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POINT-OF-CARE ANALYSIS OF INTRAOSSEOUS SAMPLES

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ACADEMIC DISSERTATION

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ABSTRACT

Background

Clinical decisions in prehospital critical care are often based on limited information about the patient's medical history and the events preceding the acute illness. In addition to physical examination, point-of-care (POC) laboratory diagnostics can provide information for decision-making. Unfortunately, obtaining blood samples for POC analysis from critically ill patients can sometimes be difficult, thus diminishing the usefulness of POC analyses in prehospital critical care.

Intraosseous (IO) access is used as an optional vascular route for fluid and medication administration for emergency patients when difficulties with venous access are encountered. Using IO access as a source of POC laboratory samples is interesting; however, evidence regarding the feasibility and agreement of these samples with arterial and venous samples is scarce. We therefore designed a series of studies to obtain more information on the POC analysis of IO samples.

Materials and methods

First, we performed a systematic literature review of the studies addressing the POC analyses of IO samples (Study I). We included all publications using POC or conventional laboratory methods to compare IO samples with venous or arterial samples for the parameters relevant to emergency care.

Second, we performed an observational study within 31 healthy volunteers (Study II). We evaluated the feasibility of IO analyses with an i-STAT® POC device and the agreement of IO values with arterial and venous values. The agreement was evaluated using the Bland–Altman method. In addition, we examined the necessity to draw waste blood before taking the actual IO sample for POC analysis.

Third, we studied the usability of IO POC values under unstable haemodynamic conditions with an experimental porcine (n = 23) resuscitation model (Study III). The model simulated a real-life course of cardiac arrest (CA) and cardiopulmonary resuscitation (CPR). Four repeated samples were simultaneously drawn from the IO access, artery and central vein during CA and CPR, and were instantly analysed with an i-STAT® POC device. The laboratory values were plotted as a function of time to demonstrate their development during CA and CPR. The arterial, venous and IO samples which were taken during CPR were compared with the arterial baseline samples to observe how they resemble the pre-arrest state.

Last, in an observational study, we analysed the agreement between IO and arterial samples obtained from 35 critically ill prehospital patients, using the

Bland–Altman method (Study IV). Moreover, we administered a questionnaire about acceptable biases in clinical practice to 16 experienced emergency physicians and compared our results with their responses.

Results

The 27 reviewed studies had heterogeneous populations: healthy volunteers, haemodynamically stable and unstable animals, adult and paediatric haematologic patients, and emergency patients. Only three of these studies followed the recommended guidelines for method comparison studies. The sample sizes were relatively small (n = 14–20) and the populations were heterogeneous in these three studies precluding the combining of results for meta-analysis. However, in IO samples, potassium values were generally higher than in arterial or venous samples.

In the observational studies, we found that the POC analyses of IO samples were often feasible. Higher failure rates were associated with higher age. Agreement of IO values with arterial values appeared acceptable for base excess, pH, standard bicarbonate, lactate, glucose, ionised calcium and sodium within healthy volunteers and critically ill emergency patients. Potassium values from IO samples were systematically higher than arterial and venous values (biases 1.8–2.2 mmol/l). Agreement within haemoglobin and haematocrit measurements showed very large variety (95% limits of agreement in the bias of haemoglobin up to 95 g/l). However, the sample sizes were too small to unequivocally prove the agreement.

Using the resuscitation model, we discovered that IO, arterial and venous values changed differently from one another during CA and CPR. Acidaemia was detectable in IO samples during untreated ventricular fibrillation, but in arterial samples the acidaemia was evident only after the initiation of CPR. The average potassium values during CPR from IO, arterial and venous samples were 4.4, 3.3, and 2.8 mmol/l higher than the pre-arrest arterial values, respectively.

Conclusions

When obtaining vascular access is challenging, IO access can be used for emergency POC analyses; however, the results of IO POC analyses should be interpreted with care. Waste blood does not need to be taken before the sample. POC analyses of IO samples may fail for older patients. In general, potassium values from IO samples are usually higher than those from arterial and venous samples, haemoglobin and haematocrit measurements from IO access are not reliable, and partial pressure measurements of oxygen and carbon dioxide from IO samples represent venous rather than arterial values.

ORIGINAL PUBLICATIONS

I Jousi M, Laukkanen-Nevala P, Nurmi J. Analysing blood from intraosseous access: a systematic review. Eur J Emerg Med 2019;26:77-85

II Jousi M, Saikko S, Nurmi J. Intraosseous blood samples for point-of-care analysis: agreement between intraosseous and arterial analyses. Scand J Trauma Resusc Emerg Med 2017;25:92

III Jousi M, Skrifvars MB, Nelskylä A, Ristagno G, Schramko A, Nurmi J. Point-of-care laboratory analyses of intraosseous, arterial and central venous samples during experimental cardiopulmonary resuscitation. Resuscitation 2019;137:124-132

IV Jousi M, Björkman J, Nurmi J. Point-of-care analyses of blood samples from intraosseous access in pre-hospital critical care. Acta Anaesthesiol Scand 2019;63:1419-1425

ABBREVIATIONS

$AaDCO_2$	arterio-alveolar carbon dioxide difference
ABG	arterial blood gases
ALS	advanced life support
AMI	acute myocardial infarction
art	arterial
BE	base excess
BP	blood pressure
CA	cardiac arrest
CCU	coronary care unit
CI	confidence interval
CLSI	Clinical and Laboratory Standards Institute
CO_2	carbon dioxide
CPR	cardiopulmonary resuscitation
CV	central venous
d	difference
EMS	emergency medical service
ED	emergency department
ERC	European Resuscitation Council
g	gram
GCS	Glasgow Coma Score
GFAP	plasma glial fibrillary acidic protein
h	hour
Hb	haemoglobin
HCO_3	standard bicarbonate
Hct	haematocrit
HEMS	helicopter emergency medical service
iCa	ionised calcium
ICC	intraclass correlation coefficient
ICH	intracerebral haemorrhage
INR	international normalised ratio
IO	intraosseous
IQR	interquartile range
IV	intravenous
K	potassium
kg	kilogram
kPa	kilopascal
l	litre

LOA	limits of agreement
MeSH	Medical Subject Headings
ml	millilitre
mmHg	millimetre of mercury
mmol/l	millimoles per litre
n	sample size
Na	sodium
ng	nanogram
OHCA	out-of-hospital cardiac arrest
paO_2	partial pressure of arterial oxygen
pCO_2	partial pressure of carbon dioxide
pH	pH (measure of acidity and basicity of an aqueous solution)
pO_2	partial pressure of oxygen
POC	point-of-care
POCT	point-of-care testing
ROSC	return of spontaneous circulation
SD	standard deviation
SE	standard error
SpO_2	peripheral oxygen saturation
VF	ventricular fibrillation

1 INTRODUCTION

An increasing number of critically ill patients receive advanced emergency care before arriving at the hospital owing to the progress in prehospital emergency care during the past decades. The progress is seen in terms of not only the training and skills of the emergency care providers but also the equipment. The methods and devices commonly used inside hospitals for examining, monitoring and treating the patients have developed to meet the needs of the prehospital environment.

New methods are eagerly adopted in prehospital systems. Emergency care providers are generally highly educated and motivated and are often forced to make decisions under challenging conditions with very limited information. Adjuncts for decision-making can reduce uncertainty and support the providers to give better care for the emergency patients. However, all new methods and devices must be properly validated before implementing them in the emergency system. The decision to start using a new method should be based on evidence-based scientific information because questioning the justification of an already established method is difficult.

Intraosseous (IO) access is used in prehospital and in-hospital care as an alternative vascular access, when difficulties in establishing an intravenous (IV) access are encountered, such as among drug abusers, burn patients, small children or patients in cold environments or in shock (1,2). Currently, IO access is used for administering fluids and medication.

Likewise, point-of-care (POC) laboratory analyses are used in prehospital care and emergency departments (ED) to save time or to compensate for missing laboratory facilities (3,4). Resuscitation guidelines suggest screening and correcting the reversible causes of cardiac arrest (CA) during cardiopulmonary resuscitation (CPR), for example, using laboratory analyses (5,6). Information about pre-arrest pathologies, such as electrolyte disorders, acid–base balance disturbances or bleeding, could be obtained via POC laboratory analyses. Obtaining a blood sample from the artery or vein of a critically ill patient can sometimes be difficult owing to vasoconstriction or cold environment. A question of whether IO access could be used as a source of emergency laboratory samples in such cases has arisen.

Whether IO samples better resemble arterial, venous or capillary blood is not self-evident. Likewise, the influence of bleeding and hypovolaemia on the bone marrow circulation (and thus IO analyses) is unclear.

Some previous studies have concluded that IO blood samples can be used as a substitute for arterial or venous blood samples (7–9). However, this information is based on studies with very different populations (healthy volunteers, haemodynamically stable haematologic patients or laboratory animals) than

emergency patients, who are the most likely target group of the POC analysis of IO samples. Unfortunately, the studies with unstable patients are scarce.

The statistical methods used in previous studies might mislead the readers. Correlation, linear regression and comparison of grouped means are the commonly used methods in previous studies. None of the methods indicate agreement, which is the relevant measure to determine whether the IO and arterial or venous values can be used interchangeably (10). False interpretation of IO values can lead to the misdiagnosis and mistreatment of a critically ill patient. To avoid such situations, limitations of the previous studies and the method of POC analysis of IO samples have to be identified.

The purpose of this dissertation is to provide understanding of how POC analyses of IO samples can be used to facilitate the assessment and treatment of emergency patients. This study reports on the existing literature of IO sample analyses and the feasibility and reliability of IO POC analyses within healthy volunteers and prehospital emergency patients. It also demonstrates how IO values act in relation to arterial and venous values during experimental CPR.

2 LITERATURE REVIEW

2.1 POINT-OF-CARE TESTING

2.1.1 DESCRIPTION OF POINT-OF-CARE TESTING

Point-of-care testing (POCT), also known as near-patient testing or bedside testing, is intended to provide test results more rapidly than those achieved in institutional laboratory settings. The key objective of POCT is to quickly provide a result so that proper treatment can be implemented faster, thus aiming for improved treatment outcome (3). Although POCT is particularly important in critical care areas, such as prehospital emergency medical services (EMS), it can also be useful in skilled nursing facilities and home health care delivery. POCT can be used in ED to optimise the turnover times of the patient flow (11). It can also provide diagnostic services to remote health care units, which otherwise lack complete laboratory services. POCT should facilitate the treatment decisions of clinicians and / or provide convenience for the patient.

Results obtained from POCT should be comparable with those from the institutional laboratory. Because unreliable test results can potentially cause serious consequences, it is vital that the obtained results from POCT are trustworthy. POCT is often performed by the personnel who are not trained in medical laboratory practice. Pre-analytic, analytic and post-analytic factors can influence the results owing to many patient-related or provider-related reasons. The analytical performance of POCT in terms of imprecision and bias, compared with that of standard laboratory analyses, is a key measure of POCT reliability (4,12). Implementation, training, quality control and surveillance of the POCT should be an integral part of the management system of the health care unit (4). Few studies suggest good agreement between POCT and an institutional laboratory testing among emergency patients (13-15). However, their results cannot be generalised for all patient groups and all devices.

Several outcome measures can be used for estimating the benefits of POCT (16). One main clinical outcome could be the proportion of patients for whom POCT changed the management or shortened the time to the initiation of treatment (11,17). Patient, physician, paramedic or nurse satisfaction, length of ED and hospital stay, mortality or economic outcomes should also be considered (18,19).

2.1.2 POINT-OF-CARE TESTING IN PREHOSPITAL CRITICAL CARE

Decision-making in prehospital critical care is based on patient and event history, current clinical findings and existing resources. The information is often limited, and additional support for decision-making can be obtained using POCT (18,19). Glucose measurement, coagulation tests, cardiac enzymes, blood-gas analyses and electrolyte and haemoglobin (Hb) measurements could provide useful information to EMS providers. Although the majority of the research concerning POCT within emergency patients addresses ED patients, a few interesting studies from the prehospital setting can be mentioned (20-29).

Prehospital lactate measurements have been shown to predict in-hospital mortality and the need of in-hospital resuscitative care. In a prospective, multi-centre study, prehospital lactate levels were measured in 387 trauma patients whose prehospital systolic blood pressure was between 71 and 100 mmHg. Elevated prehospital lactate level (≥ 2.5 mmol/l) was associated with the need for resuscitative care during the following six hours or death. Resuscitative care was defined as blood transfusions (≥ 5 units) or an intervention for haemorrhage (surgery, pelvic fixation or embolisation). Lactate was superior to blood pressure (BP) or shock index (heart rate/systolic blood pressure) in predicting the need for resuscitative care (20).

In another prospective study with 673 prehospital patients, elevated prehospital lactate levels (≥ 2 mmol/l) were strongly associated with in-hospital mortality, after adjustment for known covariates (21). The conclusions were complementary in a third study that evaluated the prognostic value of prehospital lactate within 124 patients with abnormal vital signs [systolic BP < 100 mmHg or respiratory rate < 10 or > 29 per min or Glasgow coma score (GCS) < 14]. Two prehospital lactate measurements were performed in this study: the first on arrival at the scene and the second just before arrival at the hospital. Mortality was significantly higher in patients with lactate levels ≥ 3.5 mmol/l than in those with lactate levels < 3.5 mmol/l, measured at both time points; the delta lactate level was a significant independent predictor of in-hospital mortality (22).

A prospective, randomised, controlled trial for evaluating the value of prehospital arterial blood-gas (ABG) measurements in the accuracy of diagnostics and quality of treatment was conducted in Denmark during 2016–2018 (23). In the study, 222 patients were randomised to have either standard treatment or additional prehospital ABG measurements available. In majority of the cases (78/102) with ABG measurements, the prehospital physician reported that ABG analysis results increased their perceived diagnostic precision. In the non-ABG group, the lack of ABG analysis results was perceived to have decreased the diagnostic accuracy in majority of the cases (81/120). ABG analyses increased the probability of targeting specific prehospital therapeutic interventions. However, difference in the outcome was not observed.

Coagulation in 103 prehospital emergency patients was measured using a POC device in a prospective observational study design (24). The assessment of international normalised ratio (INR) with a POC coagulometer was found feasible, and the prehospital POC INR values were in good agreement with the results of conventional in-hospital assessment. The emergency physicians in the hospital considered the prehospital measurements to be of high or medium (30%) value among all the patients, and the value was considerably higher (63%) among neurological patients.

The diagnostic value of prehospital POC Hb measurements to predict significant haemorrhage was studied in a French multi-centre study with 6402 patients (25). Prehospital POC Hb measurement could predict significant haemorrhage. The difference between prehospital and initial hospital Hb values was predictive for major haemorrhage among trauma patients – with same volume of fluids infused, larger decrease in Hb values indicated significant haemorrhage.

Future implications of prehospital POCT could be in streamlining the diagnostics and treatment of acute myocardial infarction (AMI) or stroke. Prehospital collection of blood biomarkers for increasing the speed and accuracy of diagnostics of ischemic stroke was found to be feasible and timesaving in a study within 430 thrombolysis candidates (26). In a prehospital study from Berlin, plasma glial fibrillary acidic protein (GFAP) measurements were performed in a mobile emergency stroke unit to confirm whether determining GFAP can support in differential diagnosis between acute ischemic stroke and intracerebral haemorrhage (ICH) (27). GFAP levels above the cut-off level (0.29 ng/ml) were able to confirm the diagnosis of ICH, but the sensitivity of the marker was low, particularly in smaller haemorrhages. In the editorial of *Biomarkers in Medicine*, Lindsberg et al. discuss the potentials of prehospital POCT in differential diagnostics and decreasing the prehospital delays within stroke patients (28). If patients with brain haemorrhage could be differentiated with prehospital biomarkers, they could benefit from rapid normalisation of coagulation and transfer to a hospital with neurosurgical facilities.

A protocol of an interesting Danish, multi-centre, randomised clinical trial with 4800 patients was recently published in *Trials* (29). The study aims to discover whether adding prehospital copeptin measurements to the diagnostic path of suspected AMI patient would optimise the diagnostics and length of stay in the ED. The results have not been published yet.

2.1.3 POINT-OF-CARE TESTING DURING CARDIOPULMONARY RESUSCITATION

The advanced life support (ALS) guidelines instruct to actively search for and correct the reversible causes of CA during CPR (**Figure 1**) (5). According to the European Resuscitation Council (ERC) Guidelines for Resuscitation 2015, electrolyte and

metabolic disorders can be screened with laboratory tests during CPR (6). In practice, the first POC analysis would most likely occur after the initiation of ALS when opening a vascular access.

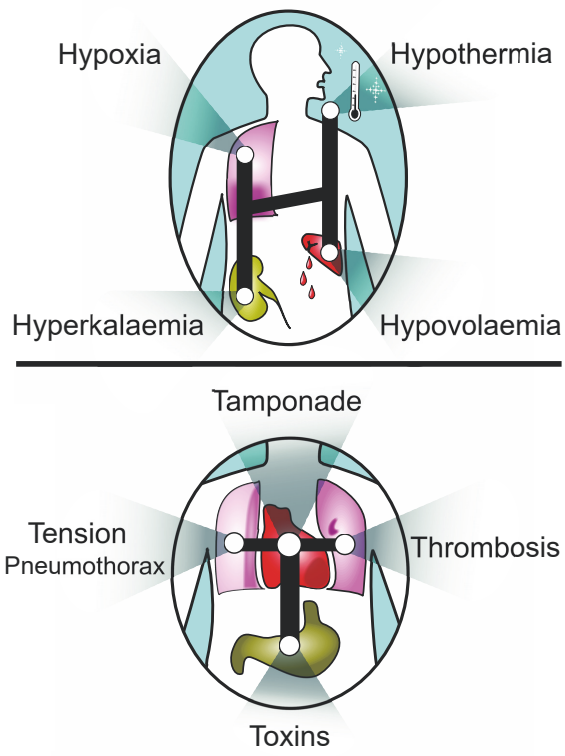


Figure 1. The 4 H's and 4 T's mnemonic to remember the reversible causes of cardiac arrest. (Copyright European Resuscitation Council – www.erc.edu – 2019_NGL_012)

The resuscitation guidelines state that blood gases are difficult to interpret during CA. The ABG may be misleading during CA; instead, central venous samples might provide better estimation of tissue pH. These arguments are based on studies by Grundler and Weil et al. in 1986 (30,31). The first was an experimental porcine resuscitation study with sequential blood sample analyses from the artery and central vein. The researchers concluded that ABG fail as indicators of systemic acid–base status and tissue acidosis (30). In the second study, Weil et al. investigated the acid–base balance of arterial and mixed venous blood during CPR of 16 critically ill coronary care unit (CCU) patients. They noted that arterial pH, partial pressure of carbon dioxide (pCO_2) and standard bicarbonate (HCO_3) did not significantly change during CA compared with the values taken before CA. However, significant changes in pH and pCO_2 were demonstrated in mixed venous blood. Weil et al. concluded that ABG may fail as appropriate guides for acid–base management

during CPR (31). However, the patient population was very different from the out-of-hospital cardiac arrest (OHCA) patients nowadays. All the study patients had major haemodynamic derangements before CA and none of the patients survived.

Tucker et al. showed similar findings in 1994 in their experimental study on 52 pigs undergoing CA and CPR. They reported that untreated CA may be accompanied by normal arterial and mixed venous blood-gas levels. Tissue acidosis was only revealed after tissue perfusion was restored, and it was better shown in mixed venous blood samples (32). Acidosis is a common finding during CA and CPR (33); however, the use of buffers is currently recommended only in cases of severe tricyclic antidepressant intoxications or CA associated with hyperkalaemia (5).

The resuscitation guidelines propose screening electrolyte abnormalities as reversible causes of CA (6). Hyperkalaemia as a possible cause of CA can be screened with POCT during CPR. During CA and CPR, the reduction in oxygen transport results in anaerobic metabolism, leading to metabolic lactic acidosis. This in turn shifts potassium from the intracellular space to the extracellular space. Therefore, the estimation of pre-arrest potassium levels, based on the intra-arrest POC measurements, is challenging and might result in false positive results. Besides, according to the resuscitation guidelines, little or no evidence supports the treatment of electrolyte abnormalities during CA (6).

The use of POC analyses during CPR for prognostication is an interesting subject that still needs further research. In a prospective observation study, Spindelboeck et al. measured ABG during CPR among 115 OHCA patients. They reported a trend towards higher partial pressure of arterial oxygen (paO_2) values in patients who reached sustained return of spontaneous circulation (ROSC). Moreover, they found out that the arterio-alveolar carbon dioxide (CO_2) difference ($AaDCO_2$) was associated with sustained ROSC, with the survivors having lower $AaDCO_2$ values (33).

2.2 INTRAOSSEOUS ACCESS

2.2.1 ANATOMY AND PHYSIOLOGY OF BONE MARROW

Highly vascularised and innervated bone marrow is located within the cavities of bones (**Figure 2**). The stromal cells (reticular cells, fibroblasts, endothelial cells and adipocytes) and the parenchymal cells (haematopoietic cells) are the two major cellular elements of bone marrow and represent its two primary functions: reticuloendothelial function and haemopoiesis. Several mechanisms regulate haemopoiesis: nervous system, humoral growth and inhibitory factors, and cell–cell interaction between marrow cells (34-36).

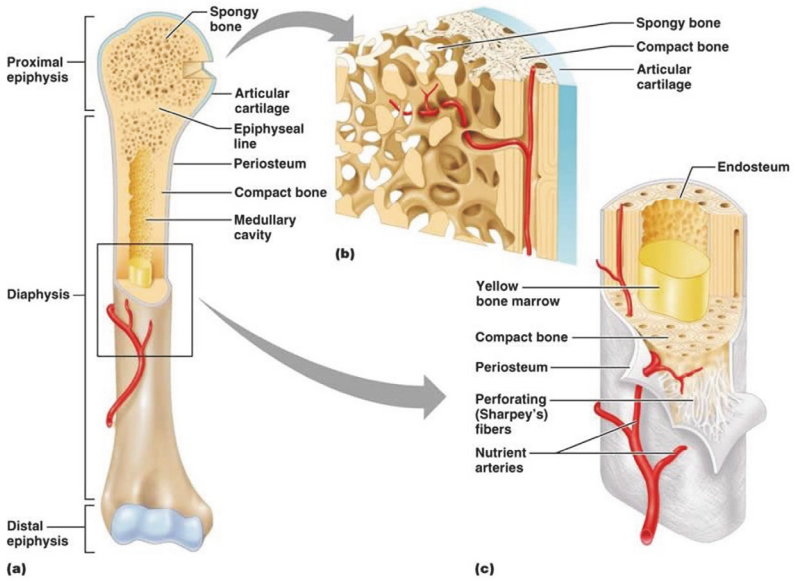


Figure 2. Structure of a long bone. The surface is compact bone, which is thicker in the diaphysis and thinner in the epiphyseal regions. Cancellous bone filled with red marrow is inside the epiphysis. The diaphysis is filled with yellow bone marrow which mainly comprises of adipocytes. (Figure reproduced with the permission of Anuj Kumar) (37)

In infancy, all the bone marrow is haemopoietic. During childhood, progressive fatty replacement of marrow occurs throughout the long bones. In adult life, only the bone marrow of the vertebrae, ribs, sternum, skull, sacrum, pelvis and the proximal ends of the femur, tibia and humerus is haemopoietic (**Figure 3**) (35,36). Their marrow becomes less productive by age. The adipose bone marrow is capable of reversion to haemopoiesis on demand. In experimental animal studies, marrow blood flow has been shown to significantly increase as a result of normovolaemic and hypovolaemic anaemia (38,39). Medullary venous channels and nutrient and emissary veins connect the bone marrow to the vascular system. The bone marrow cavity does not collapse owing to the compact bone and the presence of bone trabeculae (**Figure 4**). This provides a non-collapsible, highly vascularised space for fluid and medication administration via emergency IO access even in profound shock (2).

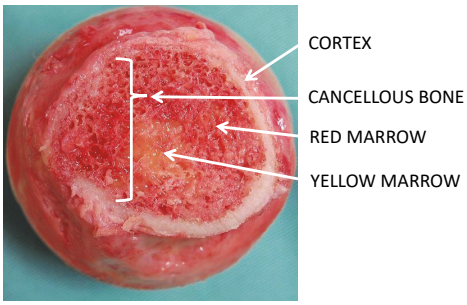


Figure 3. The structure of a proximal end of a long bone. The cortex is thin, enabling IO needle penetration. The stability of the bone is achieved with the trabecular structure of the cancellous bone. The spaces between the bone trabeculae are filled with red bone marrow, which is gradually replaced by yellow marrow with aging. (Figure source: Wikimedia common)

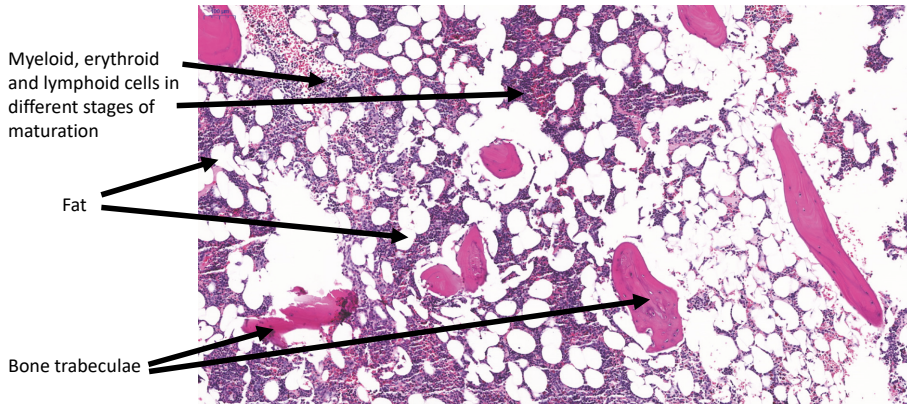


Figure 4. Histologic picture of bone marrow biopsy. Haematoxylin-eosin stain. (Figure by Johannes Dunkel)

2.2.2 USE OF INTRAOSSEOUS ACCESS IN EMERGENCY CARE

IO access can be considered a life-saving bridge to definitive vascular access. It is recommended when vascular access is impossible, difficult or likely to lead to delayed treatment in emergency situations (5,40). In general, candidates for IO access are burn patients, children, IV drug abusers and patients in shock or in cold environment. IO access can be used as a primary or rescue vascular access after failed venous access attempts (41). The local protocol in the EMS system should determine the indications and threshold for IO insertion (1,42,43).

Currently, three different methods for introducing IO needles are available: manual, impact-driven and powered drill (1). Manual needles are hollow steel needles with removable trocars that prevent the bone fragments plugging the needle

during insertion. They are manually screwed into the target bone with twisting motion. Impact-driven needles require pressure which releases a spring-loaded mechanism for the penetration of the needle into the sternum or other target bone. The newer powered drill insertion technique (EZ-IO® automated intraosseous infusion system) is likely to provide faster insertion and better success rate than the previously used manual and spring-loaded devices (1,44-46).

The insertion site is selected based on the patient's anatomy, patient's age, how one can access the patient, presenting condition and the experience of the practitioner. Recommended locations for IO access for adults are the proximal ends of humerus and tibia or the distal end of tibia; for children, the recommended locations are the proximal or distal ends of tibia or the distal end of femur (41,47). IO access can also be inserted into the sternum, but devices specially designed for this purpose are required.

Bone marrow is highly vascularised; therefore, IO access is currently used for fluid and medication administration. Within healthy volunteers and experimental animal studies, the absorption rates of fluids and medications have been shown to be comparable to those achieved by IV administration (48-52). Few case reports and experimental studies support blood product transfusion (53-56). Bjerkvig et al. have studied fresh whole-blood transfusion through the sternal IO route among voluntary military personnel (57). The study suggests the method to be safe and reliable and to provide sufficient flow rates.

2.2.3 INTRAOSSEOUS ACCESS AND CARDIOPULMONARY RESUSCITATION

The resuscitation guidelines recommend using an IO access as an optional vascular access during CPR for both adults and children (5,40). Until recently, the research on using IO access in resuscitation has focused on the success rates and procedural times of IO insertion. In a prospective, randomised trial with 182 non-traumatic OHCA patients, the first-attempt success rate of tibial IO access was higher than that of humeral IO or peripheral venous routes (91% vs. 51% and 43%, respectively) (58). Leidel et al. reported that the first-attempt success rate was higher and the procedure time was faster with IO access than that with central venous (CV) access during medical and trauma resuscitation in an ED (IO: 85%, 2 min; CV: 60%, 8 min) (45).

The recent studies focus on CPR outcomes. Questioning whether resuscitation drug administration routes (IO vs. IV) affect outcome is relevant. A small experimental study with 21 piglets showed that the adrenaline administration route (humeral IO vs. IV) did not affect ROSC (50). Another experimental study with 30 swine compared the effects of early administration of adrenaline via IO access (1 min after the start of the CPR) with the delayed administration of adrenaline via

IV route (8 min after the start of CPR) in prolonged ventricular fibrillation (VF). Early IO adrenaline administration improved 24h-survival compared with delayed IV adrenalin (100% vs. 40%). Early IO adrenaline administration seemed to result more often (60% vs. 30%) in survival with good neurological outcome, but the difference was not statistically significant (59).

The results of four larger register-based studies are not consistent with their outcomes. Clemency et al. retrospectively reviewed 1310 OHCA patients, out of whom 40% had an IO access as a primary vascular route. Based on the end point ROSC at time of ED arrival, an IO first approach was not inferior to an IV access (60). In a multivariate adjusted analysis of a retrospective register of 1800 OHCA patients, Feinstein et al. found that although access type was not associated with survival to discharge, IO access was associated with lower likelihood of ROSC and survival to hospitalisation (61). Two recent large register-based retrospective studies report opposing conclusions. In a study of 13,155 OHCA patients, out of whom 5% had an IO access as a primary vascular route, IO access was associated with poorer neurologic outcomes than IV access (62). In an analysis of 19,731 OHCA patients, out of whom 15.5% had primarily attempted IO access, there was (after adjustment) no difference in hospital survival or survival with favourable neurologic function; however, IO access was associated with lower rates of sustained ROSC (63). It has to be emphasised that in observational studies, the causality regarding the association between vascular access and outcome is unclear.

2.2.4 CONTRAINDICATIONS AND COMPLICATIONS

Contraindications for IO access insertion include fracture in the target bone, excessive tissue or absence of adequate anatomical landmarks, infection on the area of planned insertion site, previous significant orthopaedic procedure at site or previous IO access to the target bone within the past 48 h (41,47,64).

Complications associated with IO access are rare: less than 1% (2,42). Reported incidence of osteomyelitis is 0.4%–0.6% and it is generally associated with bacteraemia, peripheral vascular disease, local skin infection or prolonged infusion (45,65,66). Another possible complication is extravasation, which if undetected, might lead to compartment syndrome (67). Other rare complications are local infections, penetration through the posterior cortex of the bone (68), catheter bending and difficulty in removing the IO device, which can all be prevented using a proper insertion technique and by frequent monitoring of the infusion site. To avoid complications, the insertion of IO access must be performed under sterile conditions and the length of usage should be limited to a maximum of 24 h (47).

2.3 LABORATORY ANALYSES OF INTRAOSSEOUS SAMPLES

2.3.1 FEASIBILITY OF THE ANALYSES

Many previous study protocols have not reported on the success rates of the analyses (69-71). In studies wherein difficulties were described, the major problem seemed to be clotting of the samples (72,73). In the experimental studies with sequential samples over time, the aspirates were easily obtained in the beginning; however, the aspiration became more difficult over time (74-76). Haemolysis was reported in two studies with the IO samples of cats and dogs (77,78). Haemolysis could be one probable explanation for the elevated potassium values in the IO samples (**Figure 5**).

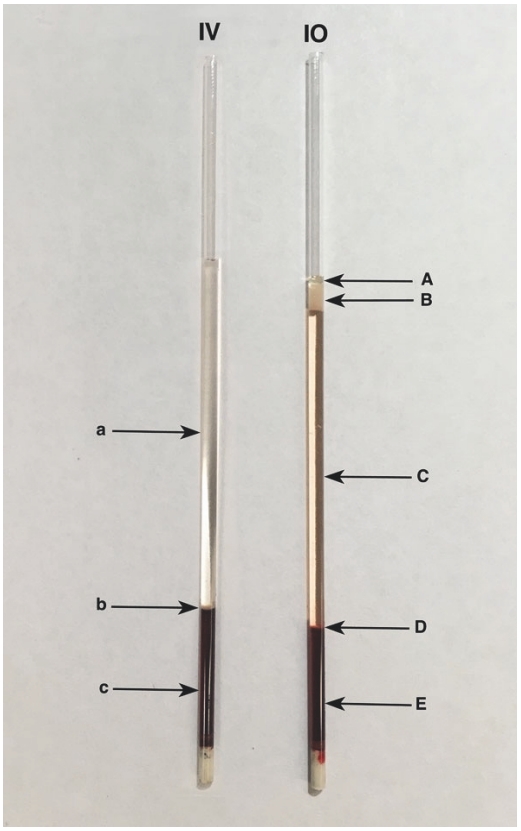


Figure 5. Micro-haematocrit capillary tubes for the analyses of intravenous haematocrit (left) and intraosseous myelocrit (right) from a study of dogs undergoing elective surgery.

Intravenous sample **(IV, left)**: plasma (a), buffy coat (b), and red blood cells (c).

Intraosseous sample **(IO, right)**: fat (A), bone marrow particles (B), plasma (C), buffy coat (D), and red blood cells (E). (Figure reproduced with the permission of Søren Boysen and the publisher) (78)

2.3.2 COMPARISON OF INTRAOSSEOUS SAMPLES WITH CONVENTIONAL BLOOD SAMPLES

Because bone marrow is a metabolically active tissue that is different from circulating blood, and located somehow between arterial and venous circulation, several physiological differences can be expected when comparing IO samples with arterial and venous samples.

The first experimental study comparing IO samples with arterial and venous samples in 10 dogs was published in 1989 (79). The samples were analysed using an institutional laboratory analyser for a large variety of parameters. To the same article was integrated a blood culture study of 24 human patients with suspected sepsis. The 15 patients with positive blood cultures for bacteria or fungus had the same microbes present in the IO samples. The reliability of the IO analyses cannot be interpreted from the study owing to insufficient statistical methods, which has been the challenge in many of the following studies.

Several experimental studies have used haemodynamically unstable animal models. Kissoon et al. compared the acid–base status between venous and IO samples of nine piglets during CPR, concluding that during longer periods of CPR, IO blood may reflect local acidosis and yield lower pCO_2 and higher pH values than the central venous blood (71). Studies with models of endotoxemic shock, hypothermia, hypovolaemia and hypoxia have concluded that IO samples were similar to venous samples (70,73,80-83). The conclusions have been drawn by comparing the mean values of IO measurements to the mean values of venous measurements; however, this method does not measure the intra-individual agreement. A recent study using proper statistical methods was performed within dogs in the Faculty of Veterinary Medicine of the University of Calgary (78). In the study, 12 dogs of different breeds underwent elective knee operations. IV and IO samples were compared for various parameters [blood urea nitrogen, glucose, packed cell volume, total plasma protein, lactate, sodium (Na), potassium (K), chloride (Cl), urea, glucose, pH, anion gap, haematocrit (Hct), pO_2 and pCO_2]. The conclusion was that the IO aspirates, excluding K and Hct measurements, appear to be a reliable alternative.

Montez et al. and Miller at al. performed studies within 15 and 10 healthy volunteers, respectively (9,84). Both studies used correlation and comparison of grouped means as statistical methods. Funding from an IO device manufacturer was reported for both. The studies concluded that there was a significant correlation between IO and venous samples for lactate (Montez) and glucose, urea, creatinine, Cl, total protein, albumin, red blood cell count, Hb and Hct but not for platelet count, white cell count, Na, K, CO_2 and ionised calcium (iCa) (Miller). The used statistical methods do not report the agreement between IO and venous samples.

In four studies, the agreement between IO and venous samples within haematologic or oncologic haemodynamically stable patients, either adults or

children, had been assessed (7,8,85,86). Veldhoen et al. compared the venous and IO samples of 20 children presented for scheduled diagnostic bone marrow aspiration (86). An i-STAT® POC analyser was used for analyses. The statistical methods used in the study for assessing the agreement followed the recommended guidelines. The agreement was found to be clinically acceptable for pH, base excess (BE), Na, iCa and glucose. Clinically relevant differences were found for pCO_2, pO_2, HCO_3 and K. The other studies with haemodynamically stable patients have used Pearson's correlation or comparison of grouped means as statistical methods, and the results concerning agreement are conflicting. Hurren et al. and Grisham et al. reported that K values in IO samples were elevated (8,85).

The agreement between IO and IV samples within emergency patients has been analysed in only one previous study (69). Seventeen critically ill patients needing resuscitation and IO access at the ED of a hospital were included in the study. Of these, 16 patients arrived in the ED in CA, and the total in-hospital mortality in the study group was 76%. IO and IV samples were obtained within 5 min and analysed using an EPOC® POC analyser. The Bland–Altman method was used for comparison. Reasonable agreement for pH, HCO_3, Na and BE, moderate agreement for lactate and poor agreement for K, pO_2, pCO_2, and glucose were reported in this study population.

Only five studies, published until 2019, have followed the recommended statistical methods for comparing IO samples with venous or arterial samples (**Table 1**). These studies have very different populations: critically ill emergency patients (69), cardiovascular stable, anesthetised haematologic patients (86), pigs with sequentially changing cardiac outputs (82,87), and anesthetised dogs undergoing elective knee surgery (78).

Table 1. Details of the studies with recommended statistical methods.

	Subjects	n	Circulation	Comparison	Analyser
Kissoon et al. (82)	pigs	14	sequential changes in cardiac output	mixed venous	Laboratory
Veldhoen et al. (86)	humans	20	cardiovascular stable, anaesthetised	peripheral venous	POC (i-STAT®)
Tallman et al. (69)	humans	17	cardiac arrest /critically ill	peripheral venous	POC (EPOC®)
Ackert et al. (78)	dogs	12	general anaesthesia for elective surgery	jugular venous	POC (i-STAT®, and other) and laboratory
Strandberg et al. (87)	pigs	12	pigs during stable circulation and after haemorrhage of 20% and 40% of the blood volume	arterial, central venous	POC (i-STAT®, and other) and laboratory

2.3.3 RELEVANT PARAMETERS

A large variety of parameters from IO samples have been studied (**Table 2**). Measurements of electrolytes, acid–base balance, blood gases and Hb could provide important information about an unstable patient for decision-making, and these could be parameters of interest within IO samples. Although measurements of opioid concentration, troponin, creatinine, amylase or liver enzymes from IO samples provide academically interesting information, they are not vital for fast decision making in critical care (72,75). These analyses could often be performed after venous access has been achieved.

Blood typing and screening from IO samples is interesting owing to the establishment of prehospital transfusion protocols. Before transfusing O-negative red blood cells, drawing a blood sample for blood type screening and cross-matching is important. Successful ABO- and Rh-blood typing from IO access has been reported in two studies with 28 and 71 patients admitted to haemato-oncological service for bone marrow examination (88,89).

Table 2. Parameters for which the agreement of IO and arterial or venous samples has been studied.

	pH	HCO_3	BE	Na	K	pO_2	pCO_2	Glucose	Lactate	iCa	Hb	Hct	ALAT	ASAT	GGT	AFOS	Crea	Urea	WBC	RBC	Trom	Cl	Albumin	Total protein	CK	LD	Blood typing	Other
Bäckman*(88)																											x	
Strandberg (87)	x	x	x	x	x	x	x			x	x	x	x	x	x	x	x								x			
Ackert (78)	x	x		x	x		x	x	x		x	x						x				x		x				x[1]
Tallman* (69)	x	x	x	x	x	x	x	x	x																			
Eriksson (76)													x	x	x	x									x	x		
Strandberg (72)																												x[2]
Eriksson (75)																												x[3]
Strandberg (74)																	x											
Veldhoen* (86)	x	x	x	x	x	x	x	x		x																		
Strandberg. (90)	x	x	x			x	x	x	x		x																	
Montez*(84)									x																			
Larsson (91)																												x[4]
Strandberg (83)	x	x	x	x	x	x	x		x	x	x																	
Miller* (9)				x	x		x	x		x	x	x					x	x	x	x	x	x	x	x				
Greco (92)				x	x			x			x	x	x	x	x		x	x	x	x	x	x						x[5]
Hurren * (85)				x				x		x	x	x					x	x	x		x							
Voelckel (70)	x		x				x		x																			
Johnson (80)				x	x			x	x	x	x																	x[6]
Abdelmoneim (73)	x						x																					
Kissoon (71)	x						x																					
Dhein (77)				x	x			x	x				x			x	x	x				x	x	x				x[7]
Ummenhofer* (7)	x	x		x	x	x	x	x			x	x	x	x		x	x	x	x		x	x		x				x[8]
Kissoon (82)	x						x																					
Kissoon (81)	x	x				x	x																					
Jennings (93)	x			x	x	x						x											x	x				
Brickman* (89)																											x	
Grisham* (8)	x	x	x	x	x	x	x	x		x		x										x						x[9]
Orlowski** (79)				x	x		x	x		x				x		x		x				x				x		x[10]
Brickman (94)	x					x	x																					

* human study
** combined animal and human study

[1] anion gap, packed cell volume
[2] thromboelastography, PT, APTT, fibrinogen
[3] TnI
[4] plasma level of morphine
[5] MCH, MCHC, MCV
[6] Mg
[7] cholesterol, phosphate
[8] bilirubin
[9] SpO_2
[10] microbial culture studies, bilirubin, phosphorus

ABG arterial blood gases
AFOS alkaline phosphatase
ALAT alanine aminotransferase
APTT partial thromboplastin time
ASAT aspartate aminotransferase
BE base excess
CK creatine kinase
Cl chloride
Crea creatinine
GGT gamma-glutamyl transferase
Hb haemoglobin
HCO_3^- standard bicarbonate
Hct haematocrit
iCa ionised calcium
INR international normalised ratio
K potassium
LD lactate dehydrogenase
MCH mean corpuscular haemoglobin
MCHC mean corpuscular haemoglobin concentration
MCV mean corpuscular volume
Mg magnesium
Na sodium
paO_2 partial pressure of arterial oxygen
pCO_2 partial pressure of carbon dioxide
pO_2 partial pressure of oxygen
PT prothrombin time
RBC red blood cell count
SpO_2 peripheral oxygen saturation
TnI troponin I
Trom thrombocyte count
WBC white blood cell count

2.4 VALIDATION OF NEW MEASUREMENT TECHNIQUES

2.4.1 METHOD COMPARISON TECHNIQUES

Prior to implementing a new measurement technique into clinical practice, it needs proper validation. Clinical and Laboratory Standards Institute (CLSI), a global leader in the standardisation and harmonisation of laboratory standards, creates guidelines of specialty areas. The Standard document *EP09 Measurement Procedure Comparison and Bias Estimation Using Patient Samples* describes methods for comparison and determining the bias between two measurement procedures (95).

Method comparison is a common technique applied by device manufacturers and laboratories to estimate the bias of the results from the new method compared with those from a standard method. The assumption that the new measurement procedure provides 'true' values requires bias estimation. Bias means the systematic measurement error of the new method compared with the comparator (95).

Tietz Textbook of Clinical Chemistry and Molecular Diagnostics recommends statistical approaches to objectively analyse the data for the differences between the measurements (96). The recommended methods are (a) *DoD plot* (distribution of differences, a frequency plot or histogram of the distribution of differences with measures of central tendency and dispersion); (b) *Bland–Altman method* (a difference/bias plot, which shows differences as a function of the averages of measurements); and (c) *regression analysis*.

2.4.2 BLAND–ALTMAN METHOD

The Bland–Altman method, which we used in our studies, is widely used for evaluating method comparison data. It is a simple and illustrative method to interpret. It was created by statisticians Martin Bland and Douglas Altman for comparing measurements in clinical medicine; however, it has also been widely adopted in clinical chemistry (10). The Bland–Altman graph is a plot of differences against the average of the results by the two methods (**Figure 6**). It provides information on the differences and their dispersion, which is useful in evaluating whether agreement problems exist over the entire range or only in certain ranges of measurements. The Bland–Altman graph demonstrates the bias and the limits of agreement (LOA). The bias is the average of the individual differences of paired measurements and represents the systematic error. LOA is the ± 1.96 standard deviation (SD) interval, within which 95% of the differences fall, and it represents the random error between the measurement methods (97). It is very important to emphasise that the Bland–Altman method only defines the calculated agreement

intervals; the acceptable limits in practice should always be based on clinical estimation (98).

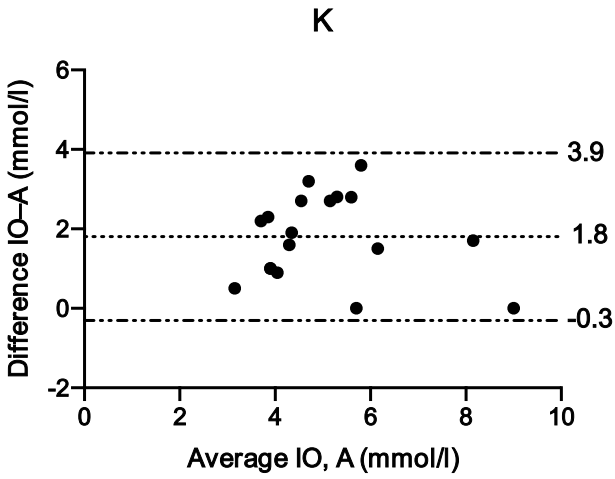


Figure 6. An example of a Bland–Altman graph. The average of the paired measurements by two different methods is presented on the X-axis, and the difference of the paired measurements by two different methods is presented on the Y-axis. The centremost dotted line represents bias (average difference of all the paired measurements), and the outermost dot/dash lines represent the 95% limits of agreement (LOA) of the bias (± 1.96 standard deviation (SD)). K, IO, A and mmol/l represent potassium, intraosseous, arterial and millimoles per litre, respectively.

2.4.3 INSUFFICIENT STATISTICAL METHODS FOR METHOD COMPARISON

A commonly used measure of agreement is the Pearson product moment correlation coefficient (99). Even high correlation does not necessarily indicate good agreement. Correlation generally indicates dependence (mainly a linear relationship) between the variables. A correlation coefficient reflects neither the slope of the correlation line nor the intercept deviation from zero. Thus, even in situations with perfect correlation, the agreement can though be poor. Only a 'line of identity' (also called 'line of equality' or '1:1 line') in which the correlation line leaves at a 45° angle from the origin in x,y coordinates would represent perfect agreement (**Figure 7**) (99).

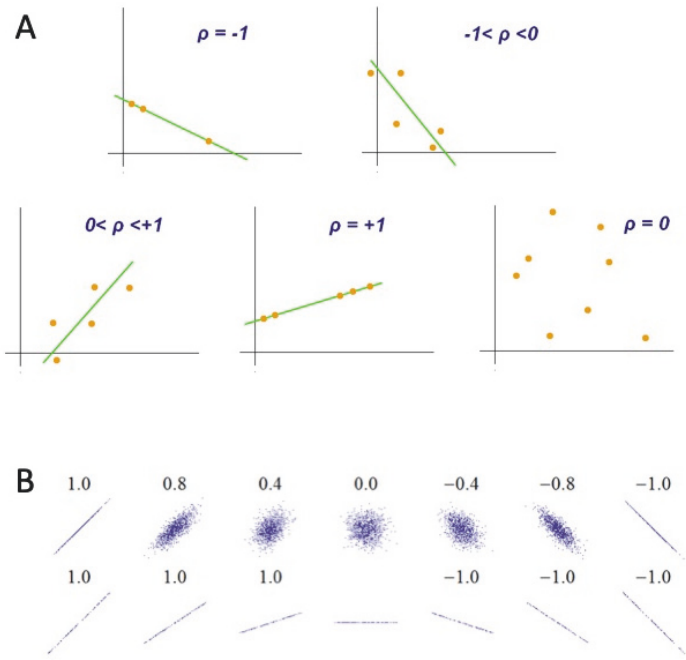


Figure 7. The Pearson product moment correlation coefficient (ρ) can range between –1 (total negative correlation) and +1 (total positive correlation) (A). A value of 1 implies that a linear equation perfectly describes the relationship between X and Y, with all data points lying on a line for which Y increases as X increases. It does not reflect the slope of the relationship (B). (Figure source: Wikimedia commons)

Another inadequately used statistical method for method comparison studies is the *comparison of grouped means* (96). The average value of all individuals' results obtained using the new method is compared with an average value of the same individuals' results measured using the standard method. If the averages of the groups are similar, this has been erroneously interpreted to indicate good agreement and interchangeability between the methods. This method ignores the intra-individual bias between the measurement methods and leads to false assumptions.

Statistical significance testing with a *paired sample t-test* should also not be used to determine the equivalency of measurements (100). Paired *t*-test measures the average bias and does not indicate the equivalency of measurements throughout the analytical measurement range (**Figure 8**).

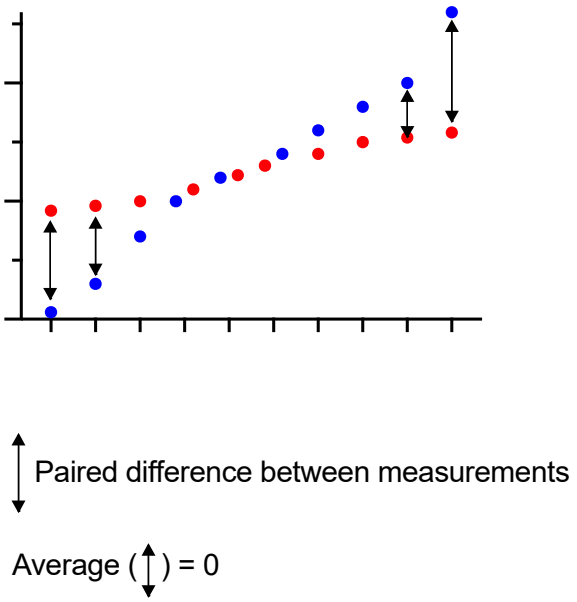


Figure 8. An example of a paired sample *t*-test. Measurements with two different methods (blue and red points). An arrow indicates the difference between paired measurements. Compared with the standard method (red points), the new method (blue points) can give lower values in the lower measurement range and higher values in the upper range. The average of the differences can be zero (equalling large p-value), leading to a false interpretation of no difference between measurements.

3 STUDY QUESTIONS

This study aims to provide knowledge to support or contradict the use of POC analyses of IO samples within emergency patients and to find proper indications for using them to support decision-making in emergency care.

The following are the specific objectives:

What is the scientific evidence concerning the analyses of IO samples?
(I)

Are the analyses of IO samples feasible with an i-STAT® point-of-care analyser?
(II, III, IV)

How do the POC values from IO samples agree with those from arterial and venous blood samples? Does the agreement persist in critical illness and cardiac arrest?
(II, III, IV)

How IO, arterial and venous samples taken during CPR reflect the pre-arrest state?
(III)

4 MATERIALS AND METHODS

4.1 STUDY DESIGN

The dissertation comprises four studies approaching the study question from different perspectives: a systematic review of the literature on analysing blood samples from IO access (Study I) and three observational prospective studies evaluating the feasibility and reliability of IO POC analyses in three distinct cohorts, including healthy volunteers (Study II), animals being resuscitated from VF (Study III) and prehospital critical care patients (Study IV) (**Table 3**).

Table 3. Characteristics of the observational studies of the dissertation.

	n	Material	IO insertion site	Comparator
Study II	31	healthy volunteers	proximal tibia	arterial and peripheral venous blood
Study III	23	anesthetised pigs	proximal tibia	arterial and central venous blood
Study IV	33	prehospital critical care patients	proximal humerus	arterial blood

4.2 METHODS OF THE SYSTEMATIC LITERATURE REVIEW

The review protocol was registered in the International prospective register of systematic reviews (PROSPERO) database (CRD42017064194), and the data were collected and reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. We searched for articles comparing IO blood samples with venous or arterial blood samples for parameters relevant to emergency care. The search was performed from Medline and Embase databases, Cochrane Library and the clinicaltrials.gov registry. Data published until 8 September 2017 were included without language restrictions. Because of the small number of human POC studies, we included both animal and human studies and the studies using conventional laboratory analyses and POC devices. Data search was performed using terms covering both 'Bone marrow' and 'Laboratory analysis' fields (**Table 4**).

Table 4. Search terms used in the literature search of the systematic literature review (Study I).

	Bone marrow	**Laboratory analysis**
MeSH major topic	bone and bones / analysis bone and bones / chemistry bone and bones / diagnosis bone marrow / analysis bone marrow / chemistry bone marrow / diagnosis bone marrow examination intraosseous infusion	blood gas analysis point-of-care systems point-of-care testing blood chemical analysis / methods
Search terms Title/Abstract	intraosseous bone marrow	point-of-care laboratory analysis blood gases chemistry

The selection process was independently performed by two authors (Jousi and Nurmi) by first screening the titles and abstracts, and if needed, the full text versions of the articles. The selected articles were assessed for eligibility in relation to the inclusion criteria. The references of the selected articles were reviewed to find additional relevant articles. From the selected articles, the data were sought for the following variables: the target population, sample size, equipment used in sampling and analyses, sample site, success rate of the analyses, blood discarded before sample withdrawal, analysed parameters, statistical methods, funding sources and findings.

For the quality assessment of the included studies, two authors (Jousi and Laukkanen-Nevala) individually assessed the studies and rated them on a self-created scale (3–0), focusing on the use of proper statistical methods in the studies (**Table 5**). *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics* (96) and *CLSI EP09-A3 Measurement Procedure Comparison and Bias Estimation using Patient Samples* (95) were used as references.

Table 5. The quality assessment of the studies in the systematic literature review.

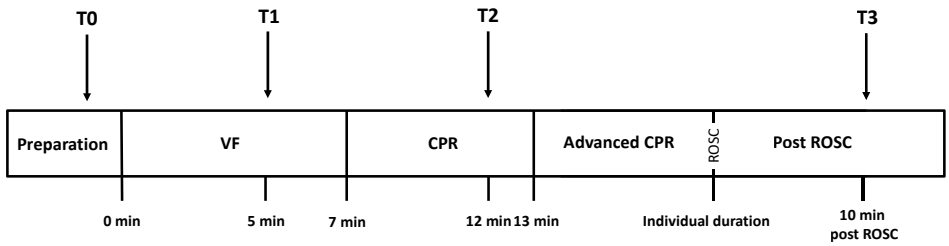
3	The Bland–Altman method has been used and the limits of agreement have been reported
2	The Bland–Altman method (proper or modified) has been used but the information about variation is absent
1	Statistical methods are insufficient for method comparison studies (such as correlation or comparison of grouped means)
0	Information for evaluating the statistical methods is insufficient

4.3 STUDY SETTINGS AND COHORTS OF THE OBSERVATIONAL STUDIES

In the first observational study (Study II), we studied the feasibility and reliability of IO POC analyses within healthy individuals. For the study, 31 voluntary, healthy paramedic students from *Saimaa University of Applied Sciences* were recruited.

We organised, based on the students' own wish, a training session where paramedic students practiced IO needle insertion on each other under supervision. During the training session, we collected blood samples from IO access, radial artery and antecubital vein from each participant. We used sterile technique including facial masks, sterile gloves and surgical skin disinfection with alcohol to reduce the risk of infection for the volunteers. The volunteers were all healthy and over 18 years of age. After risk assessment, we decided to perform IO needle insertion to the proximal tibia to minimise the risk of complications. Exclusion criteria were immunocompromising condition or medication, skin infection around the puncture site, pregnancy and breast feeding.

In the second observational study (Study III), we studied using an experimental animal model the relation of IO blood samples compared with arterial and venous samples during different phases of CA and CPR. Because studying the subject in real-life situations is not possible, we participated in an experimental CA model designed for examining the effect of 50% compared with 100% inspired oxygen fraction on brain oxygenation and post-CA mitochondrial function (101). We took blood samples at four pre-defined time points from the IO space, the femoral artery and the central vein from 23 anesthetised pigs (**Figure 9**).



T0	Blood sample at baseline before induction of VF
T1	Blood sample 5 minutes after induction of VF
T2	Blood sample 5 minutes after initiation of CPR
T3	Blood sample 10 minutes after ROSC
VF	Ventricular fibrillation
CPR	Ventilation (FiO_2 50% or 100%) and chest compressions (100/min) with LUCAS®
Advanced CPR	Defibrillation every 2 min, adrenalin 1 mg every 2 min until ROSC or at least 20 min
ROSC	Return of spontaneous circulation

Figure 9. Timeline of the experiment (Figure from Study III, reprinted with permission from *Resuscitation*).

For the protocol, CA (VF) was electrically induced in the pigs with a 4-V electrical current via a pacing wire inserted into the right ventricular wall. VF was left untreated for 7 minutes to simulate a real-life scenario. After 7 minutes, CPR was initiated with mechanical chest compressions (LUCAS®) at a frequency of 100 per minute and manual bag valve ventilations at a frequency of 10 per minute. After

6 minutes of CPR, defibrillation was performed. If sinus rhythm was not achieved, resuscitation was continued with defibrillations (if still in a shockable rhythm) and boluses of adrenaline every 2 minutes until ROSC or for at least 20 minutes, i.e. 27 minutes from CA. In the end, the pigs were euthanised with a lethal dose of potassium chloride (40 mmol).

In the third observational study (Study IV), we took IO and arterial samples from 33 emergency patients treated by the physician-staffed Helicopter Emergency Medical Services (HEMS) unit during routine missions. Two out of 35 patients were excluded owing to a reversal of consent. The included patients were critically ill adults (minimum age of 18 years) who needed prehospital POC laboratory diagnostics and an IO access for the treatment of acute illness or accident, based on the treating physician's assessment. The IO access was never inserted only for taking blood samples. Patients in CA were excluded from this study protocol, but the inclusion after ROSC was possible. The blood samples were taken within maximum a 5-min (pO_2 and pCO_2) or 15-min (all the other values) time gap, and significant physiological changes were not allowed to have happened between the two samples. No medications or fluids were allowed to be infused to the IO access before taking the sample. The study protocol was registered beforehand in the Clinical Trials database (NCT03746496).

4.4 BLOOD AND INTRAOSSEOUS SAMPLES AND POINT-OF-CARE ANALYSES

To obtain samples from the IO space, we inserted 15G 25-mm (to the proximal tibia, Studies II and III) or 45-mm (to the proximal humerus, Study IV) IO needles using the Arrow® EZ-IO® device. In the Study III, we inserted a new needle for each repeated sample. We drew the initial 0.5–2 ml blood (Studies III and IV) from the IO space without discarding any waste blood. In Study II, we took two repeated IO samples (the first was the initial 0.5 ml and the second was taken after the removal of 2-ml waste blood) to evaluate the need for discarding waste blood before actual sampling.

The control samples for Study II were taken from the radial artery and antecubital vein. For the Study III, the control samples were taken via catheters in the internal jugular vein and femoral artery. For the Study IV, the control samples were taken from the radial, brachial or femoral artery of the patient through a 20G arterial cannula or via a single puncture.

For all samples, 3-ml dry heparin (70 IU) blood gas syringes were used. If difficulties with drawing an IO sample from the IO space using a dry heparin syringe were encountered, a normal 2-ml syringe was used for aspiration, and the sample was immediately injected into a heparinised syringe.

All samples were analysed using an i-STAT® Handheld or i-STAT® Alinity (in the Study IV from 15.1.2018 onwards owing to a change in the equipment of the HEMS unit) POC device with CG4+ and CG8+ cartridges (in the Study IV, only CG8+ cartridges were used) (**Table 6**). We analysed the following parameters that we consider to be the values of interest in critically ill patients and during CA: pO_2, pCO_2, BE, HCO_3, pH, lactate, Na, K, iCa, glucose, Hb and Hct.

Table 6. Laboratory parameters in the CG4+ and CG8+ i-STAT® test cartridges used in the studies. pCO_2, partial pressure of carbon dioxide; pO_2, partial pressure of oxygen; TCO_2, total carbon dioxide; HCO_3, standard bicarbonate; BE, base excess; sO_2, oxygen saturation; Na, sodium; K, potassium; iCa, ionised calcium; Hct, haematocrit; Hb, haemoglobin. *calculated

CG4+	CG8+
lactate	Na
pH	K
pCO_2	iCa
pO_2	glucose
TCO_2*	Hct
HCO_3*	Hb*
BE*	pH
sO_2*	pCO_2
	pO_2
	TCO_2*
	HCO_3*
	BE*
	sO_2*

4.5 QUESTIONNAIRE

For Study IV, we wanted to define the maximal acceptable bias between IO samples and arterial samples in clinical practice. We sent a questionnaire via e-mail to 16 experienced prehospital emergency physicians from different parts of Finland. We asked them the extent of the bias they would be ready to accept when treating a critically ill prehospital patient if they only had the POC IO analysis results available without having a possibility to obtain arterial or venous analyses. We compared their answers with the measured biases in our study population and then evaluated whether the measured biases in the study setting would be acceptable in clinical practice.

4.6 STATISTICAL METHODS

In the Studies II and IV, we used the Bland–Altman method to calculate the bias and the 95% LOA between IO and blood sample values. The Bland–Altman method yields an informative graph regarding the agreement, wherein the individual differences between the results measured using the two different methods are plotted against the mean values of the measurements to display the average bias and the LOA. In general, the bias represents systematic error, and the LOA represent the random error describing the variation of the differences (**Figure 10**).

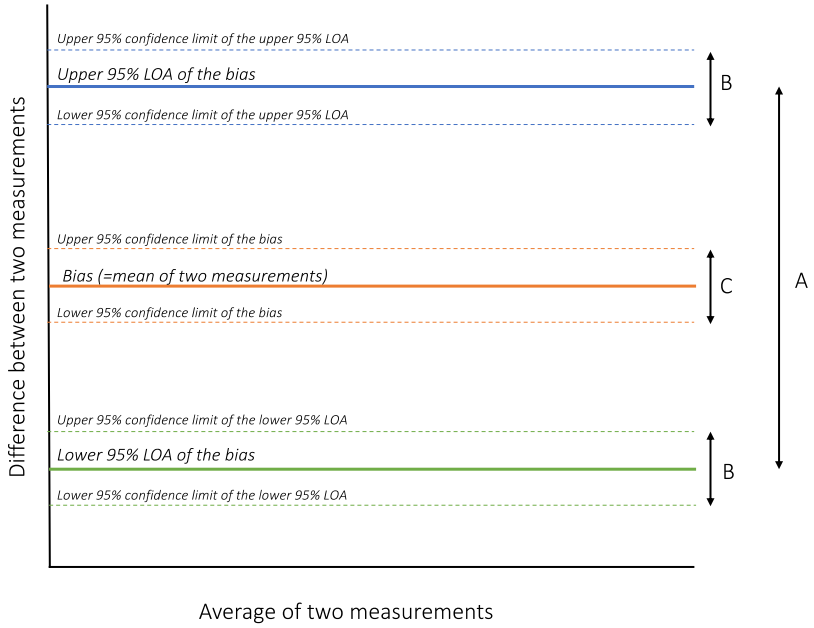


Figure 10. A diagram explaining the principles of the Bland–Altman graph.

A Agreement interval (±1.96 standard deviation of the bias, should contain 95% of the future bias measurements)

B 95% confidence interval of LOA (±1.96 standard error of the limit of agreement, reports the precision of estimation)

C 95% confidence interval of bias (±1.96 standard error of the bias, reports the precision of estimation)

LOA Limit of agreement

To assess the reliability of the measurements in Study II, we calculated intraclass correlation coefficients (ICC) with 95% confidence intervals (CI) based on a single measurement, consistency, 2-way mixed-effects model. ICC is a reliability index usually used in test-retest, intra-rater and inter-rater reliability analyses (102). There are 10 forms of ICCs, each involving distinct assumptions in their calculation and

leading to different interpretations. The reporting of ICCs should always include information about the type, model and definition. The reliability can be interpreted (based on the 95% CI of the ICC estimate) as follows (**Table 7**):

Table 7. Intraclass correlation coefficient (ICC) values and how they indicate the reliability of the analyses.

ICC	
<0.5	poor
0.5–0.75	moderate
0.75–0.9	good
>0.9	excellent

In Study III, we plotted the laboratory parameters at different time points to demonstrate the development of the values during CA and CPR and presented the data in the plots as means with 95% CIs. We also calculated the individual differences between resuscitation samples (IO, artery and vein) and the pre-arrest arterial samples and represented the differences as boxplots to demonstrate the change of laboratory values from the pre-arrest state.

In Study IV, we evaluated the factors related to the technical failures of the POC IO analyses by comparing the data from patients with failed analyses with that from the patients with successful analyses. We calculated the statistical significance of the differences of the demographic factors using the Mann-Whitney U-test for continuous variables and χ^2 -test for categorical variables. For proportions, we calculated the 95% CI using the modified Wald method.

All graphs are produced with GraphPad Prism (versions 7.0 a–8.1.0). Sample size calculations were a compromise between achieving clinically sufficient CI for the bias and the LOAs and the difficulty with sample collection. Bland et al. recommend sample sizes up to 100 – 200 for method comparison studies; however, this would have been impossible to achieve in our study settings (10). In general, sample size calculation for the Bland–Altman method is based on the SD of the differences (d) between the two measurement methods and the desired CI of the LOA (103). The standard error (SE) of the 95% LOA is approximately

$$\sqrt{\frac{3s^2}{n}}$$

where s is the SD of the differences (d) and n is the sample size. The confidence interval is $d \pm 1.96\, s \pm 1.96$ SE. When the desired accuracy of the LOA is determined, the sample size can be calculated using this formula (**Table 8**).

Table 8. Examples of the effect of different sample sizes on the confidence interval of the bias and the limits of agreement. (CI, confidence interval; SD, standard deviation)

Sample size	95% CI
10	± 1.1 SD
12	± 1 SD
30	±0.6 SD
50	±0.5 SD
100	± 0.3 SD
200	±0.2 SD
300	±0.1 SD

The sample size in Study II was estimated based on the sample sizes in the previous publications because the SD of the biases between the IO and arterial/venous samples was unknown. The sample size in Study IV was calculated a priori based on the SD of the measured biases between the IO and arterial measurements in Study II because information about the SD of the biases from critically ill patients was not available. By including 35 patients in the study, we were prepared for the possible missing data from five patients. With a sample size of 30 patients, the confidence marginals of the 95% LOA for the laboratory parameters were considered sufficient (**Table 9**).

Table 9. Confidence marginal (±) of the 95% LOA calculated with the sample size of 30 patients based on the SD of the values in Study II. (Na, sodium; K, potassium; iCa, ionised calcium; BE, base excess; HCO_3, standard bicarbonate; pO_2, partial pressure of oxygen; pCO_2, partial pressure of carbon dioxide; Hb haemoglobin; Hct, haematocrit)

Parameter	Confidence marginal
Na (mmol/l)	1.76
K (mmol/l)	0.63
iCa (mmol/l)	0.043
BE (mmol/l)	0.72
HCO_3 (mmol/l)	0.9
pO_2 (kPa)	1.23
pCO_2 (kPa)	0.37
Hb (g/l)	25.3
Hct (g/l)	7.45
Glucose (mmol/l)	0.26
Lactate (mmol/l)	0.20

4.7 ETHICAL ASPECTS

All the research projects were investigator-initiated and conducted according to the principles of the Declaration of Helsinki.

The study protocol for Study II was approved by the Coordinating Ethics Committee of Helsinki University Hospital (250/13/03/00/15, 13.10.2015) and the Helsinki University Hospital Board (§67-11/2015, 11.11.2015). A written informed consent was obtained from all the participants and a follow-up was organised in case of complications.

The study protocol for Study III was approved by the Finnish National Animal Experiment Board (ESAVI/1077/04.10.07/2016) and the Helsinki University Hospital Board (HUS/215/2016, §7 30.3.2016). The study adhered to the *Animal Research: Reporting of In Vivo Experiments (ARRIVE)* guidelines.

The study protocol for Study IV was approved by the Ethics Committee of the Helsinki University Hospital (31§/HUS/439/2017, 7.3.2017) and the Helsinki University Hospital Board (§17HUS/513/2017, 5.4.2017). Delayed informed consent of the patients or the next of kin was obtained before including the laboratory results in the analyses, as approved by the Ethics Committee.

5 RESULTS

5.1 PARTICIPANTS

The two observational studies and the experimental study contain 87 participants in total, 23 of them being animals (**Table 10** and **Figure 11**).

Table 10. Characteristics of the study participants. Continuous variables presented as medians (IQR). * at T0 before induction of VF. (IQR, interquartile range; GCS, Glasgow coma score; BP, blood pressure; SpO_2, peripheral oxygen saturation; Hb, haemoglobin)

	Study II	**Study III**	**Study IV**
n	31	23	33
Characteristics	healthy voluntary paramedic students	healthy landrace pigs	critically ill prehospital patients
Age	24 (22–27)	young	58 (39–70)
Weight, range (kg)		26–38	
Gender (male)	42%	both	61%
GCS			3 (3–4)
Systolic BP (mmHg)			121 (96–142)
Heart rate (min^{-1})			89 (70–114)
SpO_2 (%)			97 (95–100)
pH art	7.39 (7.41–7.43)	7.56 (7.50–7.61)*	7.30 (7.22–7.38)
Hb art (g/l)	145 (134–159)	88 (82–92)*	136 (126–146)

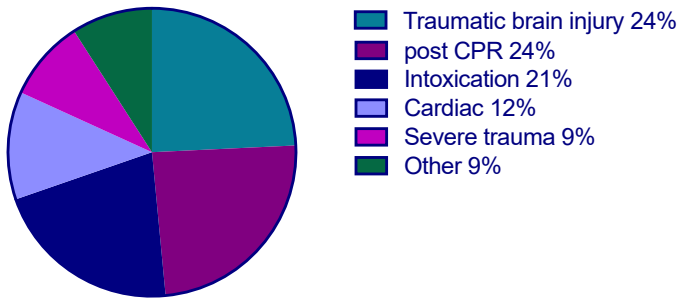


Figure 11. The medical conditions of the patients included in Study IV. (CPR, cardiopulmonary resuscitation)

5.2 SYSTEMATIC LITERATURE REVIEW

The systematic search of the articles comparing the agreement of IO samples with arterial or venous samples yielded 27 articles to be included in the qualitative synthesis (**Figure 12**).

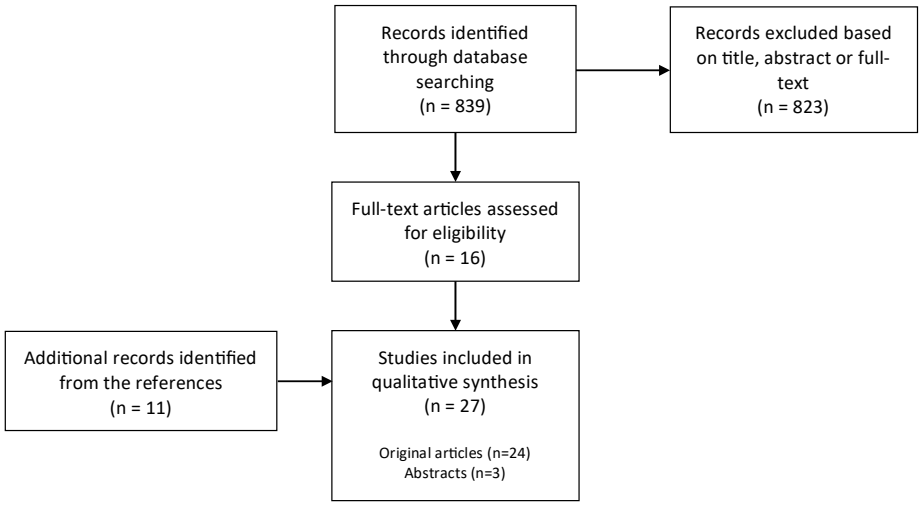


Figure 12. The study selection.

The included studies were heterogenic by populations, the haemodynamic state of the participants, sample sizes and statistical methods (**Table 11**).

Table 11. Characteristics of the 27 studies included in the systematic literature review.
* n > 27 because in some studies, several statistical methods were used. (POC, point-of-care; IO, intraosseous)

Study population	humans *n* = 8 animals *n* = 18 both *n* = 1
Haemodynamic state	unstable animals *n* = 9 unstable humans *n* = 1 stable animals *n* = 9 stable humans *n* = 8
Sample size	mean 15 median 12 range 5–34
Comparator	venous blood *n* = 18 arterial blood *n* = 2 both *n* = 7
Analyser	POC *n* = 5 laboratory *n* = 22
Waste blood	reported having discarded 1.5–2 ml *n* = 12
Conflicting interests with an IO device manufacturer	reported *n* = 6
Statistical methods used for comparison *	correlation *n* = 6 comparison of group means *n* = 14 bias without variation *n* = 9 bias with variation *n* = 3

Three studies included in the systematic review followed the recommended statistical guidelines for method comparison studies, which include the calculation of bias and the reporting of the variation of the differences (69,82,86). The populations in the three studies were heterogeneous, comprising haemodynamically unstable animals (n = 14), critically ill emergency patients (n = 17) and haemodynamically stable, anaesthetised paediatric haematologic patients (n = 20). In two of these studies, POC analysers were used for analyses.

5.3 FEASIBILITY OF THE ANALYSES

Analyses of the IO samples were technically successful in 90% (CI 74–97) of the healthy voluntary paramedic student population (Study II). We observed a trend towards lower overall success rate (70%, CI 53–83) in the critically ill prehospital patient study population (Study IV), $p = 0.06$. The patients whose IO sample analyses were technically unsuccessful were older and had higher GCS than the ones whose analyses were successful (**Table 12**).

Table 12. Characteristics of the patients with technically unsuccessful and successful IO sample analyses in Study IV.

Data are presented as medians (IQR) or proportions. p values are calculated with χ^2 test for categorical variables and the Mann-Whitney U-test for continuous variables. (GCS, Glasgow coma score; BP, blood pressure; SpO_2, peripheral oxygen saturation; Hb, haemoglobin; art, arterial)

	Technically unsuccessful IO sample analysis (n = 7)	Successful IO sample analysis (n = 23)	p value
Male	4 (57%)	17 (74%)	0.3966
Age (years)	74 (66–83)	47 (30–63)	0.0008
GCS	13 (3–15)	3 (3–3)	0.0056
Systolic BP (mmHg)	105 (89–147)	125 (103–142)	0.4855
Pulse rate (min^{-1})	84 (51–120)	86 (70–115)	0.4715
SpO_2 (%)	95 (88–96)	98 (95–100)	0.0985
pH art	7.31 (7.25–7.40)	7.30 (7.21–7.37)	0.5011
Hb art (g/l)	133 (126–161)	138 (130–145)	0.9453

The necessity to draw waste blood before obtaining the actual IO sample was studied in Study II by comparing the initial blood sample with the second sample taken after discarding 2 ml of waste blood. The agreement between the initial IO sample and the second sample was tested using the Bland–Altman method. The biases were clinically insignificant, except for Hb, Hct and potassium. In case of potassium, the initial IO sample had better agreement with arterial and venous samples than the second one (**Table 13**).

Table 13. Bias (95% CI) between the results of the initial and second IO samples (after 2 ml of waste blood was discarded) calculated using the Bland–Altman method. (CI, confidence interval; Na, sodium; K, potassium; iCa, ionised calcium; BE, base excess; HCO_3, standard bicarbonate; pO_2, partial pressure of oxygen; pCO_2, partial pressure of carbon dioxide; Hb, haemoglobin; Hct, haematocrit)

	Bias (95% CI)	
Na (mmol/l)	−0.14	(−1.17; 0.88)
K (mmol/l)	−0.77	(−1.17; −0.38)
iCa (mmol/l)	0.02	(−0.01; 0.05)
pH	−0.02	(−0.03; −0.01)
BE (mmol/l)	−0.32	(−0.69; 0.04)
HCO_3 (mmol/l)	0.2	(−0.15; 0.55)
pO_2 (kPa)	−0.26	(−1.06; 0.54)
pCO_2 (kPa)	0.25	(0.11; 0.39)
Hb (g/l)	11.52	(−3.69; 26.72)
Hct (%)	3.41	(−1.07; 7.89)
Glucose (mmol/l)	0.07	(0; 0.13)
Lactate (mmol/l)	−0.05	(−0.12; 0.01)

5.4 AGREEMENT OF INTRAOSSEOUS SAMPLES WITH ARTERIAL AND VENOUS SAMPLES

We defined the agreement of IO values with arterial and venous values using the Bland–Altman statistical method in three studies (Studies II–IV). The following figures present the synthesis of the agreement data from several sources: the observational studies (Studies II and IV), experimental study (Study III), three methodologically correct studies from the systematic literature review (Study I) and two recent methodologically correct studies that have been published after the data collection period of the systematic review (**Figure 13**).

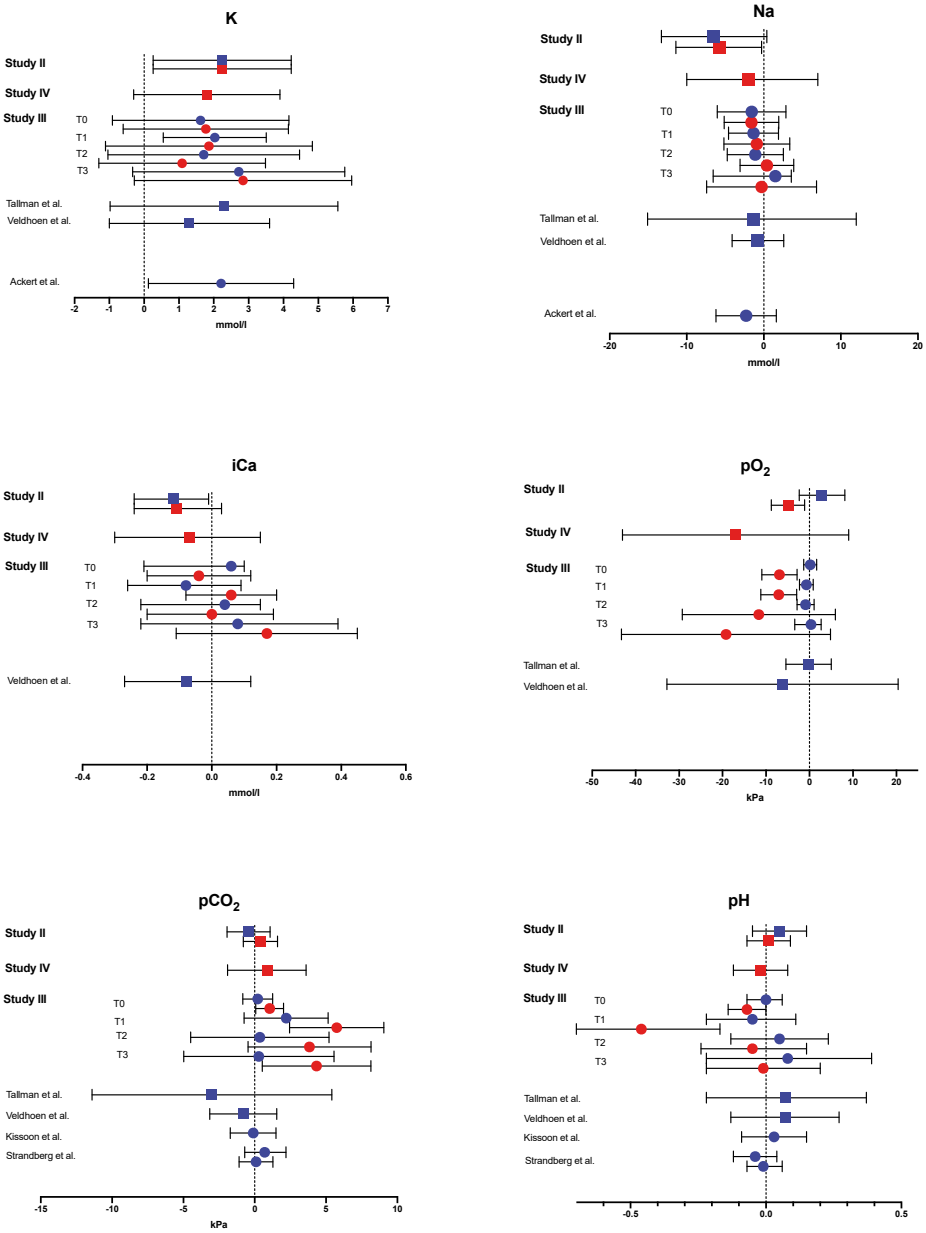
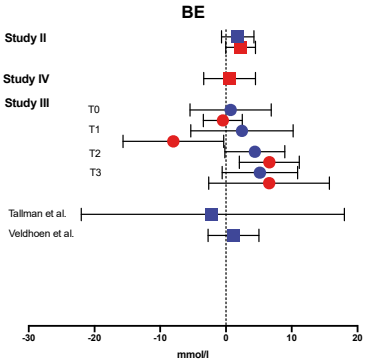
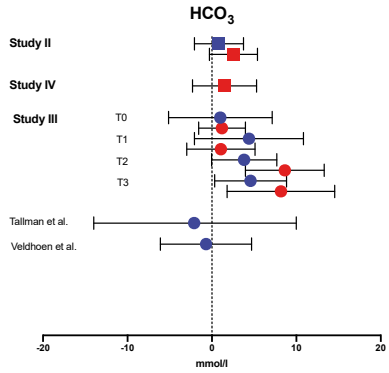
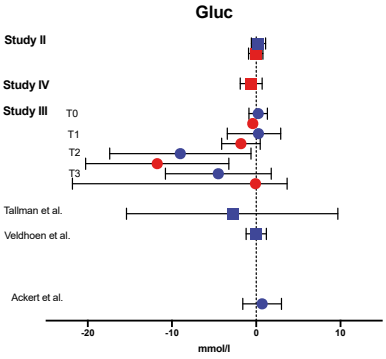


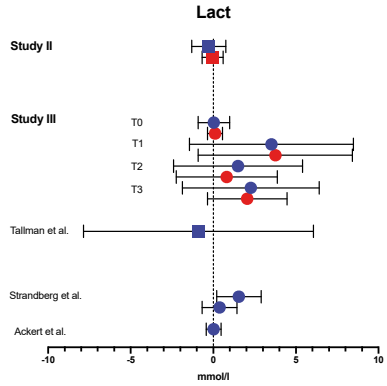
Figure 13. Combined data about the agreement between IO samples and arterial or venous samples from the following studies: the observational studies of the dissertation (Study II–IV), methodologically correct studies from the systematic literature review (Study I) (Tallman et al., Veldhoen et al., Kissoon et al.) and the methodologically correct studies published after the data collection period of the systematic literature review. (Ackert et al., Strandberg et al.) (69,78,82,86,87)

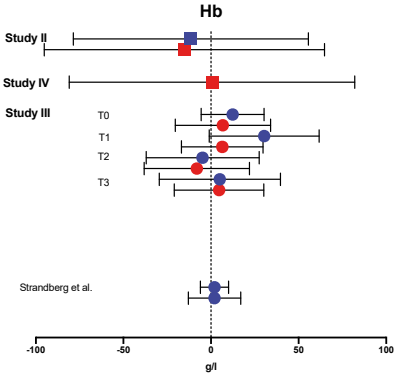
Each row displays the bias and the 95% LOA of the measured parameter. The squares represent human studies and the dots represent animal studies. The blue colour indicates agreement of the IO samples with venous samples and the red colour indicates the agreement with arterial samples.

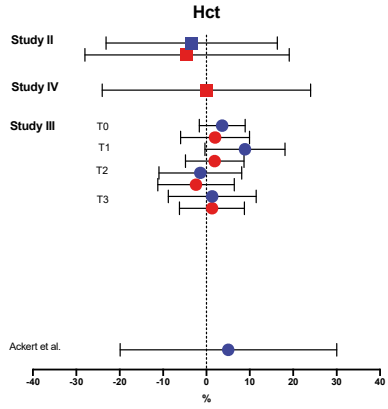




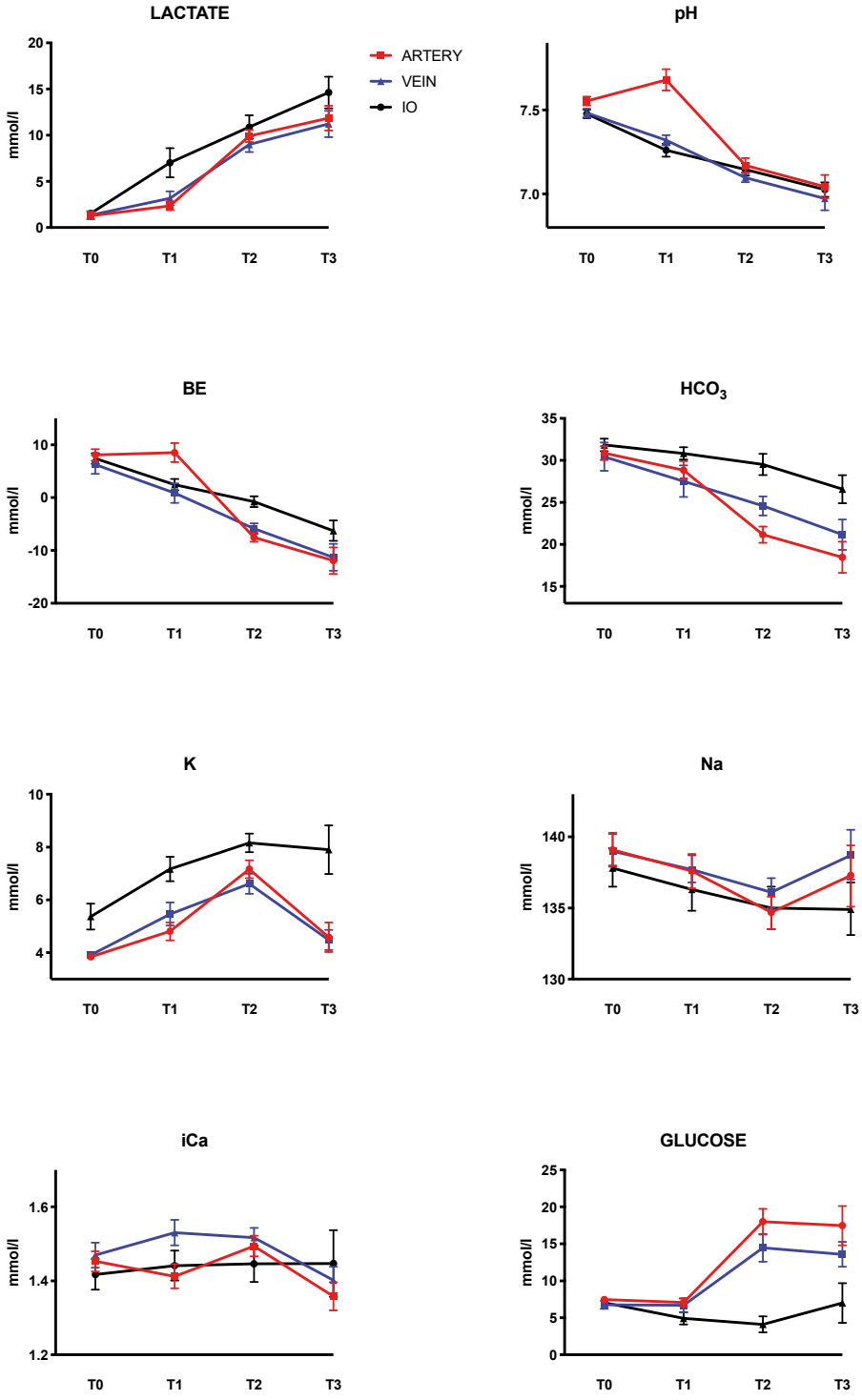








For the Study III, the following timepoints of blood samples are represented:
T0 before CA
T1 five minutes after the initiation of VF
T2 five minutes after the initiation of CPR
T3 ten minutes after ROSC


LACTATE
ARTERY
VEIN
IO
mmol/l
20
15
10
5
0
T0
T1
T2
T3
pH
7.5
7.0
BE
10
0
-10
-20
HCO3
35
30
25
20
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K
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Na
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135
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iCa
1.6
1.4
1.2
GLUCOSE
25

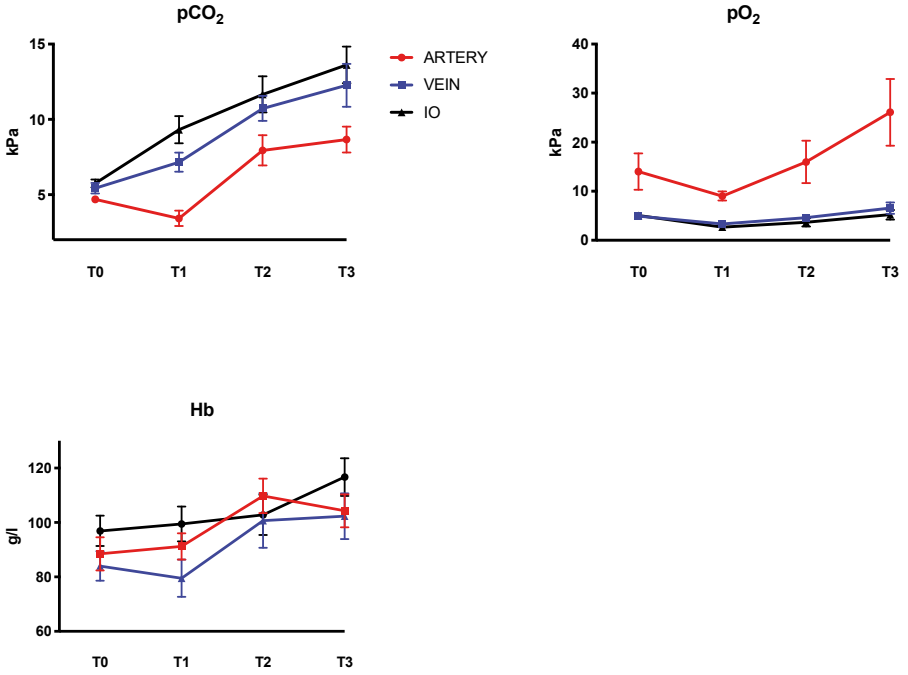

Figure 14. The dynamic change of the POC values from IO, arterial and venous samples. Data are presented as means (95% CI). **T0** = baseline, before cardiac arrest, **T1** = after five minutes of untreated VF, **T2** = five minutes after the initiation of cardiopulmonary resuscitation, **T3** = ten minutes after return of spontaneous circulation. (Figure from Study III, reprinted with permission from *Resuscitation*)

The clinical relevance of the bias, based on our questionnaire to 16 prehospital critical care physicians, is highly dependent on the clinical context, specific patient and laboratory parameters in question. Determining an acceptable standard bias level without clinical context was considered to be difficult. In answer to a generalised question about an acceptable bias level between IO and arterial POC measurement in the treatment of a critically ill patient when only the IO POC results are available, the following bias values were received (**Table 14**).

Table 14. Prehospital critical care physicians' (*n* = 16) opinions on the largest acceptable bias between IO and arterial/venous values. Data presented as medians (IQR). (IQR, interquartile range; Na, sodium; K, potassium; iCa, ionised calcium; BE, base excess; HCO_3, standard bicarbonate; pO_2, partial pressure of oxygen; pCO_2, partial pressure of carbon dioxide; Hb, haemoglobin; Hct, haematocrit)

	Maximum accepted bias	
Na (mmol/l)	5	(4.3, 7.5)
K (mmol/l)	0.5	(0.2, 0.6)
iCa (mmol/l)	0.1	(0.05, 0.16)
pH	0.05	(0.02, 0.08)
BE (mmol/l)	2.0	(1.0, 3.0)
HCO_3 (mmol/l)	3.0	(2.0, 5.0)
pO_2 (kPa)	1	(0.6, 1.5)
pCO_2 (kPa)	0.5	(0.3, 1.0)
Hb (g/l)	10	(10, 14)
Hct (%)	5	(3, 5)
Glucose (mmol/l)	0.6	(0.5, 1.0)

5.5 POINT-OF-CARE ANALYSIS OF INTRAOSSEOUS SAMPLES DURING CARDIAC ARREST AND RESUSCITATION

In Study III, we demonstrated the dynamic change of the common laboratory values during resuscitation and that IO, arterial and venous values change differently from another (**Figure 14**).

We studied how the samples taken during CPR represent the pre-arrest state and whether there are differences in the samples obtained from different sampling sites. The differences between arterial, IO and venous CPR samples compared with the 'golden standard' arterial pre-arrest sample are displayed in **Table 15**.

Table 15. Differences between the values of the samples drawn during cardiopulmonary resuscitation (T2) compared with the arterial value before cardiac arrest (T0). Parameters are presented as medians (IQR).

ART = Arterial value during cardiopulmonary resuscitation (T2) *minus* arterial value before cardiac arrest (T0).
IO = IO value during cardiopulmonary resuscitation (T2) *minus* arterial value before cardiac arrest (T0).
VEIN = Venous value during cardiopulmonary resuscitation (T2) *minus* arterial value before cardiac arrest (T0).

(BE, base excess; HCO_3, standard bicarbonate; K, potassium; Na, sodium; pCO_2, partial pressure of carbon dioxide; pO_2, partial pressure of oxygen; iCa, ionised calcium; Hb, haemoglobin)

	ART	IO	VEIN
Lactate (mmol/l)	8.6 (10.0 – 7.5)	9.3 (11.3 – 8.1)	7.7 (9.3 – 6.0)
pH	–0.38 (–0.33 – –0.46)	–0.41 (–0.35 – –0.46)	–0.45 (–0.38 – –0.53)
BE (mmol/l)	–15 (–14 – –18)	–9 (–7 – –11)	–14 (–12 – –17)
HCO_3 (mmol/l)	–9.8 (–8.3 – –12.8)	–0.8 (0.4 – –3.2)	–6.7 (–3.8 – –7.8)
K (mmol/l)	3.3 (4.0 – 2.7)	4.4 (4.9 – 3.9)	2.8 (3.7 – 1.9)
Na (mmol/l)	–5 (–4 – –5)	–3 (–2 – –6)	–3 (–2 – –4)
pCO_2 (kPa)	2.9 (4.6 – 1.9)	6.5 (9.2 – 5.9)	6.2 (7.4 – 4.4)
pO_2 (kPa)	1.1 (10.6 – –1.6)	–8.6 (–7.5 – –10.3)	–7.3 (–6.3 – –8.8)
Glucose (mmol/l)	11.7 (13.0 – 8.9)	–3.8 (–2.6 – –5.4)	8.2 (10.6 – 4.5)
iCa (mmol/l)	0.05 (0.07 – 0.02)	0.0 (0.1 – –0.1)	0.1 (0.1 – 0.0)
Hb (g/l)	24 (27 – 17)	17 (24 – 10)	21 (24 – 0)

The main findings of point-of-care analyses during CPR:

- Acidaemia was detectable in IO and venous samples during untreated VF but in arterial samples only after the initiation of CPR.
- The potassium values from the IO, arterial and venous samples were higher during CPR than the pre-arrest values [mean elevations of 4.4 mmol/l (SD 0.72), 3.3 mmol/l (SD 0.78) and 2.8 mmol/l (SD 0.94), respectively].
- The elevated glucose values were detected in arterial and venous samples during CPR but not in IO samples.
- The pO_2 and pCO_2 values from IO samples closely followed those from the venous samples.

6 DISCUSSION

6.1 RATIONAL BASIS OF THE STUDIES

Several previous studies suggest good correlation between IO and venous/arterial samples (7-9,82,84). Accordingly, one of the leading IO device manufacturers reports statistically significant correlations between IO and venous samples for several parameters, including Hb and Hct measurements (47). However, a review of the previous studies presents conflicting results for different parameters. This might be a result of various, partly insufficient statistical methods used in the studies.

Method comparison is common in laboratory medicine, where equipment and method development require regular comparison between the new and the existing, golden standard method. A fundamental publication in *Lancet*, by Bland and Altman in 1986 provides important statistical guidelines for assessing the agreement between two methods of clinical measurement (10). Most of the earlier studies evaluating the analyses of IO samples have not followed the recommended statistical methods; the conclusions are based on determining the correlation or comparison of grouped means.

In general, it is very unlikely that two different methods would exactly agree and provide identical results for all individuals (10). POC testing carries an error marginal by the method itself as well as the comparison of arterial with venous blood-gas measurements (104,105). Important measures are the amount and direction that the new method differs from the old one and whether the measurement bias is systematically higher/lower or varies within the measurement range. By defining the bias level and type, adjustments can be made for using the new method.

All published studies comparing IO samples with arterial or venous samples, including our own studies, fail to provide an unequivocal answer for whether IO samples can be used for laboratory analyses. They can suggest a direction, depending on the parameter and situation, but they cannot provide an explicit solution, mainly owing to the insufficient sample sizes and thus power of the studies. The correct way to plan a method comparison study is to first pre-specify the largest clinically acceptable bias between the measurements: for example, by a Delphi-method with clinical experts (103). This is difficult, because all variables and various clinical scenarios need to be considered. The second step is to define the null hypothesis; for example, a hypothesis that the two methods agree. In practice, this means that the maximum allowed difference between the measurement methods (Δ) would fall outside the 95% CI of the LOA (**Figure 15**). The sample size can then be calculated based on the expected bias, SD of the individual biases and maximum allowed difference (Δ), considering the desired alpha and beta error levels. A problem here

is the lack of previous information about the SD of the individual biases from the study population.

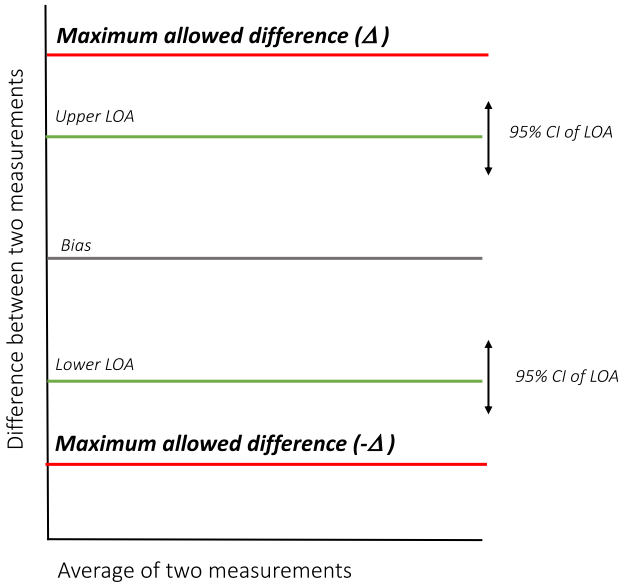

Figure 15. Defining the sample size for the Bland–Altman method. The maximum allowed difference should fall outside the 95% CI of the LOA. (LOA, limit of agreement; CI, confidence interval).

In practice, achieving studies with required sample sizes, especially within critically ill patients, is demanding; therefore, the possibility of proving the null hypothesis is theoretically almost impossible. Rejecting the null hypothesis owing to insufficient sample sizes and concluding that IO samples cannot be used for POC analyses would mean abandoning a potentially useful method because of inadequate statistical power. However, the subject needs more research, and especially using the Bland–Altman method instead of correlation. A collection of studies with small sample sizes might though be more beneficial than no studies or large studies with incorrect methods.

Our first observational study (Study II) was primarily planned to assess the feasibility of the IO POC analyses within healthy volunteers. Two earlier studies using healthy voluntary subjects for IO sample analyses have been published; however, the comparison of the values was performed using correlation or comparison of grouped means (9,84). The problem with using laboratory values of healthy voluntary subjects is that the values are usually within the reference range and the information about agreement outside of the normal levels remains unknown.

In practice, POC analysis of IO samples would most likely be applied within the critically ill patients for whom IV access cannot be achieved and POC analyses

are needed. Our prehospital study is the second one to assess IO sample analyses within critically ill patients, apart from a study in the hospital ED (69). In the hospital study, the patients were more severely ill: non-traumatic CA patients and other critically ill patients requiring immediate resuscitation, resulting in 47% mortality in the ED and 76% during hospitalisation. So far, no other studies have been conducted with unstable human patients. Important insights into the effects of unstable haemodynamics in the POC analyses of IO samples can be gathered from the unstable animal models (70-75,81,82,90). However, their results might not be applicable to the population of critically ill patients.

6.2 FEASIBILITY OF THE ANALYSES

In our first observational study (Study II), the success rate was high in the analyses of young healthy volunteers (90%, CI 74–97). Surprisingly, the success rate was lower (70%, CI 53–83) in the analyses of critically ill prehospital patients (Study IV). Tallman et al. reported in their study with critically ill hospital patients that they did not have significant technical difficulty in obtaining marrow samples and analysing them using the EPOC® POC analyser (69). They did not report the success rates, and one of the inclusion criteria was that a marrow sample could be obtained. Some patients might not have been included owing to the difficulty in obtaining an IO sample. The median age of the included patients in their study was 67 years (range of 12–93 years). The median (range) ages of our patient samples in the Studies II and IV were 24 (19–36) and 58 (18–90) years, respectively.

Reporting of the success rates varies within the other previous studies. Eriksson et al. reported in their studies with porcine models with repeated samples that IO aspirates were easily obtained during the first hours of the experiment, although aspiration became more difficult over time (15,16,88). Montez et al. reported in their study of IO sample analysis using i-STAT® in a population of healthy volunteers a success rate of 23/30 subjects, the rest being excluded owing to clotting of the samples (84). In the study with patients subjected for diagnostic bone marrow examinations, Hurren reported having excluded 2/15 samples because of problems caused by fat and clotting (85). Abdelmoneim et al. reported that 4% of the samples within an unstable animal model could not be analysed owing to the clotting of blood in the analyser or difficulties in withdrawing the samples (73). The analyses in the studies by Hurren and Abdelmoneim et al. were performed with conventional laboratory equipment.

We first speculated that hypercoagulability during acute illness, environmental factors such as temperature or challenges in the sample analyses during the concomitant treatment of a critically ill patient could have contributed to the lower success rate. In our own studies, the IO samples from the prehospital patients were

taken from the proximal humerus, whereas the samples from the healthy volunteers were taken from the proximal tibia. We do not assume the sampling site to affect the success rate. The post-hoc analysis revealed that the patients with failed analyses were older than the ones with successful analyses. This could be explained by the gradual adiposity of the bone marrow with age. In clinical practice, this could indicate that the method of POC analysis of IO samples might not be applicable within elderly patients. Another statistically significant difference between the patient groups of failed and successful analyses was the level of consciousness. The reason and clinical relevance of this finding remain unknown.

6.3 AGREEMENT ANALYSES

Before new methods are adopted in clinical practice, their agreement with the established 'golden standard' method has to be verified. The general amount of bias, its direction and distribution throughout the measurement range should be examined. Considering whether the bias would make a clinical difference is important. Several previous studies assessing the usability of IO samples have made their conclusions based on insufficient statistical methods and small sample sizes. Often correlation has been interpreted as agreement.

We found that the reliability of potassium analyses from IO samples was insufficient owing to a systematic bias between IO and arterial and venous samples. Higher potassium values in the IO samples have been reported in several previous studies as well, which is consistent with our findings (8,9,77,78,85,86,92,106). Two previous studies have reported opposing results, but these studies did not use the Bland–Altman method for agreement analyses (7,79). One potential reason for hyperkalaemia in IO sample analyses could be haemolysis owing to the higher negative pressure needed for aspirations (**Figure 16**).

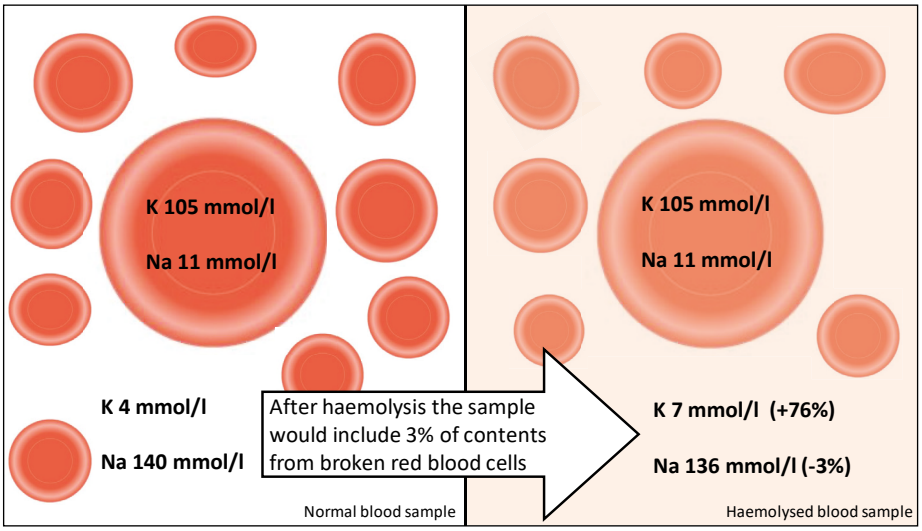

Figure 16. Schematic calculation of the effect of haemolysis on plasma sodium and potassium levels.

If the haemolysed sample would include 3% of contents from broken red blood cells , the plasma potassium level would increase as much as 76% (plasma [K] after haemolysis = 97% × 4 mmol/l + 3% × 105 mmol/l = 7 mmol/l) while the plasma sodium level would decrease by only 3% (plasma [Na] after haemolysis = 97% × 140 mmol/l + 3% × 11 mmol/l = 136 mmol/l) (96,107).

Our finding of unreliability within the Hb and Hct measurements has been confirmed only in one other study (78). On the contrary, several studies report that the Hb and Hct measurements from IO samples are highly predictive or reliable (7-9,85,92). Again, all these studies used other methods than the Bland–Altman. All three studies using proper statistical methods, included in our systematic literature review, did not analyse Hb or Hct values (69,82,86). In our studies, the general biases (the average of all paired measurement biases) for Hb and Hct were close to zero. If this bias value is interpreted without taking the LOA into account, the conclusions are false. For example, in our own studies, the bias in a single Hb measurement could be up to 50 g/l between IO samples and arterial/venous samples. If all the Hb measurements from IO samples would be pooled and compared with the pooled results of arterial Hb measurements, the averages might be equal even if the paired measurements for one individual might have a significant difference. In the case of Hb and Hct measurements, the wide LOA makes the agreement poor in general and contradicts analysing them from IO samples. This finding was constant in all our observational studies and may be attributable to the pre-analytical problems within POC analyses, such as coagulation of the sample before analysis. More likely explanations are the haematopoietic function of the bone marrow and the different cell compositions of peripheral blood and bone marrow.

6.4 POINT-OF-CARE TESTING DURING CARDIAC ARREST

Screening the reversible causes of CA is an important part of resuscitation protocols. A limited number of methods are available to achieve this in the prehospital setting. Investigating the medical history of the patient and the events and complaints preceding the CA is most important. POC ultrasound examination and POC laboratory analyses can be used to supplement the collected information. The knowledge of what the laboratory values during CA indicate or could be used to screen for is surprisingly limited. Moreover, the treatment options for electrolyte and acid–base balance abnormalities or the impact of their correction on the outcome lack evidence (6,108).

In general, the metabolites and electrolytes between the tissues and blood are in equilibrium, and blood sample analyses provide useful information about the tissues and metabolism. The low-flow state during CPR differs from stable circulation, and the assumptions from a stable circulation do not apply during CPR. The circulation and blood pressure during standard CPR markedly varies between individuals (109). The effect of mechanical chest compression devices should also be taken into account. It can be assumed that during CPR, the blood samples do not reflect the tissues in the same way as during stable circulation. Moreover, the samples taken from different anatomical sites may differ owing to the redistribution of the circulation. Central venous samples have been suggested to provide better estimation of acid–base balance than arterial samples (5,31). In practice, central venous blood samples are generally unavailable in resuscitations.

The difficulties in interpreting electrolyte abnormalities during CA are demonstrated in our experimental resuscitation study (Study III). When hyperkalaemia was detected in arterial, venous or IO samples during CPR, the potassium levels were often normal before CA. This indicates that in practice, if no anamnestic information about hyperkalaemia is present (history of renal insufficiency etc.), hyperkalaemia in a POC analysis during CPR might not necessarily indicate the reason for CA.

Normally, 98% of the potassium in the body is in the intracellular space. The normal extracellular potassium concentration is 3.5–5.0 mmol/l, whereas the intracellular potassium concentration is 140–150 mmol/l. Na–K pumps in the cell membranes are constantly maintaining the potassium-gradient over the cell membrane (110). Compromised blood flow to the tissues and hypoxia during CA cause acidosis, which in turn causes changes in the potassium distribution probably by reducing the activity of the Na–K pump and elevating the extracellular potassium concentration (111). Hyperkalaemia during CA often indicates deranged balance rather than the absolute excess of potassium in the body.

In a prospective prehospital study, Spindelboeck et al. demonstrated significant arterial acidosis within patients during ongoing CPR (33). The phenomenon is supported by findings in animal studies (32). In our experimental study, we demonstrate the differences between arterial, venous and IO samples during the dynamic development of the acidosis. Lactate elevation and pH and BE decrease were detectable in IO samples during untreated VF, whereas the same changes were detectable in arterial blood only after the initiation of CPR. This is an interesting finding and suggests that IO samples could provide a better estimation of tissue acidosis during CA than arterial samples. Moreover, our study demonstrated paradoxical alkalosis in the arterial blood during untreated VF, which has also been shown in the previous experimental studies (30,31,112).

In conclusion, POC values dynamically change during CA and CPR, and different sampling sites differently display the acid–base balance. As demonstrated by our experimental resuscitation model, the timing of the samples affects the agreement of IO samples. This finding would be difficult to prove in a clinical OHCA study because of the variation in the prehospital delays, sample collection timing and challenges with protocol compliancy. Animal models currently seem to be the only feasible way to examine the agreement between IO and conventional blood samples during CA and CPR in a standardised setting.

6.5 CLINICAL IMPLICATIONS

IO access has become a standard vascular access method both in prehospital and in-hospital emergencies when difficulties with the conventional methods are encountered. Even if several studies and guidelines state that IO samples can be used for laboratory analyses, the values should still be interpreted with caution. The consideration should always be related to the particular patient and the clinical situation. Based on our observational studies and the systematic review of the previous studies, it is easier to state when POC analyses of IO samples cannot be used. They cannot be used for analysing Hb and Hct values. Similarly, conclusions should not be drawn based on the potassium values from IO samples without considering the likely positive bias of up to 2–3 mmol/l between IO samples and arterial/venous samples. Excluding hyperkalaemia from a patient by using IO samples might be reasonable.

IO samples might be used for POC analyses for assessing the acid–base status or the sodium, calcium or glucose levels of the patient. Understanding that the IO sample is different from venous and arterial samples, like arterial blood is different from venous blood, is important. Interpretations from IO samples should be based on this understanding. If arterial or venous blood samples cannot be taken, IO POC results can be used to aid in clinical decision-making.

6.6 LIMITATIONS

The most important limitation is the insufficient power of the studies. The sample sizes in the observational studies (Studies II, III and IV) were smaller than the generally recommended size for method comparison studies. In the interpretation of Bland–Altman analyses, the bias alone is not a sufficient measure of agreement; however the LOA (which describes the variance of the individual biases) and the CI of the LOA provide very important information. The small sample size causes large CIs for the bias and LOAs; this precludes us from making clear conclusions based on the study results to uniformly support or contradict the use of IO samples for diagnostic use. Recommended sample sizes of 100–200 patients might be achieved if the samples are collected during diagnostic bone marrow aspirations; however, this would not provide important information of the values outside the reference ranges or the effects of unstable haemodynamics on the agreement. Note that the sample sizes in our studies (sample sizes of 23, 31 and 33), compared with other previously published studies (sample size range of 5–34), are above average.

Sample collection was not standardised in the experimental studies and might have resulted in pre-analytical errors. The samples were taken by professionals with limited training in laboratory methods. The amount of blood or bone marrow aspirate in the syringe varied and the dry-heparin-to-sample ratio was not constant. Excessive negative pressure could have been applied during the aspiration of the IO samples, thus causing haemolysis and hyperkalaemia. Incomplete air removal from the syringes might have altered the amount of gas dilution into the sample. Delays in sample analyses could have resulted in haemolysis, acidosis and hyperkalaemia. However, this represents the general conditions during prehospital blood collection and POC analyses, and the results from our studies are applicable to prehospital surroundings in general.

There is a possible selection bias within the patient sample in Study IV. Sample collection was performed during the routine HEMS missions without any additional research assistance, and the staff always prioritised patient treatment. The most time-critical and demanding patients were likely not included in the study because of the general workload. For the same reason, several values had to be excluded from the agreement analyses because of protocol violations mostly owing to the excess pre-defined time limit between IO and arterial samples. The strict inclusion criteria of patients requiring an IO access and POC laboratory analyses for treatment prolonged the recruitment period. Because of ethical reasons, opening an IO access only for taking study samples was not allowed. The calculated sample size was 30 patients; therefore, we collected samples from 35 patients to cover the possible missing data. The final number of paired measurements for the agreement analyses

remained under 30 owing to withdrawal of consents, protocol violations and failed analyses.

6.7 FUTURE DIRECTIONS

Ideally, the method of POC analysis of IO samples could be validated and specific reference values could be set for different parameters. Different manufacturers' POC devices should be compared to determine any differences in their performance. The validation studies should be based on proper sample size calculations and should include several hundred paired measurements, which is very challenging owing to ethical and consent issues and needs multi-centre studies to be achievable.

Blood typing and crossmatching from the IO samples are feasible and reliable within hospital patients undergoing diagnostic bone marrow aspirations (88,89). A prehospital transfusion protocol is present in many EMS systems, including drawing a crossmatch sample before the transfusion of O-negative red blood cells. If the patient is so hypovolemic that venous access cannot be achieved, drawing the crossmatch sample from the IO access would be very useful. A study on the feasibility and reliability of blood typing and crossmatching from prehospital IO samples should be performed to validate the method and confirm the results of in-hospital patient material.

The evidence supporting the laboratory screening of the reversible causes of CA is very limited. It is not completely evident what the electrolyte and acid–base values taken during CPR indicate and how do they reflect the pre-arrest state. Based on our experimental study, the elevated potassium levels during CPR, compared to the pre-arrest state, involve a risk of false positive diagnoses of hyperkalaemia as a cause of the CA. More evidence is required about the reliability and consequences of the laboratory analyses during CPR and whether the correction of electrolyte disorders affects outcome.

7 CONCLUSIONS

The conclusions of this doctoral dissertation are as follows:

1. Previous scientific evidence concerning the analysis of IO samples is scarce and it is based on 27 studies with heterogenic populations and small sample sizes. Majority of the subjects have been animals or haemodynamically stable humans. Only one previous study has been performed in a critically ill human population. Recommended statistical methods for method comparison studies were used only in three of these studies.

2. The analyses of IO samples using an i-STAT® POC analyser are generally feasible in young people. The method might not be applicable to older patients owing to the gradual increase in the adiposity of the bone marrow with age.

3. Drawing waste blood before obtaining the actual sample does not bring any benefit.

4. POC analysis of IO samples can be used for supporting decision-making in critical care as long as its limitations are recognised. Providing unambiguous interpretations about the acceptable agreement between IO samples and arterial/venous samples is not possible because the tolerance to bias and precision depends on the clinical context and specific parameter. The agreement between the POC results from IO and arterial samples varies for different parameters; however, the following general findings can be stated:
   - Potassium values from IO samples are generally higher than those from arterial and venous samples.
   - pO_2 and pCO_2 values from IO samples resemble those from venous samples.
   - Hb and Hct values from IO samples do not agree with those from arterial and venous samples and should not be used.
   - Glucose and lactate values form IO samples agree with those from arterial and venous samples during normal circulation. During CA, IO glucose values resemble the pre-arrest state, whereas hyperglycaemia is detected in arterial and venous blood. Elevated lactate levels are noticed in IO samples during untreated VF; however, in arterial and venous samples, they are detected only after the initiation of CPR.
   - Na and iCa have clinically sufficient agreement with arterial and venous samples.

- pH, BE and HCO_3 measurements from IO samples agree relatively well with those from arterial and venous samples, except during CA, when tissue acidosis develops differently in different blood compartments.

5. The POC laboratory values dynamically change during CA and CPR. The following remarks about how the IO, arterial and venous samples during CPR reflect the pre-arrest state can be pointed out:
   - Potassium values taken during CPR are higher than pre-arrest values in the venous, arterial and IO samples, leading to a risk of false interpretation of hyperkalaemia as a cause of the CA.
   - Developing acidaemia during CA is detectable in IO samples during untreated VF but in arterial samples only after the initiation of CPR.
   - During CPR, the lactate, pH, Na and iCa values from IO samples are similar to those from arterial and venous samples; thus providing an alternative to arterial and venous samples.

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