khan TM, Saldajeno V, Goldstein J, Murphy SP. Intraosseous infusion and pulmonary fat embolism. Pediatric critical care medicine : a journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies. 2001;2(2):133-8.
40. Rubal BJ, Meyers BL, Kramer SA, Hanson MA, Andrews JM, DeLorenzo RA. Fat Intravasation from Intraosseous Flush and Infusion Procedures. Prehospital emergency care : official journal of the National Association of EMS Physicians and the National Association of State EMS Directors. 2015;19(3):376-90.
41. Claudet I, Baunin C, Laporte-Turpin E, Marcoux MO, Grouteau E, Cahuzac JP. Long-term effects on tibial growth after intraosseous infusion: a prospective, radiographic analysis. Pediatric emergency care. 2003;19(6):397-401.
42. Philbeck TE, Miller LJ, Montez D, Puga T. Hurts so good. Easing IO pain and pressure. JEMS : a journal of emergency medical services. 2010;35(9):58-62, 5-6, 8; quiz 9.
43. Pasley J, Miller CH, DuBose JJ, Shackelford SA, Fang R, Boswell K, et al. Intraosseous infusion rates under high pressure: a cadaveric comparison of anatomic sites. The journal of trauma and acute care surgery. 2015;78(2):295-9.
44. Orlowski JP, Porembka DT, Gallagher JM, Lockrem JD, VanLente F. Comparison study of intraosseous, central intravenous, and peripheral intravenous infusions of emergency drugs. American journal of diseases of children. 1990;144(1):112-7.
45. Burgert J, Gegel B, Loughren M, Ceremuga T, Desai M, Schlicher M, et al. Comparison of tibial intraosseous, sternal intraosseous, and intravenous routes of administration on pharmacokinetics of epinephrine during cardiac arrest: a pilot study. AANA journal. 2012;80(4 Suppl):S6-10.
46. Yost J, Baldwin P, Bellenger S, Bradshaw F, Causapin E, Demotica R, et al. The pharmacokinetics of intraosseous atropine in hypovolemic swine. American journal of disaster medicine. 2015;10(3):217-22.
47. Hurren JS. Can blood taken from intraosseous cannulations be used for blood analysis? Burns : journal of the International Society for Burn Injuries. 2000;26(8):727-30.

48. Ummenhofer W, Frei FJ, Urwyler A, Drewe J. Are laboratory values in bone marrow aspirate predictable for venous blood in paediatric patients? Resuscitation. 1994;27(2):123-8.
49. Abdelmoneim T, Kissoon N, Johnson L, Fiallos M, Murphy S. Acid-base status of blood from intraosseous and mixed venous sites during prolonged cardiopulmonary resuscitation and drug infusions. Critical care medicine. 1999;27(9):1923-8.
50. Giuliano KK, Grant ME. Blood analysis at the point of care: issues in application for use in critically ill patients. AACN clinical issues. 2002;13(2):204-20.
51. Di Serio F, Petronelli MA, Sammartino E. Laboratory testing during critical care transport: point-of-care testing in air ambulances. Clinical chemistry and laboratory medicine : CCLM / FESCC. 2010;48(7):955-61.
52. Larsson A, Greig-Pylypczuk R, Huisman A. The state of point-of-care testing: a European perspective. Upsala journal of medical sciences. 2015;120(1):1-10.
53. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. Intensive care medicine. 2013;39(2):165-228.
54. Annane D, Bellissant E, Cavaillon JM. Septic shock. Lancet. 2005;365(9453):63-78.
55. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). Jama. 2016;315(8):801-10.
56. Vincent JL, Moreno R, Takala J, Willatts S, De Mendonca A, Bruining H, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. Intensive care medicine. 1996;22(7):707-10.
57. Angus DC, van der Poll T. Severe sepsis and septic shock. The New England journal of medicine. 2013;369(21):2063.
58. Mayr FB, Yende S, Angus DC. Epidemiology of severe sepsis. Virulence. 2014;5(1):4-11.
59. Kumar G, Kumar N, Taneja A, Kaleekal T, Tarima S, McGinley E, et al. Nationwide trends of severe sepsis in the 21st century (2000-2007). Chest. 2011;140(5):1223-31.
60. Kaukonen KM, Bailey M, Suzuki S, Pilcher D, Bellomo R. Mortality related to severe sepsis and septic shock among critically ill patients in Australia and New Zealand, 2000-2012. Jama. 2014;311(13):1308-16.
61. Antonucci E, Fiaccadori E, Donadello K, Taccone FS, Franchi F, Scolletta S. Myocardial depression in sepsis: from pathogenesis to clinical manifestations and treatment. Journal of critical care. 2014;29(4):500-11.
62. Kimmoun A, Ducrocq N, Levy B. Mechanisms of vascular hyporesponsiveness in septic shock. Current vascular pharmacology. 2013;11(2):139-49.
63. Ince C, Mayeux PR, Nguyen T, Gomez H, Kellum JA, Ospina-Tascon GA, et al. The Endothelium in Sepsis. Shock. 2016;45(3):259-70.
64. Fortin CF, McDonald PP, Fulop T, Lesur O. Sepsis, leukocytes, and nitric oxide (NO): an intricate affair. Shock. 2010;33(4):344-52.
65. Sharawy N. Vasoplegia in septic shock: do we really fight the right enemy? Journal of critical care. 2014;29(1):83-7.
66. Zanotti-Cavazzoni SL, Hollenberg SM. Cardiac dysfunction in severe sepsis and septic shock. Current opinion in critical care. 2009;15(5):392-7.
67. Walley KR. Deeper understanding of mechanisms contributing to sepsis-induced myocardial dysfunction. Critical care. 2014;18(3):137.

68. Ahrens T. Hemodynamics in sepsis. AACN Adv Crit Care. 2006;17(4):435-45.
69. Ceneviva G, Paschall JA, Maffei F, Carcillo JA. Hemodynamic support in fluid-refractory pediatric septic shock. Pediatrics. 1998;102(2):e19.
70. Rivers E, Nguyen B, Havstad S, Ressler J, Muzzin A, Knoblich B, et al. Early goal-directed therapy in the treatment of severe sepsis and septic shock. The New England journal of medicine. 2001;345(19):1368-77.
71. Mouncey PR, Osborn TM, Power GS, Harrison DA, Sadique MZ, Grieve RD, et al. Trial of early, goal-directed resuscitation for septic shock. The New England journal of medicine. 2015;372(14):1301-11.
72. Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Critical care medicine. 2006;34(6):1589-96.
73. Mikkelsen ME, Miltiades AN, Gaieski DF, Goyal M, Fuchs BD, Shah CV, et al. Serum lactate is associated with mortality in severe sepsis independent of organ failure and shock. Critical care medicine. 2009;37(5):1670-7.
74. Marty P, Roquilly A, Vallee F, Luzi A, Ferre F, Fourcade O, et al. Lactate clearance for death prediction in severe sepsis or septic shock patients during the first 24 hours in Intensive Care Unit: an observational study. Annals of intensive care. 2013;3(1):3.
75. Hanberger H. Empirisk antibiotikabehandling vid intensivvårdskrävende infektioner 2012 [cited 2014 2014-10-04]. Available from: www.internetmedicin.se.
76. Levison ME, Levison JH. Pharmacokinetics and pharmacodynamics of antibacterial agents. Infectious disease clinics of North America. 2009;23(4):791-815, vii.
77. Gutierrez G, Reines HD, Wulf-Gutierrez ME. Clinical review: hemorrhagic shock. Critical care. 2004;8(5):373-81.
78. Dyson A, Ekbal N, Stotz M, Barnes S, Carre J, Tully S, et al. Component reductions in oxygen delivery generate variable haemodynamic and stress hormone responses. British journal of anaesthesia. 2014;113(4):708-16.
79. Mutschler M, Nienaber U, Brockamp T, Wafaisade A, Wyen H, Peiniger S, et al. A critical reappraisal of the ATLS classification of hypovolaemic shock: does it really reflect clinical reality? Resuscitation. 2013;84(3):309-13.
80. Gann DS, Drucker WR. Hemorrhagic shock. The journal of trauma and acute care surgery. 2013;75(5):888-95.
81. Palta S, Saroa R, Palta A. Overview of the coagulation system. Indian journal of anaesthesia. 2014;58(5):515-23.
82. Heemskerk JW, Bevers EM, Lindhout T. Platelet activation and blood coagulation. Thrombosis and haemostasis. 2002;88(2):186-93.
83. Curry APJ. Conventional and near-patient tests of coagulation Continuing Education in Anaesthesia, Critical Care & Pain. 2007;7(2):45-50.
84. Longstaff C, Kolev K. Basic mechanisms and regulation of fibrinolysis. Journal of thrombosis and haemostasis : JTH. 2015;13 Suppl 1:S98-105.
85. Kolev K, Longstaff C. Bleeding related to disturbed fibrinolysis. British journal of haematology. 2016.
86. Semeraro N, Ammollo CT, Semeraro F, Colucci M. Coagulopathy of Acute Sepsis. Seminars in thrombosis and hemostasis. 2015;41(6):650-8.
87. Hess JR, Brohi K, Dutton RP, Hauser CJ, Holcomb JB, Kluger Y, et al. The coagulopathy of trauma: a review of mechanisms. The Journal of trauma. 2008;65(4):748-54.

88. Levi M, Hunt BJ. A critical appraisal of point-of-care coagulation testing in critically ill patients. Journal of thrombosis and haemostasis : JTH. 2015;13(11):1960-7.
89. Inaba K, Rizoli S, Veigas PV, Callum J, Davenport R, Hess J, et al. 2014 Consensus conference on viscoelastic test-based transfusion guidelines for early trauma resuscitation: Report of the panel. The journal of trauma and acute care surgery. 2015;78(6):1220-9.
90. Haase N, Ostrowski SR, Wetterslev J, Lange T, Moller MH, Tousi H, et al. Thromboelastography in patients with severe sepsis: a prospective cohort study. Intensive care medicine. 2015;41(1):77-85.
91. Hunt H, Stanworth S, Curry N, Woolley T, Cooper C, Ukoumunne O, et al. Thromboelastography (TEG) and rotational thromboelastometry (ROTEM) for trauma induced coagulopathy in adult trauma patients with bleeding. The Cochrane database of systematic reviews. 2015;2:CD010438.
92. Swindle MM, Makin A, Herron AJ, Clubb FJ, Jr., Frazier KS. Swine as models in biomedical research and toxicology testing. Veterinary pathology. 2012;49(2):344-56.
93. Lipcsey M, Carlsson M, Larsson A, Algotsson L, Eriksson M, Lukinius A, et al. Effect of a single dose of tobramycin on systemic inflammatory response-induced acute kidney injury in a 6-hour porcine model. Critical care medicine. 2009;37(10):2782-90.
94. Lipcsey M, Larsson A, Eriksson MB, Sjolin J. Inflammatory, coagulatory and circulatory responses to logarithmic increases in the endotoxin dose in the anaesthetised pig. Journal of endotoxin research. 2006;12(2):99-112.
95. Freudenberg MA, Galanos C. Bacterial lipopolysaccharides: structure, metabolism and mechanisms of action. International reviews of immunology. 1990;6(4):207-21.
96. Vaure C, Liu Y. A comparative review of toll-like receptor 4 expression and functionality in different animal species. Frontiers in immunology. 2014;5:316.
97. Lakhani SA, Bogue CW. Toll-like receptor signaling in sepsis. Current opinion in pediatrics. 2003;15(3):278-82.
98. Redl H, Bahrami S, Schlag G, Traber DL. Clinical detection of LPS and animal models of endotoxemia. Immunobiology. 1993;187(3-5):330-45.
99. Lambermont B, Kolh P, Detry O, Gerard P, Marcelle R, D'Orio V. Analysis of endotoxin effects on the intact pulmonary circulation. Cardiovascular research. 1999;41(1):275-81.
100. Deep A, Goonasekera CD, Wang Y, Brierley J. Evolution of haemodynamics and outcome of fluid-refractory septic shock in children. Intensive Care Med. 2013;39(9):1602-9.
101. Brierley J, Peters MJ. Distinct hemodynamic patterns of septic shock at presentation to pediatric intensive care. Pediatrics. 2008;122(4):752-9.
102. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986;1(8476):307-10.
103. Paxton JH, Knuth TE, Klausner HA. Proximal humerus intraosseous infusion: a preferred emergency venous access. The Journal of trauma. 2009;67(3):606-11.
104. Sheils M, Ross M, Eatough N, Caputo ND. Intraosseous access in trauma by air medical retrieval teams. Air medical journal. 2014;33(4):161-4.
105. Gluckman D FR. Intraosseous Cannulation www.emedicine.medscape.com2014 [cited 2016 09-20]. Available from: emedicine.medscape.com.
106. Veldhoen ES, de Vooght KM, Slieker MG, Versluys AB, Turner NM. Analysis of bloodgas, electrolytes and glucose from intraosseous samples using an i-STAT((R)) point-of-care analyser. Resuscitation. 2014;85(3):359-63.

107. Kissoon N, Peterson R, Murphy S, Gayle M, Ceithaml E, Harwood-Nuss A. Comparison of pH and carbon dioxide tension values of central venous and intraosseous blood during changes in cardiac output. Critical care medicine. 1994;22(6):1010-5.
108. Zhang H, Vincent JL. Arteriovenous differences in PCO2 and pH are good indicators of critical hypoperfusion. The American review of respiratory disease. 1993;148(4 Pt 1):867-71.
109. Mallat J, Lemyze M, Tronchon L, Vallet B, Thevenin D. Use of venous-to-arterial carbon dioxide tension difference to guide resuscitation therapy in septic shock. World journal of critical care medicine. 2016;5(1):47-56.
110. Ospina-Tascon GA, Umana M, Bermudez WF, Bautista-Rincon DF, Valencia JD, Madrinan HJ, et al. Can venous-to-arterial carbon dioxide differences reflect microcirculatory alterations in patients with septic shock? Intensive care medicine. 2016;42(2):211-21.
111. Antimicrobial wild type distribution of microorganisms: European Committee on Antimicrobial Susceptibility Testing; [cited 2014 2014-10-01]. Available from: http://www.eucast.org/mic_distributions/.
112. Greco SC, Talcott MR, LaRegina MC, Eisenbeis PE. Use of intraosseous blood for repeated hematologic and biochemical analyses in healthy pigs. American journal of veterinary research. 2001;62(1):43-7.
113. Wajda M. Lipid Composition of Human Bone Marrow. The Biochemical journal. 1965;95:252-5.
114. Shaw AW, Pureza VS, Sligar SG, Morrissey JH. The local phospholipid environment modulates the activation of blood clotting. The Journal of biological chemistry. 2007;282(9):6556-63.
115. Lippi G, Salvagno GL, Montagnana M, Lima-Oliveira G, Guidi GC, Favaloro EJ. Quality standards for sample collection in coagulation testing. Seminars in thrombosis and hemostasis. 2012;38(6):565-75.
116. Martini WZ, Cortez DS, Dubick MA, Park MS, Holcomb JB. Thrombelastography is better than PT, aPTT, and activated clotting time in detecting clinically relevant clotting abnormalities after hypothermia, hemorrhagic shock and resuscitation in pigs. The Journal of trauma. 2008;65(3):535-43.
117. Cap A, Hunt B. Acute traumatic coagulopathy. Current opinion in critical care. 2014;20(6):638-45.
118. Lippi G, Cervellin G, Mattiuzzi C. Critical review and meta-analysis of spurious hemolysis in blood samples collected from intravenous catheters. Biochemia medica. 2013;23(2):193-200.
119. Mishler JM, Williams DM. Alkaline phosphatase as a marker of maturity in human neutrophils. Studies in normals dosed with aetiocholanolone and prednisolone. Journal of clinical pathology. 1980;33(6):555-61.
120. Zavorsky GS, Cao J, Mayo NE, Gabbay R, Murias JM. Arterial versus capillary blood gases: a meta-analysis. Respiratory physiology & neurobiology. 2007;155(3):268-79.
121. Sabat J, Gould S, Gillego E, Hariprashad A, Wiest C, Almonte S, et al. The use of finger-stick blood to assess lactate in critically ill surgical patients. Annals of medicine and surgery. 2016;10:41-8.
122. Ueta I, Jacobs BR. Capillary and arterial blood gases in hemorrhagic shock: a comparative study. Pediatric critical care medicine : a journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies. 2002;3(4):375-7.

Acta Universitatis Upsaliensis

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 1337

Editor: The Dean of the Faculty of Medicine

A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title "Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine".)

Distribution: publications.uu.se
urn:nbn:se:uu:diva-321403

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2017